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# Airway Inflammation and Bronchial Hyperresponsiveness in Elite Cross-Country Skiers and in Patients with Newly Diagnosed Asthma: A Bronchial Biopsy Study

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ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Medicine of the University of Helsinki, for public examination in lecture room 2, Meilahti Hospital, on 13 June 2008, at 12 noon.

Helsinki 2008

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*"Those who do not make time for exercise will eventually have to make time for   illness"*

*The Earl of Derby, 1863*

# Abstract

The objective of these studies was to evaluate possible airway inflammation and remodeling at the bronchial level in cross-country skiers without a prior diagnosis of asthma, and relate the findings to patients with mild chronic asthma and patients with newly diagnosed asthma. We also studied the association of airway inflammatory changes and bronchial hyperresponsivess (BHR), and treatment effects in cross-country skiers and in patients with newly diagnosed asthma. Differences were also studied in airway inflammation between atopic and nonatopic asthmatics.

Bronchial biopsies were obtained from the subjects by flexible bronchoscopy, and the inflammatory cells (eosinophils, mast cells, T-lymphocytes, macrophages, and neutrophils) were identified by immunohistochemistry. Tenascin (Tn) immunoreactivity in the bronchial basement membrane (BM) was identified by immunofluorescence staining. Cell densities were counted and the thickness of Tn immunoreactivity was measured. Lung function was measured with spirometry, and BHR was assessed by methacholine (skiers) or histamine (asthmatics) challenges. Skiers with BHR and asthma-like symptoms were recruited to a drug-intervention study. Skiers were given treatment with placebo or budesonide (400 μg bid), and bronchial biopsies were obtained after 22 weeks' treatment. Patients with newly diagnosed asthma were given treatment for 16 weeks with placebo, salmeterol (SLM) (50 μg bid), fluticasone propionate (FP) (250 μg bid), or disodium cromoglicate (DSCG) (5 mg qid). Bronchial biopsies were obtained at baseline and at the end of the treatment period.

In the skiers a distinct airway inflammation was evident. In their bronchial biopsy specimens, T-lymphocyte, macrophage, and eosinophil counts were, respectively greater by 43-fold ($P<0.001$), 26-fold ($P<0.001$, and 2-fold ($P<0.001$) in skiers, and by 70-fold ($p>0.001$), 63-fold ($P<0.001$), and 8-fold ($P<0.001$) in asthmatic subjects than in controls. In skiers, neutrophil counts were more than 2-fold greater than in asthmatic subjects ($P<0.05$). Tn expression was higher in skiers vs. controls and lower in skiers vs. mild asthmatics. There were no significant changes between hyperresponsive and nonhyperresponsive skier in the inflammatory cell counts or Tn expression. Treatment with inhaled budesonide did not attenuate asthma-like symptoms, the inflammatory cell infiltration, or basement membrane tenascin expression in the skiers.

In newly diagnosed asthmatic patients, SLM, FP, and DSCG reduced asthma symptoms, and need for rescue medication ($P<0.04$). BHR was reduced by doubling doses 2.78, 5.22, and 1.35 respectively (all $P<0.05$). SLM and placebo had no effect on cell counts or Tn expression. FP and DSCG reduced eosinophil counts in the bronchial biopsy specimens ($P<0.02$ and $<0.048$, respectively). No significant change in tenascin expression appeared in any treatment group.

Regarding to atopy, no significant differences existed in the inflammatory cell counts in the bronchial mucosa of subjects with newly diagnosed asthma or in elite cross country skiers. Tn expression in the BM was significantly higher in atopic asthma than in those with nonatopic asthma.

Airway inflammation occurred in elite cross-country skiers with and without respiratory symptoms or BHR. Their inflammatory cell pattern differed from that in asthma. Infiltration with eosinophils, macrophages, and mast cells was milder, but lymphocyte counts did not differ from counts in asthmatic airways. Neutrophilic infiltration was more extensive in skiers than in asthmatics. Remodeling took place in the

skiers' airways, as reflected by increased expression of BM tenascin These inflammatory changes and Tn expression may be caused by prolonged exposure of the lower airways to inadequately humidified cold air.

Inflammatory changes and remodeling were not reversed with anti-inflammatory treatment. In contrast, in patients with newly diagnosed asthma, anti-inflammatory treatment did attenuate eosinophilic inflammation in the bronchial mucosa. In skiers, anti-inflammatory treatment did not attenuate BHR as it did in asthmatic patients. The BHR in skiers was attenuated spontaneously during placebo treatment, with no difference from budesonide treatment. Lower training intensity during the treatment period may explain this spontaneous decrease in BHR. The origin of BHR probably differs in skiers and in asthmatics. No significant association between BHR and inflammatory cell counts or between BHR and Tn expression was evident in cross-country skiers or asthmatic subjects. Airway remodeling differed between atopic and nonatopic asthma. As opposed to nonatopic asthma, Tn expression was higher in atopic asthma and is related to inflammatory cell densities.

# Contents

# Original publications

This thesis is based on the following publications:

I         Karjalainen EM, Laitinen A, Sue-Chu M, Altraja A, Bjermer L, Laitinen LA. Evidence of airway inflammation and remodeling in ski athletes with and without bronchial hyperresponsiveness. Am J Respir Crit Care Med 2000;161:2086-91 *

II       Sue-Chu M, Karjalainen EM, Laitinen A, Larsson L, Laitinen LA, Bjermer L. Placebo-controlled study of inhaled budesonide on indices of airways inflammation in bronchoalveolar lavage fluid and bronchial biopsies in cross country skiers. Respiration 2000;67:417-25  *

III      Karjalainen EM, Lindqvist A, Laitinen LA, Kava T, Altraja A, Halme M, Laitinen A. Airway inflammation and basement membrane tenascin in newly diagnosed atopic and nonatopic asthma. Respir Med 2003;97:1045-51

IV      Lindqvist A, Karjalainen EM, Laitinen LA, Kava T, Altraja A, Pulkkinen M, Halme M, Laitinen A. Salmeterol resolves airway obstruction but does not possess anti-eosinophil efficacy in newly diagnosed asthma: a randomized, double-blind, parallel group biopsy study comparing the effects of salmeterol, fluticasone propionate, and disodium cromoglycate. J Allergy Clin Immunol. 2003;112:23-8

The publications are referred to in the text by their roman numerals. The original publications are reprinted with permission of the copyright holders.

* This publication has also appeared in the thesis of Malcolm Sue-Chu 2000, Trondheim, Norway

# Abbreviations

| | |
|---|---|
| ASM | airway smooth muscle |
| BAL | bronchoalveolar lavage |
| bid | twice daily |
| BHR | bronchial hyperresponsiveness |
| BM | basement membrane |
| CI | confidence intervals |
| DD | doubling dose units |
| DSCG | disodium cromglycate |
| EIA | exercise-induced asthma |
| EIB | exercise-induced bronchoconstriction |
| ECP | eosinophil cationic protein |
| ECM | extracellular matrix |
| EPO | eosinophil peroxidase |
| EVH | eucapnic hyperventilation |
| FEV1 | forced expiratory volume in one second |
| FP | fluticasone propionate |
| FVC | forced vital capacity |
| IGE | immunoglobulin E |
| IL | interleukin |
| IQr | interquartal range |
| mAb | monoclonal antibody |
| $PC_{20}FEV_1$ | provocative consentration inducing a 20% fall in $FEV_1$ |
| $PD_{15}FEV_1$ | provocative dose inducing a fall of 15% in $FEV_1$ |
| PEF | peak flow |
| PL | placebo |
| pMDI | pressurised metered dose inhaler |
| qid | four times daily |
| SLM | salmeterol |
| Tn | tenascin |

# 1 Introduction and review of the litterature

## 1.1 Definitions of asthma and bronchial hyperresponsiveness

Asthma is a disease with many clinical phenotypes. An international consensus definition for asthma has been developed to characterize asthma (www.ginasthma.com), the main characteristics of which are reversible airflow obstruction and hyperresponsiveness of the airways to various stimuli. Intermittent airflow obstruction leads to one of the main clinical symptoms of asthma: attacks of breathlessness; cough, excessive mucus secretion, and wheeze are some others. Distinct morphological changes are visble in the asthmatic bronchial mucosa. Infiltration of inflammatory cells increases, especially eosinophils and T-lymphocytes in the submucosa and the epithelium. Thickening of the epithelial basement membrane (BM) occurs even in mild asthma. Epithelial integrity is lost due to epithelial shedding, and the number of mucus-secreting goblet cells rises. Edema results from leakage of plasma from the microvasculature. Airway remodeling is also an important characteristic of asthma. (Bateman et al 2008)

Bronchial hyperresponsiveness (BHR), a major pathophysiological feature of asthma, is an abnormal tendency of the bronchi to contract following the exposure to a variety of stimuli, including allergens, cold air, exercise, and non-physiological stimuli. However, BHR is not specific for asthma. Even 70% of subjects with BHR report no respiratory symptoms (Kolnaar et al 1997). Asymptomatic BHR, however, is a risk factor for asthma (Hopp et al 1990, Laprise and Boulet 1997), and degree of BHR may predict the outcome of asthma (Gerritsen et al 1989). Patients with various lung disorders like chronic obstructive pulmonary disease (COPD), tuberculosis, bronchiectases, or farmer's lung can also present with BHR (Tashkin et al 1992, Laitinen et al 1974, Varpela et al 1978, Mönkäre et al 1981). Viral infections can also cause a transient BHR in nonasthmatic subjects (Empey et al 1976, Laitinen et al 1980).

BHR is measured by challenging airways to bronchoconstrictive stimuli that induce airflow limitation directly or indirectly. Direct stimuli act on effector cells such as smooth muscle cells, causing bronchoconstriction. The most frequently used agents in BHR testing in research and clinical settings are methacholine and histamine, which are direct stimuli (Foos 2003). Indirect stimuli cause bronchoconstriction by acting on other than effector cells, the so-called intermediary cells, by releasing inflammatory mediators, or by stimulating neural pathways (Van Schoor et al 2005). Indirect stimuli can be pharmacological, such as adenosine, or they can be physiological, such as exercise. In asthmatic subjects, the degree of BHR is clearly much more pronounced than in nonasthmatic subjects (Sovijärvi et al 1993), and asthmatic subjects are at least 1000 times more sensitive to bronchoconstricting stimuli. The magnitude of BHR to histamine or methacholine correlates with other signs of asthma like the degree of diurnal variation of Peak Flow (PEF) (Ryan et al 1982, Gibson et al 1995).

## 1.2 Airway inflammation and remodeling in asthma

The inflammatory character of asthma has been recognized since the 1990s. Earlier, the airway obstruction was considered to be caused by contraction of smooth muscle and by mucus secretion. Introduction of flexible bronchoscopy gave new insights into the disease pathology (Diamant et al 2007), and made it possible to investigate larger patient populations and study the complex inflammatory reaction at the bronchial wall level. Bronchial biopsy remains the standard investigative method to determine inflammation and remodeling in the airways (Jeffery et al 2000). As flexible bronchoscopy is an invasive method – though mostly well tolerated by asthmatics (Busse et al 2005) – new non-invasive techniques have been developed to study inflammation and remodeling in asthma such as induced sputum and exhaled breath condensate. These methods make it possible to study a greater number of subjects, but for studying inflammatory alterations in the airway wall, samples acquired by these methods cannot totally replace endobronchial biopsies (Bergeron et al 2007).

Remodeling occurs in a wide range of tissues and organs and reflects the healing response to an injury (Bergeron et al 2007). Airway remodeling describes the structural changes in the airway wall that develop in response, by repair and restoration, to a chronic inflammatory process in asthmatic airways. Remodeling in asthma includes inflammatory cell infiltration in the submucosa, destruction of the epithelium, thickening of the basement membrane (BM) and changes in the extracellular matrix, hypertrophy and hyperplasia of smooth muscle, increased vascularity, mucus metaplasia, and the promotion of a cholinergic phenotype in the airway nerves (Beckett and Howarth 2003, Durcan et al 2006). Acute inflammation usually resolves without any pathologic changes remaining in the structures, but when inflammation does not resolve, remodeling is unavoidable (McParland et al 2003).

Evidence is extensive for chronic inflammatory cell infiltration in the bronchial mucosa (Lundgren et al 1988, Beasley et al 1989, Jeffery et al 1989, Ohashi et al 1992, Bentley et al 1992, Ackerman et al 1994). Inflammatory cell infiltration occurs in both chronic (Laitinen et al 1997) and newly diagnosed asthma (Laitinen et al 1993a). Compared with control subjects, patients with newly diagnosed and chronic asthma show an increased number of eosinophils, lymphocytes, macrophages, and mast cells in the bronchial mucosa of (Jeffery et al 1989, Foresi et al 1990, Bentley et al 1992, Laitinen et al 1997, Hoshino et al 1998a). Neutrophilic inflammation occurs principally in severe asthma (Wentzel et al 1999). Inflammatory cell infiltration in the bronchial mucosa is seen also in patients with asthma in clinical remission of asthma (Foresi et al 1990), but inflammatory cell counts decrease after treatment with inhaled corticosteroids and this is accompanied by improvement in lung function and a reduction in asthma symptoms (Laitinen et al 1992, Djukanović et al 1992, Trigg et al 1994, Laitinen et al 1997).

The epithelium in the airways forms an outer cellular barrier and is in the front line of defense against triggers of asthma like aeroallergens or viral infections. It is therefore not surprising that it is altered in asthma. Bronchial biopsies from asthmatic patients show extensive epithelial damage consisting of epithelial shedding (Dunnill 1960, Laitinen et al

1985, Jeffery et al 1989, Laitinen et al 1993a). Such epithelial damage may be caused by proteases and granule products released by eosinophils and mast cells (Motojima et al 1989, Pesci et al 1993, Venaille et al 1995). Numbers of ciliated cells decrease, and of goblet cells increase (Laitinen et al 1993a). The shed columnar epithelial cells are quickly replaced by basal cells (Erjefält et al 1997), but the barrier structure is still weak, and intraepithelial nerves can be more exposed to stimuli than in a normal intact epithelium (Laitinen et al 1985). Epithelial denudation may be due to the trauma caused by biopsy forceps (Söderberg et al 1990, Ordoñez et al 2000a). This theory is not supported by the studies performed with a rigid bronchoscope, which yields representative biopsy specimens with proper airway epithelium (Laitinen et al 1985, Laitinen et al 1993a). Epithelial shedding is evident even in mild asthma (Lozewicz et al 1990).

Why the epithelium in asthmatic airways is fragile is still unclear. The attachment of the epithelium to the inner epithelial wall may be defective. In an electron microscopic study, Ohashi et al (1992) showed widening of intercellular spaces and tight junctions in the bronchial epithelium of asthmatic subjects as a possible mechanism of epithelial shedding and BHR. Epithelial cells can be injured by mediators released by inflammatory cells, or they can be denuded from the underlying BM due to a repair process. It is also possible that the cells undergo apoptosis (Vignola et al 2000), but the airway epithelial cells in asthmatic subjects possess antiapoptotic properties (Vignola et al 2001). The bronchial epithelial cell is not a passive element. Epithelial cells communicate with the submucosal matrix to promote the normal epithelial repair, but in asthma this function is abnormal (Holgate 2007). Epithelium plays an active role in the repair process and is a source of growth factors like epidermal growth factor (EGFR) and transforming growth factor β (TGFβ), which are more intensively expressed in the epithelium of asthmatic subjects than in that of control subjects (Vignola et al 1997, Amishima et al 1998, Redington et al 1998, Fedorov et al 2005). The bronchial epithelium secretes various mediators of inflammation as response to epithelial injury and orchestrates the remodeling process in the airway mucosa (Holgate 2007). Epithelial damage and inflammatory reactions in the epithelium and submucosa may be considered as an airway wound-repair process. With anti-inflammatory treatment, the damaged epithelium and inflammation in the submucosa may be totally restored (Laitinen et al 1992).

In asthma, the epithelial repair process is altered and probably causes the subepithelial fibrosis and thickening of the BM seen in asthma (Roche et al 1989, Chetta 1997, Ward 2002). Reports are conflicting concerning the degree of BM thickening as correlated with asthma severity, deterioration of lung function, and submucosal inflammatory cell infiltration. Chetta and coworkers found that BM thickness correlated positively with asthma symptoms and daily peak expiratory flow (PEF) variability and correlated negatively with baseline lung function (Chetta et al 1997). In a recent study, BM thickness was greater in patients with severe asthma than in those with mild asthma (Bourdin et al 2007). Other studies have found no correlation between BM thickness and lung function, sex, age, or asthma duration (Payne et al 2003, Kim et al 2007). BM thickening occurs even in children with asthma (Cokugras et al 2001, Payne et al 2003, Kim et al 2007). The association of BM thickening and inflammation is obscure. In recent reports number of eosinophils in the submucosa of asthmatic bronchi did not correlate with BM thickness

(Fedorov et al 2005, Kim et al 2007). In another study, BM thickness correlated significantly with the percentage of mast cells in the BAL fluid (Ward et al 2002).

Bronchial epithelium may be altered by physical exercise. In mice, intensive training reduced the quantity of ciliated epithelial cells and induced apoptosis of bronchial epithelial cells (Chimenti et al 2007). It is possible that similar epithelial damage is caused by intensive training in humans. In long-distance runners the number of apoptotic bronchial epithelial cells in induced sputum after the race was increased (Bonsignore et al 2006).

The extracellular matrix (ECM) in epithelial tissues consists of the BM and the loose connective tissue that lies beneath the bronchial BM. The ECM is made up of macromolecules that form an intricate network providing mechanical support for airway structure. It is not only an inert framework in the airway wall but also an active regulator of the functions of cells embedded in the ECM, e.g. differentiation, migration, proliferation, and survival (Ingber et al 1994). The BM is a thin layer of specialized ECM, composed of a basal lamina and the lamina fibroreticularis (Merker 1994). Its border at the epithelial side can be seen clearly, but at the stromal side, a gradual change to the stromal matrix makes it difficult to determine its inner border. The main components of BMs are type IV collagen, laminin, nidogen, and proteoglycans (Merker et al. 1994). Changes in the BM are mainly described in the lamina fibroreticularis (Roche et al 1989, Jeffery et al 1992), but changes are seen also in the components of the basal lamina. In a morphometric study, Altraja and coworkers showed expression of lamininα2 and lamininβ2 chains in adult asthma. These chains are expressed only during lung morphogenesis (Virtanen et al 1996), and their reappearance in asthma may be the result of airway inflammation and may reflect remodeling (Altraja et al 1996a). The increased thickness of the lamina fibroreticularis in asthma is mainly caused by excess deposition of several ECM proteins like proteoglycans tenascin (Tn) (Laitinen et al 1997), lumican, byglycan, and vesican (Huang et al 1999) and of collagens (Roche et al 1989, Jeffery et al 1992).

One hallmark of this remodeling is the increase in bronchial smooth muscle mass. Enlargement of smooth muscle mass consists of hypertrophy (Carroll et al 1993) or hyperplasia (Cohen et al 1997). Because contraction of airway smooth muscle (ASM) is largely responsible for the acute airway narrowing seen in asthma, ASM may play an important role in BHR. In animal experiments, repeated challenge to an allergen causes a marked increase in ASM mass (Sapienza et al 1991, Ramos-Barbón et al 2005), suggesting that in ASM inflammation induces structural changes. ASM has also been shown to increase in association with asthma severity: Greater smooth muscle mass is evident in severe asthma than in moderate asthma (Pepe et al 2005). Airway remodeling with thickened BM and deposition of ECM proteins, and an increased ASM mass along with vascular hyperplasia, mucosal edema, and mucus hypersecretion is the main reason for the chronic airflow obstruction in asthma (Becket and Howarth 2003).

### 1.2.1. Inflammatory cells

*The eosinophil* is the key effector cell in asthma, and eosinophilic inflammation in asthmatic airways correlates with asthma severity (Bousquet el al 1990). Airway eosinophilia is detectable in most asthma patients, but a non-eosinophilic phenotype of asthma is known (Lemiére et al 2006). Eosinophils are present early in the airways even before the diagnosis of asthma (Pohunek et al 2005). Airway eosinophilia is not a unique feature of asthma, but also occurs in other diseases of the airways such as in chronic bronchitis (Lacoste et al 1993). Eosinophils are recruited to the airway mucosa from the circulation (Wardlaw 1999) by several chemokines like RANTES (Alam et al 1993) and eotaxins (Garcia-Zepeda et al 1996). Eosinophils contain dense intracellular granules filled with cytotoxic, eosinophil-specific granule proteins (ESGP): eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), eosinophil protein-X (EPX), and major basic protein (MBP) (Venge et al 1988). By releasing these proteins into the airway mucosa, eosinophils can cause extensive damage in the bronchial epithelium (Gleich et al 1988) and start the injury-repair process leading to remodeling. In addition to the direct cytotoxic effects of ESGPs, eosinophils also induce apoptosis of the bronchial epithelial cells (Trautmann et al 2002). Interestingly, Wenzel et al (1999) found that in patients with severe asthma, the subgroup of patients without airway eosinophilia had a thinner BM than did patients with airway eosinophilia.

Eosinophils play an important role in neural remodeling by releasing EPO, MBP, eosinophil-derived neurotoxin (EDN), and nerve growth factor (NGF), which is a member of the neurotrophin family. Eosinophils localized to cholinergic nerves promote the action of cholinergic nerves in the airways by increased expression of the muscarinic type 2 receptor and enhanced action of acetylcholine (Durcan et al 2006).

Eosinophils modulate their own accumulation and survival in tissues by expressing cell-surface receptors and at the same time secreting activators of these receptors that are important to their own activation, recruitment, and survival. Eosinophils secrete several mediators of inflammation like cytokines (several interleukins and eotaxins), growth factors like granulocyte-macrophage colony-stimulating factor (GM-CSF), transforming growth factor-beta (TGF-ß) and, cysteinyl leukotrienes (Jacobsen et al 2007). With these mediators, eosinophils not only regulate their own existence and function but also influence the function and survival of resident tissue cells (epithelial cells, fibroblasts, smooth muscle cells) and inflammatory cells (T-lymphocytes, mast cells, dendritic cells, neutrophils, and macrophages). However, eosinophils are not self-sufficient; they are also regulated by other cells in the same environment, like T-lymphocytes, for their initial recruitment to sites of inflammation (Jacobsen et al 2007).

*Mast cells* are normal resident cells in the airways; they arise in the bone marrow and travel to the airways. They contain granules filled with histamine, proteolytic enzymes (tryptase, chymase), proteoglycans, cytokines ( IL-4, IL-5, IL-13), and lipid mediators (leukotriene C4, prostaglandin D2) (Warner and Kroegel 1994). Mast cells have receptors in their cell surface, and the activation of these receptors causes mast cells to become partly or totally degranulated (Pesci et al 1993). In asthma, the number of mast cells may be increased, especially in the airway epithelium, but the main difference from

nonasthmatics is a greater degree of mast cell degranulation (Bradley et al 1991, Laitinen et al 1993a, Pesci et al 1993). Degranulation is related to disease severity (Carroll et al 2002a) and to mucus secretion (Carroll et al 2002b). Allergen challenge causes a rapid degranulation of the mast cells by activating the high-affinity receptor for immunoglobulin E (FcεRI) on the cell surface (Warner and Kroegel 1994). Mast-cell derived products act as the main factors in the acute response to allergen by causing bronchoconstriction and mucosal edema (Wenzel et al 1991, Bradding et al 2006). Mast cell infiltration in the bronchial smooth muscle occurs in symptomatic asthmatic in BHR subjects but not in subjects with eosinophilic bronchitis with chronic cough or in normal controls, suggesting that in asthma, mast cells may be a key factor in BHR and in the development of variable airflow obstruction (Brightling et al 2002, Begueret et al 2007). However, in one recent study, neither an increase nor significant differences appeared in mast cell densities in the airway smooth muscle between asthmatic subjects with BHR and control subjects or subjects with chronic obstructive pulmonary disease (COPD) (Liesker et al 2007). Furthermore, in contrary to findings by Brightling et al (2002), there was no correlation between mast cell density and BHR to adenosine monophosphate (Liesker et al 2007).

*Lymphocytes* play a crucial role in asthma pathogenesis. In the asthmatic airway mucosa, numbers of T-lymphocytes are increased (Azzawi et al 1990, Bentley et al 1992, Poston et al 1992, Laitinen et al 1993 a, Ohashi et al 1998). T-lymphocytes are divided into two major groups by the receptors they bear: CD4+ and CD8+ lymphocytes. In asthma, the majority of T-lymphocytes bear a CD4+ cell surface antigen and are thus called helper T-cells. CD4+ cells are divided into TH1- and TH2types, the TH2type being predominant (Corrigan et al 1995). T-lymphocytes play a regulatory role in asthmatic airway inflammation. They orchestrate the inflammatory process by releasing cytokines, particularly IL-4 and IL-5, leading to accumulation of eosinophils and mast-cell activation in the bronchial mucosa (Bradley et al 1991, Durham et al 2000). Lymphocytes may also participate in airway remodeling by interacting with airway smooth muscle, leading to hypertrophy, at least in atopic asthma (Ramos-Barbon et al 2005, Begueret et al 2007).

*Macrophages* are the most prevalent cells in the bronchoalveolar space in both asthmatic and nonasthmatic subjects (Beasley et al 1989). They are present also in the asthmatic airway wall (Bentley et al 1992, Poston et al 1992, Laitinen et al 1993, Ohashi et al 1998) and may be involved in asthma pathogenesis. Macrophages may also be involved in the remodeling process of asthma by secreting growth factors and proinflammatory cytokines including IL-1, TNF-α, IL-6, interferon γ, and GM-CSF (John et al 1998, Hamid et al 2003). Some of these cytokines may prolong eosinophil survival. Macrophages may also perpetuate mast cell activation in asthma and the late-phase response to allergens (Hamid et al 2003). Alveolar macrophages in asthmatics release TGF-β, suggesting that macrophages take part in airway remodeling (Vignola et al 1996).

*Neutrophils*. The role of neutrophils in asthma is still unclear. Neutrophils are polymorphonuclear granulocytes with the potential to cause damage in tissues when activated by releasing enzymes like neutrophil elastase, reactive oxygen compounds, cytokines, and lipid mediators. The number of neutrophils in the bronchial mucosa is not pronounced in clinically stable asthma (Poston et al 1992), but, in severe asthma, neutrophils seem to play an important role (Kamath et al 2005). Neutrophil concentration

in the bronchoalveolar lavage fluid and neutrophil numbers in endobronchial biopsies are higher in patients with severe asthma than in patients with moderate asthma (Wenzel et al 1997). Neutrophils are increased also in patients with nocturnal asthma (Martin et al 1991) and in acute severe asthma (Ordonez et al 2000b, Qiu et al 2007) as well as in occupational asthma (Park et al 1998). In sudden-onset fatal asthma, neutrophilic infiltration in the airway submucosa was significantly increased over that with slow-onset fatal asthma (Sur et al 1993).

## 1.2.2 Tenascin in the bronchial basement membrane zone

Tenascin is a family of oligometric glycoproteins of the ECM that have adhesive and antiadhesive properties. Tns include the isoforms Tn-C, Tn-N, Tn-R, Tn-X, Tn-Y, and Tn-W, and they are first expressed during embryonic development, particularly in neural development, skeletogenesis, and vasculogenesis (Jones and Jones 2000). Tns are reexpressed in the adult during normal processes like wound healing (Mackie et al 1988) and nerve regeneration (Probstmeier et al 2000) and in pathological states including tumorgenesis and metastasis (Juuti et al 2004, Orend and Chiquet-Ehrismann 2006). In breast cancer tumors, expression of tenascin expression is associated with a significantly worse prognosis than for tenascin-negative cancers (Ioachim et al 2002).  Tns play an important role in the developing embryo, and in the adult when remodeling processes occur, but their specific functions still remain poorly understood. Tns play a regulatory role, and they are involved in modulation of cell-matrix interactions and mediation of matrix attachment to the environment (Hsia and Schwarzbauer 2005). Tn knock-out mice were originally thought to develop normally (Saga et al 1992), but in further studies have shown subtle abnormalities in wound healing, brain chemistry, and in the neuromuscular junction, as well as in behavior (Mackie and Tucker 1999).

In the lung, Tn is normally expressed during embryonic development of the conducting airway and alveoli and may participate in epithelial-mesenchymal interaction during organogenesis of the lung (Zhao 1999, Kaarteenaho-Wiik 2001). Tn expression in the adult lung has been described in several pathological states like pulmonary fibrosis (Pääkkö et al 2000), sarcoidosis, atypical mycobacteriosis, and tuberculosis of the lung (Kaarteenaho-Wiik 2007). In asthmatic subjects, increased Tn expression is apparent in the bronchial BM in patients with chronic asthma, in patients with seasonal asthma (Laitinen et al 1997, Hoshino et al 1998a), and in those with occupational asthma (Laitinen et al 1996). In a placebo-controlled study, anti-inflammatory treatment with the inhaled corticosteroid budesonide was shown in asthma patients to reduce the BM accumulation of Tn. Simultaneously there occurs a significant reduction of number of T-lymphocytes in the bronchial mucosa in budesonide-treated asthmatics, suggesting that in asthma, Tn is associated with disease activity and airway inflammation (Laitinen et al 1997). However, Altraja and coworkers (1999) failed to show any reduction in Tn expression in chronic asthmatics treated with the weak anti-inflammatory drug nedocromil sodium, but found a reduction in Tn expression in patients treated with the β2-agonist albuterol. These authors hypothesized that the reduction in Tn expression may be derived

from albuterol's property of inhibiting the release of proinflammatory cytokines like interferon γ and tumor necrosis factor α. In a recent study, Amin and coworkers demonstrated that Tn layer thickness was increased parallel with lower epithelial integrity in bronchial biopsy specimens from asymptomatic smokers compared with those from asymptomatic never-smokers (Amin et al 2003). They also showed a significant correlation between Tn layer thickness and eosinophils and mast cells in the bronchial mucosa, which was not seen in asthmatic subjects (Laitinen et al 1997). The role of Tn in asthma has been studied in Tn-C-knock-out mice. That these mice were protected from allergen-induced airway inflammation and goblet cell increase supports the view that Tn plays an important role in asthma (Nakahara et al 2006).

## 1.3 Bronchial hyperresponsiveness and airway inflammation

BHR is a fundamental abnormality in asthma, representing both structural and inflammatory changes in the airways due to the disease process. The chronic airflow limitation due to airway remodeling forms a structural component which is irreversible. The variable and potentially reversible component reflects the fact that an inflammatory component of BHR may be reversed by treatment (Cockcroft and Davis 2006). Perhaps BHR could be a surrogate marker for airway inflammation (Laprise et al 1999). However, the causal relationship between BHR and airway inflammation is still unclear (Brusasco et al 1998). Reports are conflicting concerning the relationship between inflammatory and structural changes in the airways in asthma and degree of BHR (Table 1). One reason for this may be the wide use of direct challenges to assess BHR: They are not as closely correlated with airway inflammation as are indirect stimuli (Van Den Berge et al 2001, Prosperini G et al 2002).

Anti-inflammatory treatment with inhaled corticosteroids reduces BHR markedly and in parallel, it improves asthma symptoms (Juniper et al 1990), which supports the view that BHR and inflammation may be associated. Treatment with inhaled corticosteroids improves the structure of the damaged airway epithelium (Laitinen et al 1992) and reduces the infiltration of inflammatory cells into the bronchial mucosa, a process which may be linked to the reduction in BHR (Burke et al 1996, Boulet et al 2000). Sovijärvi et al (2003) showed, in asthmatic subjects, that an inhaled corticosteroid, fluticasone 250 µg bid, caused a very rapid attenuation of BHR. This same kind of rapid effect has been evident with budenosine. A single dose of 2400 µg of budesonide has produced a 2.2-fold improvement in BHR to hypertonic saline with a simultaneous significant reduction in sputum eosinophils (Gibson et al 2001). However, even after long-term treatment with inhaled corticosteroids and despite the absence of airway inflammation and reduced epithelial damage, BHR persists (Lundgren et al 1988). Furthermore, the bronchial biopsies obtained from healthy nonasthmatic subjects with BHR have shown no signs of airway inflammation or remodeling (Power et al 1993).

Recent observations are that nerve growth factor (NGF) and other members of the neurotrophin family may induce BHR in animals, and NGF appeared in BAL fluid from asthmatic subjects (Frossard et al 2004). Neurotrophins are produced by epithelial cells

and inflammatory cells in the lung. Their expression is increased during allergic inflammation, and they take part in the inflammatory process by prolonging the survival of eosinophils (Hahn et al 2006).

**Table 1.** *Correlations between BHR to direct stimuli and histopathologic findings in bronchial biopsies from asthmatic subjects.*

| Author (year) | N | Method | Histologic variable | correlation with BHR |
|---|---|---|---|---|
| Roche et al 1989 | 8 | $PC_{20}$histamine | BM thickening | no |
| Jeffery et al 1989 | 21 | $PC_{20}$methacholine | epithelial intergity[a] | yes |
| Losewicz et al 1990 | 19 | $PC_{20}$methacholine | epithelial damage | no[b] |
| Foresi et al 1990 | 13 | $PC_{20}$methacholine | cell infiltration in the epithelium | yes |
| Djucanovic et al 1990 | 11 | $PC_{20}$methacholine | eosinophil and mast cell numbers | no |
| Ollerenshaw & Woolcock 1992 | 10 | $PC_{20}$methacholine | inflammatory cell numbers | no |
| Ohashi et al 1992 | 19 | $PC_{20}$acetylcholine | opening of tight junctions, widening of intracellular spaces | yes |
| Bentely et al 1992 | 17 | $PC_{20}$methacholine | eosinophil numbers | yes |
| Ackerman et al 1994 | 28 | $PC_{20}$methacholine | EG2+ cells, mast cells, CD3+ cells, CD25 + cells | yes |
| Sont et al 1996 | 26 | $PC_{20}$methacholine | eosinophils, mast cells, leukocytes, CD8+ cells | yes |
| Cho et al 1996 | 13 | $PC_{20}$methacholine | epithelial denudation, BM thickness, inflammatory cell numbers | yes |
| Roisman et al 1996 | 18 | $PC_{20}$methacholine | eosinophil numbers | no[c] |
| Chetta et al 1996 | 27 | $PC_{20}$methacholine | inflammatory cell numbers in epithelium, BM thickness | yes |
| Chetta et al 1997 | 34 | $PC_{20}$methacholine | BM thickness | yes |
| Boulet et al 1997 | 80[d] | $PC_{20}$methacholine | subepithelial fibrosis intensity, epithelial desquamation | no[e] |
| Crimi et al 1998 | 20 | $PD_{15}$methacholine | inflammatory cell numbers | no |
| Hoshino et al 1998b | 25 | $PD_{15}$methacholine | BM collegen III, collagen IV, and tenascin | yes |
| Möller et al 1999 | 20 | $logPD_{20}$methacholine | EG1+ and EG2+ cells, BM thickness | no |
| Gibson et al 2000 | 20 | $PD_{20}$methacholine | metachromatic cells (mast cells) | yes |
| Milanese et al 2001 | 11 | $PD_{20}$methacholine | BM thickness | yes |
| van den Toorn et al 2001 | 37 | $PD_{20}$methacholine | BM thickness and MBD density | no[f] |
| Ward et al 2002 | 35 | $PD_{20}$methacholine | BM thickness | yes |
| Shiba et al 2002 | 36 | $PC_{20}$methacholine | BM thickness | yes |

[a]Percentage of epithelium covering BM correlated positively with PC20 methacholine, [b]no difference in the epithelial structures between normal subjects and asthmatics with BHR, [c]a significant negative correlation between eosinophils and $PD_{15}FEV_1$ to bradykinin, [d]38 subjects were asthmatics, [e]a significant correlation between $PC_{20}$methacholine and fibrosis found only in normoreactive subjects, [f]a significant inverse correlation between PD20 AMP and BM thickness or MBP density.

## 1.4 Bronchial hyperresponsiveness and asthma in athletes

### 1.4.1 Prevalence of bronchial hyperresponsiveness, respiratory symptoms, and asthma in elite athletes

Several studies have reported a high prevalence of asthma, asthma-like respiratory symptoms, and bronchial hyperresponsiveness accompanied by a frequent use of asthma medication among highly trained competitive athletes (Weiler et al 1986, Larsson et al 1993, Heir and Oseid 1994, Mannix et al 1996, Sue-Chu et al 1996, Langdeau et al 2000). The definition of asthma in these studies on athlete's asthma varies: from self-reported asthma to physician-reported asthma; diagnostic methods were also variable (Langdeau and Boulet 2001). Especially endurance sports like long-distance running, cross-country skiing, and swimming are associated with an increased frequency of asthma (Larsson et al 1993, Tikkanen and Helenius 1994, Potts 1996). Atopy is a risk factor for developing asthma in a normal population, but among endurance athletes, atopy increases the risk for asthma substantially. Helenius and coworkers found that atopy, determined as at least one positive skin test reaction, raised the risk for asthma in swimmers 96-fold compared to that of nonatopic swimmers, who had a 6-fold higher risk for asthma compared with that of control subjects (Helenius and Haahtela 2000). Asthma and exercise-induced bronchospasm are common as well at the high-school level (Rupp et al 1992) and in Olympic-level athletes (Voy 1986, Weiler et al 1998). Turcotte and coworkers (2003) reported that nearly 50% of athletes suffer from exercise-induced symptoms.

BHR prevalence in athletes has been studied by several authors (Table 2), whose BHR testing involved challenging the athletes to inhaled histamine or methacholine or to hyperventilation of eucapnic dry air. The majority of the studies also included a sedentary control group. The prevalence of BHR among the controls was lower than for the athletes. Only a few studies showed any significant difference between athletes and controls. Variability between studies and even within sports was great. BHR is frequent in athletes with asthma-like respiratory symptoms but also in athletes without symptoms (Potts 1994, Leuppi et al 1998). However, BHR to direct stimuli like methacholine is associated with a higher risk for respiratory symptoms (Weiler et al 1986, Verges et al 2005).

The frequent use among cross-country skiers of anti-asthmatic drugs initiated a discussion about whether cross-country skiing predisposes to asthma or whether athletes use asthma medication to improve their performance. Questionnaire-based studies confirmed that physician-diagnosed asthma in cross-country skiers is significantly higher than in age-matched control subjects (Larsson et al 1994, Heir and Oseid 1994). Unlike in the controls, asthma prevalence in the skiers increased with increasing age (Heir 1994).

**Table 2.** *Prevalence of BHR in athletes.*

| author | sport | N (athletes) | N (controls) | Prevalence of BHR in athletes (%) | Prevalence of BHR in controls (%) |
|---|---|---|---|---|---|
| Weiler et al 1986 | football/basketball | 156/18 [a] | 167 | 50/25 | 41 |
| Larsson et al 1993 | cross-country skiing | 42 [a] | 29 | 79[§] | 10 |
| Potts 1996 | swimming | 35 [a] | 16 | 60 [§] | 13 |
| Sue-Chu et al 1996 | cross-country skiing | 171 [a] | NA | 23 | NA |
| Helenius et al 1998a | swimming | 29[b] | 19 | 48[§] | 16 |
| Helenius et al 1998b | swimming/long-distance running/ speed and power | 42/71/49[b] | 45 | 36[§]/9/18 | 11 |
| Leuppi et al 1998 | basketball/ice hockey | 50 [a] | NA | 21/35 | NA |
| Langdeau et al 2000 | long-distance running + biking,/cross-country skiing+skating/triathlon/ swimming | 25/25/25 /25 [a] | 50 | 32/52/76/32[§] | 28 |
| Lumme et al 2003 | ice hockey | 88[b] | 47 | 24 | 11 |
| Mannix et al 2004 | high school athletes | 79[c] | NA | 38 | NA |
| Verges et al 2005 | cross-country skiing/triathlon | 29/10[a] | 13 | 38/40 | 0 |

BHR methods: [a]methacholine provocation, [b]histamine provocation, [c]eucapnic hyperventilation
[§]significant difference (P<0.05) between athletes and controls

## 1.4.2 Exercise-induced bronchoconstriction in athletes

When intense exercise causes bronchoconstriction, the phenomenon is called exercise-induced bronchoconstriction (EIB) or exercise-induced asthma (EIA). EIB refers to bronchial obstruction after provocation by exercise test or during self-induced exercise even in the absence of a previous asthma diagnosis, and EIA infers symptoms induced directly by exercise (www.wada-ama.org 2007). The bronchoconstriction is transient, appears after cessation of the exercise, and it reaches its peak at 5-10 minutes after exercise lasting from a few minutes to several hours. EIA is one symptom of asthma, and the prevalence of EIA among asthmatics is high, ranging from 45 to 80% (Karjalainen 1991, Backer et al 1992, Bardagi et al 1993). Anti-inflammatory treatment attenuates but

does not totally prevent EIA. Of asthmatic subjects treated with inhaled corticosteroids, 50% have shown EIA (Walkaans et al 1993).

EIB is common among athletes, prevalent even in athletes without an asthma diagnosis (Rupp et al 1992, Mannix et al 1996, Schoene et al 1997). Its prevalence has been higher in healthy nonasthmatic skiers than in control subjects, 35% vs 11% (Pohjantähti et al 2005). Atopy seems to enhance the possibility of an athlete's reacting to exercise with EIB. Helenius and coworkers showed that running in a cold environment caused EIB in atopic but not in nonatopic elite runners (Helenius et al 1996). Post-exercise respiratory symptoms do not predict EIB either in summer sports (Holzer et al 2002) or in winter sports. Rundell and coworkers, comparing self-reported symptoms for EIA to postexercise lung-function test results in 158 elite winter sport athletes, found that self-reported symptoms yielded a high frequency of false positives and false negatives (Rundell et al 2001). Nor are self-reported symptoms reliable predictors of EIB in summer sports either (Holzer et al 2002).

Exercise testing can be conducted in standardized laboratory conditions using a bicycle ergometer or treadmill or outside the laboratory in field conditions. In the laboratory, EIB is documented by measuring a fall of 10% in $FEV_1$ (Sterk et al 1993), but in field conditions a fall of 15% in $FEV_1$ is required for diagnosis (Kukafka et al 1998). Bronchial responsiveness to histamine or methacholine has low sensitivity but high specificity for EIB (Holzer and Brukner 2004). Deal and coworkers found that isocapnic hyperventilation at rest can cause the same level of bronchoconstriction as does exercise (Deal et al 1979). Currently, the most appropriate method to detect responsiveness to exercise in athletes is eucapnic voluntary hyperventilation (EVH) (Anderson et al 2001, Anderson et al 2003). But EVH is insufficiently accurate to detect EIB in every athlete at risk. Mannix and coworkers (1999) compared EVH and exercise testing for 29 figure skaters, demonstrating EIB in 16 skaters, but only 5 were positive in both tests; EVH missed 4 skaters with EIB and exercise-testing performed at an ice-rink missed 7.

The main pathophysiological mechanism of EIB is heat and water loss from the airway surface resulting in bronchospasm (Anderson and Daviskas 1997). Another theory of the mechanism of EIB is the thermal theory (McFadden Jr 1990). It postulates that heat and water loss from the airways during exercise leads to cooling of the airways followed by rapid rewarming causing narrowing of the airways by hyperemia and vascular engorgement.

The severity of EIB occurring in connection with specific exercise depends on level of ventilation achieved and sustained during exercise and temperature and humidity of inspired air (Deal et al 1979). At the beginning of exercise, air enters through the nose where it is filtered, adjusted to body temperature, and humidified before it passes through the trachea. During exercise, minute ventilation increases greatly; 140 to 150 l/min in athletes (Stromme et al 2003) and even up to 200 liters in elite cross-country skiers (Rusko 2003). When minute ventilation exceeds 22 to 44 liters, a switch occurs from nose to mouth breathing (Wheatley et al 1991), and the air-conditioning function of the nose is lost. At least ten generations of airways are needed in the heating and humidification process (Daviskas et al 1990). This leads to water loss from the liquid lining the airway surface. The hyperosmolarity of this liquid due to water loss stimulates the release of

inflammatory mediators such as histamine and leukotrienes, resulting in contraction of the bronchial smooth muscle (Anderson and Holzer 2000). Inhaled mannitol induces bronchoconstriction in an asthmatic subject most probably due to hyperosmolarity in the airway surface liquid. The main mediator of hyperosmolarity-induced bronchoconstriction is prostaglandin D2 derived from mast cells (Brannan et al 2006).

Reports are conflicting concerning EIA and airway inflammation in the asthmatic population. Exercise challenge has not caused any increase in histamine or tryptase levels or in inflammatory cells in BAL fluid in subjects with EIA (Jarjour and Calhoun 1992), suggesting mechanisms other than histamine release by pulmonary mast cells as responsible for EIA. Gavreau et al (2000) found that in ten asthmatic subjects with EIA, exercise had no effect on inflammatory cells measured in blood or sputum, unlike with allergen inhalation. Evidence does, however, support the inflammatory basis of EIB. Venge and coworkers (1991) studied 13 asthmatic subjects with EIA and nine without; only in subjects with EIA did exercise cause a small transient rise in serum eosinophilic cationic protein (ECP), reflecting airway inflammation. These changes in ECP levels were not associated with EIA severity. Crimi and coworkers (1992) showed that after exercise challenge, more degranulating mast cells appeared in the bronchial mucosa, and a greater percentage of eosinophils in the BAL than after methacholine challenge. In a recent study, exercise challenge caused an increase in histamine, tryptase, and cysteinyl leukotrienes levels in induced sputum (Hallstrand et al 2005).

The cause of this increased prevalence of EIB in athletes remains obscure despite much research. Inhalation of large amounts of dry and often cold air during exercise may cause an inflammatory reaction due to dehydration, leading then to EIB (Anderson and Holzer 2000, Davis and Freed 2001). Environmental factors like temperature, aeroallergens, air pollutants, and increased activity of the parasympathetic nerves may contribute in athletes to development of EIB and BHR (Langdeau and Boulet 2001).

## 1.5 Cross-country skiing as a sport

Competitive cross-country skiing is an endurance sport requiring very high aerobic power and capacity. The most important determinant of skiing performance is maximum oxygen uptake ($VO_{2max}$). In a trained adult skier weighing 72 kilograms, $VO_{2max}$ can be 87 ml/kg/min; in an unfit adult it is only about 30 ml/kg/min. In order to achieve this very high level of oxygen uptake, top-level skiers need a high level of ventilation, which may even reach 200 l/min or more. (Rusko 2003). Elite skiers' exercise is very strenuous and demanding. Oxygen consumption and need during heavy exercise is high, but the main demand on ventilation is production of carbon dioxide ($CO_2$) from the high energy consumption. Heavy exercise is associated with metabolic acidosis resulting in a fall in arterial pH. This requires a further increase in ventilation in order to recoup acidosis by a compensatory decrease in arterial carbon dioxide tension ($P_{CO2}$). (Stromme et al 2003) The athlete must increase ventilation to achieve an acid-base balance resulting in hyperpnoea. Ventilation is increased by increasing the depth (tidal volume) and frequency of breathing until 70 to 80% of peak exercise performance, at which point ventilation is increased by

frequency (Johnson et al 1992). Breathing frequency during ski-training or racing may even reach 50 breaths per minute, which is very demanding for the humidification of the air passing rapidly into the lower the airways, meaning that air reaching the lower airways is dry and may actually cause lower-airway water loss. Highly trained athletes may achieve the mechanical limits of their lung and respiratory muscles during maximal exercise (Johnson et al 1992).

Elite cross-country skiers train intensively, with annual training hours ranging from 250 to 750 hours depending on age: Training hours increase with increasing age (Rusko 2003). Training is not restricted to the Nordic snow season from the end of October till April, as some training camps are at high altitudes. Weather conditions are cold (usually with subfreezing temperatures) during ski-races and during part of the training season. As temperature falls, air humidity also decreases, and at subfreezing temperatures the inspired air is very dry.

## 1.6. Anti-asthmatic drugs – effects on airway inflammation and bronchial hyperresponsiveness

**Inhaled glucocorticosteroids** have become the mainstay in the treatment of asthma. Corticosteroids act mainly by binding to a glucocorticoid receptor in the cytoplasm of various cells involved in the inflammatory reaction (Beato et al 1995). This steroid-bound receptor translocates into the nucleus, binds to DNA glucocorticoid response elements, and results in both suppression of inflammatory gene transcription and activation of anti-inflammatory gene transcription (Barnes and Adcock 1998, Barnes 2006).

The introduction of the first inhaled corticosteroid, beclomethasone, caused a revolution in asthma management in the early 1970's (Clark 1972). At the moment, several inhaled corticosteroids including beclomethasone, budesonide, and fluticasone are available for clinical use in Finland. Long-term treatment with inhaled corticosteroids improves both asthma symptoms and lung function and reduces BHR (Haahtela et al 1991, 1994, Sont et al 1999). Reduction in BHR is very rapid and can be reached in 3 days, but the effect tapers off within 2 weeks after cessation of treatment (Sovijärvi et al 2003). Inhaled corticosteroids are currently the most potent agents in the treatment of airway inflammation in asthma, reducing inflammatory cell infiltration into the bronchial mucosa and restoring the structure of the epithelium (Djukanovic et al 1992, Jeffery et al 1992, Laitinen et al 1992 Trigg et al 1994). This decrease in eosinophil numbers and T-cell numbers may in part be due to apoptosis induced by corticosteroids (Druilhe et al 1998, O'Sullivan et al 2004). Corticosteroids have been shown to reduce the thickness of the BM (Trigg et al 1994, Olivieri et al 1997, Hoshino 1998, Chetta et al 2003). However, one study with a limited number of patients failed to show any changes in BM thickness after short- or long-term treatment with budesonide (Jeffery et al 1992). Corticosteroids inhibit microvascular leakage (Boschetto et al 1991), and some evidence shows that at least high doses of inhaled corticosteroids reduce vascularity in asthmatic airways (Orsida et al 1999, Chetta et al 2003). Treatment with inhaled corticosteroids reduces expression of some inflammatory cytokines like GM-CSF and IL-8 (Wang et al 1994, Trigg et al 1994).

Inhaled corticosteroids are safe to use; systemic side-effects are uncommon compared with those from oral corticosteroids. The most common side-effects of are pharyngitis and oral candiasis, caused by deposition of the drug in the oropharynx. However, high doses of inhaled corticosteroids may exert the same systemic side effects as oral corticosteroids, like hypothalamic-pituitary-adrenal-axis suppression, growth suppression in children, osteoporosis, cataract, and skin thinning and bruising (reviewed by Dahl 2006).

**β$_2$-agonists** have relieved asthma symptoms for more than four decades. Being potent bronchodilators, they relax the bronchial smooth muscle by interaction with β$_2$-receptors, which are abundantly present in the lungs (Nelson 1995). Furthermore, β$_2$-agonists enhance mucociliary clearance (Devalia et al 1992), reduce vascular permeability (Erjefält and Persson 1991), inhibit release of inflammatory mediators from mast cells (Butchers et al 1991, Bissonnette and Befus 1997), eosinophils (Munoz et al 1994), and neutrophils (Busse and Sosman 1984). They also inhibit cholinergic neurotransmission, resulting in reduced cholinergic bronchoconstriction (Rhoden et al 1988). The principal side-effects of β$_2$-agonists are tremor, tachycardia, palpitations, and restlessness. Regular administration of β$_2$-agonists may induce tolerance to their bronchodilator effect and may reduce bronchoprotection (Nelson 1995, Ramage et al 1994).

**Short-acting β$_2$**-agonists are used as needed in asthma management to alleviate asthma symptoms rapidly and therefore are known as the reliever medication. Salbutamol, terbutaline, and fenoterol, the short-acting β$_2$-agonists available in Finland, are effective in preventing exercise-induced bronchoconstriction (Godfrey and Konig 1976). The onset of their action is rapid, and the duration of their effect is 3 to 6 hours (Nelson 1995). Regular treatment with short-acting β$_2$-agonists is not recommended, because this can lead to increased asthma exacerbations and BHR (Sears et al 1990, Taylor et al 1993), and worsen airway inflammation (Manolitsas et al 1995).

**Long-acting β$_2$-agonists** formoterol and salmeterol have been in clinical use since the 1990's and have longer duration of action (12 hours) than the short-acting β$_2$-agonists. Adding a long-acting β$_2$-agonist to treatment for asthma not optimally controlled with inhaled corticosteroids improves asthma symptoms, lung function, and BHR and reduces asthma exacerbations (Greening et al 1994, Pauwels et al 1997, Lemanske et al 2001). As monotherapy, long-acting β$_2$-agonists are less effective than inhaled corticosteroids in reducing BHR or in controlling symptoms (Simons and Estelle 1997) and are recommended only as add-on treatment with inhaled corticosteroids, not as monotherapy (Lazarus et al 2001). The possible anti-inflammatory effects of long-acting β$_2$-agonists have been investigated in only a few studies. In patients with mild asthma monotherapy with salmeterol for 6 weeks significantly reduced the numbers of neutrophils in the bronchial mucosa compared with fluticasone (Jeffery et al 2002). However, salmeterol lacked this same anti-inflammatory effect for eosinophils or lymphocytes like fluticasone, even though salmeterol treatment improved asthma symptoms and BHR better than did flutacasone. Formoterol as monotherapy has reduced eosinophil and mast cell numbers in the bronchial mucosa, but only in a subgroup of patients with higher baseline eosinophil counts (Wallin et al 1999). Although one suggestion is that the bronchodilator effect of long-acting β$_2$-agonists potentially masks airway inflammation (McIvor et al 1998), one bronchial biopsy study comparing a high dose of fluticasone (500 μg bid) to salmeterol

(50 µg bid) added to a low dose of fluticasone (200 µg bid) caused neither worsening nor improvement in airway inflammation (Wallin et al 2003).

After allergen challenge, salmeterol inhibits the early and the late asthmatic reaction, and inhibits the increase in serum ECP and EPX, suggesting an inhibitory effect on eosinophil activation (Pedersen et al 1993). However, following bronchial segmental allergen challenge, salmeterol failed to inhibit the inflammatory reaction at the bronchial level (Calhoun et al 2001). Thus, the evidence of anti-inflammatory effects of long-acting $\beta_2$-agonists is weak (Openheimer and Nelson 2008).

Van Schayck and coworkers (2002) studied patient-perception of histamine-induced bronchoconstriction during chronic dosing of salbutamol, salmeterol, and placebo, finding no difference in the perception between the treatments and placebo. Asthma-related deaths were earlier linked to the overuse of short-acting $\beta_2$-agonists (O'Byrne and Ädelroth 2006). Recent large trials have raised concern about the risk of deaths caused by long-acting $\beta_2$-agonists (Nelson et al 2006, Salpeter et al 2006). At least part of the mortality could be explained by the lack of inhaled corticosteroid treatment. Furthermore there is extensive evidence that combining long-acting $\beta_2$-agonists with inhaled corticosteroids reduces the risk for severe asthma exacerbations and hospitalization for asthma.

**Combination inhalers of long-acting $\beta_2$-agonists and inhaled corticosteroids** are now widely used instead of separate inhalers. Combination inhalers are easier for the patient and are at least as effective as the same agents from separate inhalers (Zetterström et al 2001). Treatment with a combination of fluticasone and salmeterol (100 µg/50 µg bid) compared with a higher dose of fluticasone alone (250 µg bid) for 24 weeks had a similar effect on airway inflammation and remodeling (Jarjour et al 2006). Results from recent in vitro studies suggest that combining long-acting $\beta_2$-agonists with corticosteroids enhances corticosteroid anti-inflammatory action (Barnes 2006).

**Antileukotrienes** are targeted antiasthmatic agents that directly inhibit a group of potent inflammatory mediators, cysteinyl leukotrienes, either by inhibiting the function of leukotriene receptors (montelukast, pranlukast, zafirlukast) or by inhibiting their synthesis (zileuton). The cysteinyl leukotrienes LTC4, LTD4, and LTE4 are derivatives of the metabolism of arachidonic acid known in the 1980's as Slow-Reacting Substance of Anaphylaxis (SRS-A) (Holgate and Dahlén 1997). Cysteinyl leukotrienes are potent bronchoconstricting agents (Dahlén et al 1980) and cause mucosal swelling by increasing vascular permeability (Rinkema et al 1984); they also cause increased mucus production (Marom et al 1982). Cysteinyl leukotrienes are involved in the recruitment of inflammatory cells, especially eosinophils, into the bronchial mucosa (Laitinen et al 2005). Inhalation of LTE4 induced a significant bronchial constriction, and at the same time a significant increase in eosinophils in the bronchial mucosa (Laitinen et al 1993b).

Antileukotrienes have a dual effect: anti-inflammatory and bronchodilating. They improve asthma symptoms and lung function (Dahlén 2006) and also have bronchoprotective properties to inhaled antigens and exercise (Currie and Lipworth 2002). Montelukast is more effective in chronic treatment of EIB than is salmeterol because of its sustained bronchoprotective efficacy (Villaran et al 1999). In a Japanese study, treatment with pranlukast for 4 weeks reduced hyperresponsiveness to methacholine and also reduced inflammatory cells in the bronchial mucosa compared with placebo (Nakamura et

al 1998). However, O'Sullivan et al (2003) showed that combining montelukast with a low dose of inhaled fluticasone had no additional effect either in reducing BHR to methacholine or in reducing numbers of inflammatory cells in the bronchial mucosa. As the clinical effects of antileukotrienes are inferior to those of inhaled corticosteroids, and their role in asthma management is not fully established (Polosa 2007), they are used mainly as add-on therapy to optimally control asthma. The advantages of antileukotrienes are oral dosing and few side-effects, mainly headache and gastrointestinal discomfort (Diamant and van der Molen 2005).

**Cromones** were introduced as a treatment for asthma in 1965 by Robert Altounyan, who made the first experiments on himself, a sufferer from chronic asthma since childhood (Edwards and Howell 2000). Two cromones are available for the treatment of asthma: disodium cromoglicate (DSCG) and nedocromil sodium, which are weak antiinflammatory inhaled medications. They have no bronchodilator effect, but have been shown to attenuate exercise-induced bronchoconstriction (Poppius et al 1970), reduce BHR (Hoag and McFadden 1991, Anderson et al 1994, Fiocchi et al 1997), and improve asthma symptoms. The mechanism of cromone action may be the blocking of chloride ion channels (Heinke et al 1995). Treatment with DSCG reduces the numbers of inflammatory cells and reduces expression of adhesion molecules at the bronchial mucosal level in patients with atopic asthma (Hoshino et and Nakamura 1997). However, this study was not blinded and was a single-agent study with no control group. Nedocromil sodium failed to reduce either inflammatory cell counts (Altraja et al 1996b) or BM tenascin thickness (Altraja et al 1999) in the bronchial mucosa of patients with chronic asthma. Compared with inhaled corticosteroids, cromones are less effective and more costly (Andersson F et al 2001). The advantage of cromones is their lack of significant side-effects, but they are rather expensive compared with inhaled corticosteroids (Barnes et al 1995).

## 1.7. Diagnosis and treatment of asthma in elite athletes

To relieve asthma symptoms and achieve normal lung function, asthma in athletes should be recognized and treated according to accepted guidelines (Bateman et al 2008), but diagnosis of asthma in elite athletes is more complex than in a normal population (Weiler et al 2007). Pulmonary function tests are often normal in athletes; in elite athletes, diagnostic procedures for asthma should also include an exercise challenge test, preferably in sport-specific conditions and environment, or EVH (Storms 1999, Anderson 2001).

An athlete may have chronic asthma from childhood, or symptoms of asthma may develop during his/her career. Some athletes suffer from asthma symptoms induced only by exercise (EIB). Major risk factors for an athlete's developing asthma during an athletic career are atopic disposition and training for an endurance sport (Helenius et al 2005). As allergic rhinitis is common among athletes and is a risk factor for asthma, rhinitic athletes should be screened for asthma (Bonini et al 2006). Allergic rhinitis in athletes needs effective treatment (Helenius et al 2005). If an athlete has had chronic asthma since childhood, management of the disease should follow normal guidelines. Regular use of inhaled corticosteroids is the cornerstone of asthma management, and inhaled $\beta_2$-agonists

when needed to treat or prevent bronchospasm. Leukotriene antagonists or chromones can also be useful for an asthmatic athlete (Storms 1999). For those with chronic asthma, symptoms of EIA may reflect poor asthma control (Weiler 2007), that should lead to review of the athletes' asthma management plan. Vocal cord dysfunction should be considered as a differential diagnosis, if an athlete suffers symptoms like EIA despite adequate asthma medication (Storms 1999).

When treating competitive athletes, physicians face the dilemma of doping regulations and asthma medication. Competitive athletes need special approval from anti-doping authorities to use asthma medications. The current list of prohibited and permitted medicines can be checked at the web-site of the World AntiDoping Agency (WADA). Inhaled $\beta_2$-agonists and glucocorticosteroids are prohibited substances. An athlete with asthma may be authorized to take these medications, if he or she applies for a Therapeutic Use Exemption (TUE) from the national anti-doping authorities (www.wada-ama.org). TUE is valid for 4 years, but the treatment plan should be confirmed annually by a respiratory physician. Currently, antihistamines, antileukotrienes, chromones, anticholinergics, and theophylline are permitted without TUE, but use of inhaled steroids and inhaled salbutamol, terbutaline, formoterol, and salmeterol require an abbreviated TUE. Interestingly, immunotherapy is permitted (www.wada-ama.org).

Recently, athletes participating in the Olympic Games have been permitted to use $\beta_2$-agonists only if they show objective documentation of asthma or EIB (Bonini et al 2004). Otherwise the athlete applying for use of a $\beta_2$-agonist should fulfill one of the following criteria: $\geq 12\%$ increase in $FEV_1$ (of the predicted $FEV_1$ value) after the administration of an inhaled $\beta_2$-agonist, $\geq 10\%$ fall in $FEV_1$ in exercise challenge or in EVH, in methacholine challenge $PC_{20} < 4$ mg/ml or $PD20 \leq 2$ µmol in a steroid-naïve athlete or $PC_{20} \leq 6.6$ mg mg/ml or $PD_{20} \leq 13.6$ µmol in athletes on inhaled steroids (Carlsen et al 2008). One non-medical treatment of EIB is a physical warm-up. It attenuates EIB in athletes and is recommended also for non-athletic asthmatics before exercise (McKenzie et al 1994).

## 1.7.1 Frequency of anti-asthmatic drug use among athletes

Asthma medication is frequently used by elite athletes (Heir et al 1994, Weiler et al 1996,). In the 1996 Summer Olympic Games, 15.3% of United States Olympic athletes had a previous diagnosis of asthma and 13.5% had used asthma medication (Weiler et al 1998). A recent Finnish study reported increased use of any physician-prescribed medication (Alaranta et al 2006). In a questionnaire sent to 494 athletes financially supported by the Finnish National Olympic Committee and to age-matched controls. 34.5% of the athletes used some type of prescribed medication. The control group used prescribed medication significantly less, 24.9% of respondents. In the athletic group, 7.0% had been prescribed anti-asthmatic medication during the previous 7 days, and the adjusted odds ratio for an athletic subject using asthma medication was 3.42.

Much concern has risen as to elite athletes using too much $\beta_2$-agonist, especially long-acting $\beta_2$-agonists, to avoid increased risk for asthma exacerbations and asthma-related

death (Sears 1990, Nelson et al 2006, Salpeter et al 2006). On the other hand, doubts exist whether these enhance an athlete's performance. This casts another shadow upon anti-asthma medication, especially $\beta_2$-agonists. Despite $\beta_2$-agonists' being the most commonly used asthma medication among elite athletes (Weiler et al 1998, Helenius et al 2000).

## 1.7.2 Effects of anti-asthmatic drugs in athletes

Few studies examine the effects of antiasthmatic medicines in the management of asthma, EIB, or asthma-like symptoms in elite athletes, with the ergogenic potential of anti-asthmatic drugs studied more extensively. In a recent meta-analysis, Kinderman reviewed 19 randomized placebo-controlled trials on the ergogenic potential of $\beta_2$-agonists in non-asthmatic athletes. He found no evidence that inhaled $\beta_2$-agonists could improve athletic performance (Kinderman 2007). Unlike the inhaled $\beta_2$-agonists, the oral formulations of these drugs do possess ergogenic effects (Martineau et al 1992).

Treatment of symptoms or bronchoconstriction that occurs only in association with exercise is not very well documented, and EIA in chronic asthmatics and athletes with EIB differ (Weiler et al 2007). Inhaled $\beta_2$-agonists, both short-acting and long-acting, are effective in preventing EIB in athletes (Ferrari et al 2000, Weiler et al 2007). Regular use of short-acting $\beta_2$-agonists worsens EIB (Indman and O'Byrne 1996), and therefore cannot be recommended. Daily use of $\beta_2$-agonists, especially without regular inhaled corticosteroids, may lead to tachyphylaxis or partial loss of efficacy (Simons et al 1997, Ramage et al 1994, Hancox 2002). $\beta_2$-agonists are also associated with the occurrence of lymphoid aggregates in the bronchial mucosa of elite cross-country skiers (Sue-Chu et al 1998). Leukotriene antagonists are effective in preventing EIA in asthmatic subjects (Leff et al 1998), but in a recent placebo-controlled study, montelukast failed to alleviate asthma-like symptoms, BHR, or airway inflammation in highly-trained ice-hockey players (Helenius et al 2004). The role of inhaled corticosteroids in treating EIB in athletes is not well studied.

## 2. AIMS OF THE STUDY

- To characterize airway wall inflammation in nonasthmatic ski athletes with and without bronchial hyperresponsiveness and mildly asthmatic subjects with bronchial hyperresponsiveness.

- To study tenascin immunoreactivity in the basement membrane zone in ski athletes and compare the findings with those of mildly asthmatic subjects and control subjects

- To relate possible inflammatory changes to lung function, symptoms, and bronchial hyperresponsiveness in ski athletes and in asthmatic subjects.

- To examine differences between airway inflammation in atopic and nonatopic asthma.

- To evaluate the effects of budesonide treatment on airway inflammation, basement membrane tenascin expression, and bronchial hyperresponsiveness in non-asthmatic ski athletes with asthma-like symptoms and bronchial hyperresponsiveness.

- To evaluate the effects of salmeterol, fluticasone, and disodium cromoglygate on airway inflammation and tenascin expression in subjects with newly diagnosed asthma and bronchial hyperresponsiveness.

- To study any differences between airway inflammation and bronchial hyperresponsiveness in nonasthmatic elite ski athletes and subjects with newly diagnosed asthma and chronic mild asthma

# 3. MATERIALS AND METHODS

## 3.1 Subjects

The study population was composed of four groups: elite nonasthmatic cross-country skiers (n=40) compared to subjects with mild asthma (n=12) and healthy nonathletic subjects (n=12) (I). The fourth group of study subjects (n=79) comprised patients with newly diagnosed asthma who participated in an intervention study comparing the effects of salmeterol, fluticasone propionate, or disodium chromoglycate (III-IV). From the skier group, subjects with bronchial hyperresponsiveness and respiratory symptoms (n=25) were recruited to a drug intervention study comparing inhaled budesonide and placebo (II).

None of these subjects had experienced any upper respiratory tract infection or asthma exacerbation 4 weeks prior to investigational bronchoscopy. All subjects and parents of those subjects younger than 18 years gave their written informed consent. The studies were approved by the local ethics committees for the participating institutions.

### 3.1.1 Cross-country skiers (I-II)

The studies enrolled 44 competitive cross-country skiing athletes without prior diagnosis of asthma from Sweden and Norway. They were attending senior secondary schools in Norway and Sweden, or serving as conscripted soldiers in a Swedish military ski-platoon, and were recruited to these studies while participating in an epidemiological study of skiing and asthma (Sue-Chu 1996). All subjects with prior anti-inflammatory asthma medication were excluded; 15% used inhaled $\beta_2$-agonists for respiratory symptoms. None of the subjects was a current or former smoker. Their annual mean duration of training was 434 hours (range 200 to 630). 30 skiers with BHR and asthmatic symptoms were asked to participate in a drug intervention study (Study II), and 25 consented to do so.

### 3.1.2 Control subjects (I)

The control non-athlete subjects were 12 medical students (Tartu, Estonia), of whom 5 were female. Their mean age was 25 years (range 22 to 29 years). They had no current respiratory symptoms or any allergy based on medical history and negative skin prick testing. All were healthy non-smokers with normal lung function in spirometry without abnormal reversibility in a bronchodilator test. PEF measurements showed no signs of abnormal diurnal variation. No subject was involved in competitive sports.

### 3.1.3 Mild asthmatics (I)

A total of 12 asthmatic subjects (Tartu, Estonia) with a well-documented medical history and symptoms of asthma, 6 of them female, had mild asthma defined as forced expiratory volume in one second ($FEV_1$) >80 % of predicted. The asthma diagnosis was confirmed in a bronchodilator test. Their asthma was considered chronic, because the diagnosis made at least 2 years earlier. They underwent an at least 6-week break in anti-inflammatory asthma medication before lung function testing and bronchoscopy. During this period asthmatic symptoms were controlled with inhaled $\beta_2$-agonists or theophylline. All of these subjects were nonsmokers.

### 3.1.4 Newly diagnosed asthmatic subjects (III-IV)

Patients with newly diagnosed, symptomatic asthma (diagnosis made within 2 years of entering the study) aged between 18 and 60 were recruited in the regions of the Helsinki University Central Hospital and North Karelia Central Hospital in Finland. Diagnosis of asthma was according to guidelines of the American Thoracic Society (ATS) (American Thoracic Society 1991) less than 2 years prior to entering the study. These criteria included: at least 15% reversibility in $FEV_1$, or at least 15% improvement in PEF, or at least 20% diurnal PEF variation. These criteria were met either at entry to the study or 12 months earlier. Other inclusion criteria were: $FEV_1$ 60 to 100% of the national predicted value (Viljanen et al 1982), moderate to severe bronchial hyperresponsiveness to histamine ($PD_{15}FEV_1$ less than 0.4 mg) at entry (Sovijärvi et al 1993), either no smoking history or  status of ex-smoker (less than 10 pack-years) for at least 2 years, no treatment with long-acting $\beta_2$-agonists or cromones for 4 weeks, no antihistamines for 2 weeks, and no inhaled or oral steroids for the last 2 months prior to the study. Subjects with uncontrolled systemic disease, and women who were currently pregnant, lactating, or of childbearing potential without adequate contraception were excluded. Patients with seasonal allergies were not studied during the season for their allergy.  During 2 weeks before the baseline bronchoscopy, all subjects recorded asthmatic symptoms (Table 3) and PEF measurements on a diary card.

*Table 3. Asthma symptom scoring.*

|   | **Daytime symptom score** |
|---|---|
| 0 | no symptoms during the day |
| 1 | symptoms for one short period during the day |
| 2 | symptoms for two or more periods during the day |
| 3 | symptoms for the most of the day; symptoms did  not affect your normal daily activities |
| 4 | symptoms for the most of the day; symptoms affected your normal daily activities |
| 5 | symptoms so severe that you could not to go to work or perform normal daily activities |
|   | **Night-time symptom score** |
| 0 | no symptoms during the night |
| 1 | symptoms causing you to wake once or wake up early |
| 2 | symptoms causing you to wake twice or more (including waking up early) |
| 3 | symptoms causing you to be awake for the most of the night |
| 4 | symptoms so severe that you did not sleep at all |

## 3.2 Design of drug intervention studies

### 3.2.1 Skiers (II)

The study design was a double-blind, block randomized, placebo-controlled parallel-group study. The 25 subjects were randomized to receive budesonide 400 µg or placebo twice daily by Turbuhaler™ (Astra Draco AB, Sweden) for a planned 12 weeks. They were skiers who were hyperresponsive and had asthmatic symptoms. Immediately before and after the treatment period, each underwent fiberoptic bronchoscopies with bronchoalveolar lavage (BAL) and bronchial biopsies. Bronchial hyperresponsiveness to methacholine was also assessed before and after the treatment period.  At the end of treatment, each subject was asked to evaluate the effect of treatment on respiratory symptoms. Compliance with study medication was assessed by asking the subject if he or she had taken the medication as instructed.

The study commenced in October/November 1993, which was prior to the start of the competitive season. Practical problems made it impossible for the skiers to attend the second bronchoscopy at 12 weeks after commencement of treatment, as this coincided with the peak period of competition, so, in fact, the mean treatment period was 22 weeks (range 10 to 32). During the treatment period, the subjects were allowed to use terbutaline 0.5 mg (Bricanyl Turbuhaler, Astra Draco AB, Sweden) as required.

### 3.2.2 Newly diagnosed asthmatics (IV)

The study had a randomized, double-blind, double-dummy, placebo-controlled parallel-group design. The patients visited the clinics (Helsinki University Central Hospital and North Karelia Central Hospital in Finland) seven times. After a run-in period of 2 weeks from the first visit, patients were randomized to receive inhaled salmeterol 50 µg (Diskhaler™) bid, fluticasone propionate 250 µg (Diskhaler™) bid, disodium cromoglycate 5 mg (pMDI) four times daily (qid), or placebo for 16 weeks. When needed, inhaled salbutamol pMDI 0.1 mg was allowed as rescue medication during the whole study period. Bronchoscopy with bronchial biopsies was performed at baseline before starting the study medication (visit 2) and after the 16-week treatment period (visit 6). During the treatment period, the patients visited the clinic twice before the second bronchoscopy (visits 3 to 5). Two weeks before each visit the patients recorded asthma symptoms (Table 3) and PEF measurements on a diary card. After the second bronchoscopy and cessation of treatment, the patients came to the clinic for a follow-up visit (visit 7). Patients measured their morning PEF before taking their morning dose of the study medication. At each clinic visit, compliance with study medication was assessed by collecting and examining the inhalers and Rotadisks.

## 3.3 Investigation methods

### 3.3.1 Lung function tests and symptom evaluation

The skiers' (I-II) lung volumes were assessed by spirometry with a Microlab 3300 Mk2 5 spirometer (Micro Medical Ltd., Rochester, UK). The control subjects and mild asthmatic subjects performed spirometry with Jaeger Flowscreen 2.1 gG spirometer (Erich Jaeger Laboratories, Würtzburg, Germany). In the patients with newly diagnosed asthma, spirometry was measured with a Vitalograph spirometer (Fleisch, Bucks, UK). For the control subjects, the bronchodilator test was performed with 3 x 0.2 mg inhaled rimiterol (Pulmadil™, 3 M Health Care Ltd., Loughborough, UK) and for the asthma patients with inhaled salbutamol (Ventoline™, Glaxo, London, UK).

In the skier group, bronchial hyperresponsiveness (BHR) was assessed with methacholine by use of a controlled tidal volume breathing technique (Nieminen et al 1988). A cumulative dose of 1.8 mg methacholine was administered, and the test was discontinued if a 20% fall in forced expiratory volume in one second ($FEV_1$) occurred, and the dose causing a 20% fall in ($FEV_1$) was determined as $PD_{20}$ $FEV_1$. Subjects with $PD_{20}$ $FEV_1 \leq 1.8$ mg methacholine were considered to be hyperresponsive, as based on earlier findings (Sue-Chu et al 1996). In all asthmatic subjects, BHR was assessed with histamine challenge according to the method described by Sovijärvi et al (1993). A cumulative dose of histamine causing a 15% fall in $PD_{20}$ FEV1 was assessed. The subject was considered as having severe BHR if the histamine dose was <0.1 mg, and moderate BHR at dose <0.4

mg. Both methacholine and histamine challenges were performed with the Spira Elektro 2 inhalation-synchronized nebulizer (Respiratory Care Center, Hämeenlinna, Finland).

The skiers completed a questionnaire about respiratory symptoms (cough, wheeze, and dyspnea at rest, during training, or at exposure to irritants), allergies, medical consultations during the previous five years, use of antiasthmatic medication, smoking, training hours, and competitive skiing experience (Larsson et al 1994). The questionnaire was in Swedish or Norwegian depending on the subjects' own language. The skiers suffering from wheeze and abnormal breathlessness or chest tightness on exertion or at rest, or on exposure to irritants during the previous year combined with bronchial hyperresponsiveness in methacholine challenge were defined as having asthmatic symptoms.

Patients with newly diagnosed asthma completed diary cards to assess symptoms and performed PEF measurements with a mini-Wright peak flow meter (Clement-Clarke, Harlow, UK) twice daily during the 2 weeks of the run-in before bronchoscopy. In the intervention study, each subject performed PEF measurements and assessed symptoms during the 2 weeks before every clinic visit (IV). Asthma symptoms (0-5, daytime; 0-4 night-time) and number of puffs of rescue medication were recorded. In the intervention study (IV), PEF measurements were performed before inhalation of the study drug. $FEV_1$ and forced vital capacity (FVC) were measured with a Vitalograph™ spirometer (Fleisch, Bucks, UK) at baseline. Histamine challenge was performed before noon at baseline during the first clinic visit (III), and after 8 and 16 weeks of treatment (IV). The last histamine challenge was performed 3 days before bronchoscopy at visit 6 (the day of the second bronchoscopy). Efficacy of the treatment was measured as a change in reaction to histamine challenge in doubling dose (DD) units.

### 3.3.2 Allergy testing and laboratory tests

Presence of atopy in the skier group (I) was tested with a Phadiotop CAP test (Pharmacia Diagnostics, Uppsala, Sweden) which detects specific IGE in the serum to eight common aeroallergens (birch, cat, cladosporium, dog, horse, house dust mite, mugwort, and timothy). In control subjects and mild asthmatic subjects (I), the presence of allergy was assessed with skin prick testing a panel of 12 common allergens (Soluprick SQ, ALK, Denmark). In subjects with newly diagnosed asthma (Studies III-IV) atopy was assessed by skin prick testing (Soluprick SQ) with a panel of ten common allergens in Finland (alder, alopecus, birch, cat, horse, house dust mite, Kentucky blue, mugwort, reed, timothy).

### 3.3.3 Fiberoptic bronchoscopy

Bronchoscopy and obtaining of bronchial specimens conformed to international guidelines (Bleecker et al 1991).

The skiers (I,II) were premedicated with a solution of 2.5 ml salbutamol (1 mg/ml) and 1 ml ipratropium bromide (0.25 mg/ml) administered by nebulization at 15 minutes prior

to bronchoscopy, followed by the intravenous administration of 0.3 to 0.5 mg glycopyrronium, 1-2 mg midazolam, and 0.25 mg alfentanil. They were bronchoscoped before the start of the skiing season in October and November at the University Hospital in Trondheim. The skiers participating in the drug intervention study were bronchoscoped the second time after the treatment period. Patients with mild asthma and control subjects (I) were premedicated with intravenous atropine (0.5 to 1 mg) and oral diazepam (5 to10 mg). They were bronchoscoped at Tartu University Hospital. None of the subjects were bronchoscoped during a respiratory infection or less than 4 weeks after one. The fiberoptic bronchoscopy was performed under local anesthesia with topical lidocaine with the Olympus BF XT20, BF IT30, and BT IT20D (Olympus Co., Tokyo, Japan). Bronchial biopsies were performed at the first and second generation bronchial carinal levels from both lungs. The forceps were Olympus F-19C, FB-20C or FB-35C (Olympus Co).

In subjects with newly diagnosed asthma, fiberoptic bronchoscopy was performed twice: before the start of the investigational medication (III) and after 16-week therapy with the investigational medication (IV). Oral oxazepam 30 mg, intramuscular atropine 0.5 mg, and inhaled salbutamol 0.2 mg served as premedication. Lidocaine served as the local anesthetic up to a maximum dose of 500 mg. The bronchoscope (Olympus BF XT20) was introduced orally, and the biopsy specimens were taken with cupped forceps (Olympus FB-36C E) from areas not yet touched by the bronchoscope at the lobar bronchial level.

Macroscopic inflammatory index was assessed by an experienced bronchoscopist on a five-point scale (0 to 4) (Van Vyve et al 1993). Friability, vascularity, and edema of the bronchial mucosa, and amount of secretions in the airways were evaluated.


## 3.4 Examination of biopsy specimens

All bronchial biopsy specimens were snap-frozen in liquid nitrogen and stored at -70ºC before processing. They were then embedded in Tissue Tek O.C.T. medium (Miles Inc., Elkhart, IN, USA), and 5-µm cryosections were cut on a Leitz 1720 Digital Cryostat (Ernst Leitz GmbH, Wetzlar, Germany). The slides were coded and evaluated by the same observer blinded to subjects' clinical data or treatment.

**Immunohistochemistry**
Sections were stained with mouse monoclonal antibodies (mAbs) to detect inflammatory cells by the alkaline phosphatase anti-alkaline phosphatase method, modified as reported earlier (Laitinen et al 1997). The following mAbs were used: EG2 (Kabi Pharmacia Diagnostic Ab, Uppsala, Sweden) to detect activated eosinophils (dilution 1:50); tryptase clone AA1 (Dako A/S, Glostrup, Denmark) to detect mast cells (dilution 1:500); Ber-Mac3 (Dako A/S) to detect macrophages (dilution 1:25); CD3+ (Dako A/S) to detect T-lymphocytes; and neutrophil elastase clone NP57 mAb to detect neutrophils (dilution 1:2000). The sections were first fixed in acetone at room temperature (20ºC) for 10 minutes, rinsed in Tris-buffered saline for 10 minutes, and then exposed to the primary mAb for 30 minutes in a moist chamber. The rabbit anti-mouse immunoglobulin (dilution

1:25, Dako A/S) and the mouse monoclonal antibody (dilution 1:25, Dako A/S) were successively applied for 30 minutes. Following each antibody application, the slides were washed in Tris-buffered saline. The color reaction was developed with New Fuchsin (Sigma, St Louis, MO, USA). The endogenous alkaline phosphatase activity was blocked with 1M levamisole (Sigma). For counterstaining, the sections were treated for 5 to10 seconds with Mayer's hemalan (dilution 1:5, E. Merck, Darmstadt, Germany) and after rinsing with water and distilled water, the slides were mounted with Glysergel (Dako A/S) and cover-slipped. In Studies III and IV, eosinophils were detected by a different method: The frozen sections were fixed with 4% paraformaldehyde in phosphate buffer at + 20$^o$C for 20 min, then exposed for 30 min to normal rabbit serum (dilution1:10) and then to primary mAbs overnight at +4$^o$C and processed for the avidin-biotin-peroxidase method in accordance with manufacturer's instructions (Vector Laboratories, Burlingame, CA, USA). Negative controls and were provided by omitting the primary antibody and replacing it with buffer. The slides were examined under Leitz Dialux 22 EB microscope.

The density of inflammatory cells in the entire sections was computed with the Autocad program 10.1 (Autodesk Inc., Sausalito, CA, USA). The entire sections were first photographed on Kodak Ektachrome EPN 100 color slide films at magnification of x16. The slides were projected onto a calibrated 42x60-inch Kurta IS/THREE digitizing tablet (Kurta Corp., Phoenix, AZ, USA). The total number of each inflammatory cell type was counted with a pointing device throughout the bronchial mucosa, disregarding damaged areas. The inflammatory cell counts were expressed as cells per mm2 of total mucosa analyzed.

**Tenascin expression in basement membrane**

To detect the Tn immunoreactive area in the BM zone, the sections were stained with mouse mAb 100EB2 (Locus Genex, Helsinki, Finland) (Balza et al 1993) by an indirect immunofluorescence technique. The cryosections were first fixed in -20ºC acetone for 10 minutes and after being rinsed in phosphate buffer saline (PBS) the sections were incubated for 30 min with the primary Mab in a moist chamber at room temperature. Then sections were thoroughly rinsed in PBS and thereafter incubated in the same way as the primary mAb with 1:150 diluted fluorescein isothiocyanate (FITC)-conjugated goat antimouse IgG (Jackson Laboratories, Wets Grove, PA, USA). The sections were then mounted in Veronal-glycerol buffer (1:1) pH 8.4 and cover-slipped. Negative controls were provided by omitting the primary Mab or replacing it with an irrelevant one.

The specimens were examined with a Leitz Aristoplan fluorescence microscope equipped with an appropriate filter. Areas containing crosssections of the immunoreactive BM were photographed with Kodak T-MAX 400/800 black and white film (Eastman Kodak Company, Rochester, NY, USA). Paper photocopies had a final magnification of 643. To quantitate Tn expression in the BM zone, the thickness of the Tn immunoreactive area in the BM was measured from the photocopies semiautomatically by the computerized image analysis Autocad program 10.1 (Autodesk Inc., Sausalito, CA, USA). The upper and lower margins of the immunostained BM were digitized with a pointing device; the mean distance (in micrometers) was regarded as thickness of the Tn in each biopsy specimen.

## 3.6 Statistical methods

In all studies, P-value <0.05 was considered statistically significant.

Data on cell counts and tenascin immunoreactivity thickness were in the skier baseline study (I) analyzed by the Mann-Whitney U-test or Kruskal-Wallis test applying Dunn's correction for multiple comparisons. Correlations were calculated with Spearman's rank correlation method.

In the skiers' intervention study, data underwent intention-to-treat analysis. To evaluate differences between groups, the Kruskall-Wallis test with Dunn's test was used. Spearman's rank correlation test was performed to evaluate any correlation between inflammatory findings and pulmonary function tests.

Student's T-test or the Mann-Whitney U-test served to assess comparisons between atopic and nonatopic asthmatics (III). Correlation coefficients were calculated with Spearman's rank method when assessing the correlations between inflammatory cells and tenascin immunoreactivity.

In the asthma intervention study, treatment effect on BHR was assessed by calculating the doubling-dose effect, with logarithmic transformed PD15 values used. Testing of significance of changes within groups during treatment was by Wilcoxon-rank sum tests. Significance of the differences in the changes during treatment between the four groups was tested with Kruskall-Wallis analysis of variance, and when appropriate, comparisons were continued between groups. Diary card data were summarized over both the run-in and the treatment period. Statistical analyses were performed with the SPSS package for Windows (version 10). Means and standard deviations and range or 95% confidence intervals (CI) were used in data presentation.

# 4. RESULTS

## 4.1. Clinical characteristics of study subjects

Lung function in all study groups was on average normal (Table 4). This was in part for safety reasons, because subjects with poor lung function ($FEV_1$ <60% of predicted) were excluded from the studies. Lung function was normal in skiers, mildly asthmatic patients, and controls, with no significant differences between these groups. Skiers were significantly younger than asthmatic subjects. All asthmatic patients were determined as hyperresponsive by a histamine challenge test and fulfilled diagnostic criteria (ATS) for asthma. They were mildly symptomatic before the investigational bronchoscopy. The majority of skiers (75%) were hyperresponsive to methacholine. Asthmatic symptoms were frequent among skiers, occurring in 26 subjects (65%). Six of the symptomatic skiers reported use of an inhaled $\beta_2$-agonist. Asthmatic symptoms were slightly more frequent in the hyperresponsive skiers than in nonhyperresponsive skiers (70% vs 50%); 18 skiers reported wheeze, 14 reported dyspnea during training or competition, and only 11 of the skiers were asymptomatic (unpublished data). Even though no exercise test was performed, dyspnea during training or competition can be suggestive of EIB.

## 4.2 Inflammatory cells in bronchial biopsy specimens (I, III)

Adequate biopsy specimens were obtained from all skiers and mild asthmatics, but not from all patients with newly diagnosed asthma. Neutrophil detection was impossible in the control group, because of a lack of biopsy material from most of the control subjects.

In skiers, cell counts were greater than in the controls, T-lymphocytes by 43-fold (P<0.001), macrophages by 26-fold (P<0.001), and eosinophils by two-fold (P<0.001). However, in skiers, the increase in inflammatory cell densities did not quite reach that in mildly asthmatic subjects, except for T-lymphocytes. Skiers had lower counts of macrophages (P<0.001), mast cells (P<0.001), and eosinophils (P<0.001) than did mildly asthmatic subjects. The T-lymphocyte count was higher than in controls in both skiers and asthmatic subjects. T-lymphocyte counts were as high in skiers as in mild asthmatics. In subjects with mild asthma, cell counts were significantly higher than in control subjects: T-lymphocytes were increased by 70-fold (P<0.001), macrophages by 63-fold (P<0.001), and eosinophils by 8-fold (P<0.001). No significant differences in mast cell counts appeared between skiers and controls, but the mast cell count was 3-fold greater in subjects with mild asthma than in controls (P<0.001) and 2-fold greater in subjects mild asthma than in skiers.

The inflammatory cell counts in subjects with newly diagnosed asthma resembled those in subjects with mild chronic asthma (Table 5). Neutrophil counts were significantly higher in skiers than in mild asthmatics (P<0.05). Skiing experience in years correlated

positively with macrophage density in the bronchial mucosa (Rho=0.42, P=0.007), but not with other cell densities.

*Table 4.* *Clinical characteristics of study subjects.*

| Group | Skiers (n=40) | Mild asthmatics (n=12) | Newly diagnosed asthmatics (n=80) | Controls (n=12) |
|---|---|---|---|---|
| Gender (men/women) | 32/8 | 6/6 | 33/46 | 7/5 |
| Age (mean, range) | 17.5 (16-20) | 39.9 (18-58) | 36 (18-61) | 25 (22-29) |
| Presence of BHR (n) | 30 | 12 | 79 | N.A. |
| BHR (median ± SD) | 1.25 µg[a] ± 0.44 | 0.06 mg ± 0.15[b] | 0.13 mg ± 0.1 [b] | N.A. |
| $FEV_1$ % of predicted ±SD | 99.2 ± 2.1 | 95.7 ± 3.22 | 80.9 ± 11.7 | 109.1 ± 4.52 |
| $FEV_1$ reversibility in % ±SD | N.A. | 29.34 ± 17.4 | 11.2 ± 8.8 | 5.39 ± 4.1 |
| Duration of asthma (years, range) | - | 8.9 (2-30) | 0.33 (0-2) | - |
| Presence of atopy (%) | 38 | 42 | 73 | 0 |
| **Study** | **I, II[c]** | **I** | **III, IV** | **I** |

Degree of bronchial hyperresponsiveness determined by [a]$PD_{20}FEV_1$ to methacholine and [b]$PD_{15}FEV_1$ to histamine. [b]25 of these subjects participated in Study II

*Table 5.* *Inflammatory cell counts as medians (interquartal range) in cells/mm$^2$ in study groups.*

| Cell type | Skiers (n=40) | Mild asthmatics (n=12) | Newly diagnosed asthmatics (n=80) | Controls (n=12) |
|---|---|---|---|---|
| Eosinophils | 21 (9-52) | 81 (61-119) | 81[b] (32,144) | 10 (5-11) |
| T-lymphocytes | 521 (315-972) | 853 (557-1106) | 409[c] (271-725) | 12 (0-44) |
| Mast cells | 65 (43-95) | 164 (89-226) | 81[c] (39-115) | 50 (27-85) |
| Macrophages | 105 (60-174) | 253 (175-382) | 204[c] (115-301) | 4 (0-9) |
| Neutrophils | 83[a] (47-119) | 31 (10-68) | 23[c] (11-53) | N.A. |

[a]data from 37 skiers, [b]data from 71 subjects, [c]data from 72 subjects

## 4.3 Tenascin in the bronchial biopsy specimens (I, III)

Tn immunoreactivity occurred in every skier and patient with asthma. In contrast to this, the Tn immunoreactivity was absent from 50% of the control subjects. In control subjects, Tn immunoreactivity showed as a thin and sometimes interrupted line underneath the epithelium, whereas in the skiers and patients with asthma the immunoreactivity was strong, showing as a distinct thick line underneath the epithelium. The Tn-specific immunoreactivity band in the BM was significantly thicker in skiers [6.7 (IQr 5.3-8.5) µm, $P<0.001$] and in mild asthmatics [8.8 (IQr 7.2-10.8) µm, $P<0.001$] than in controls [0.8 (IQr 0-3.1) µm] expressed as median and interquartile range. A significant increase in Tn expression occurred in the order: controls < nonhyperresponsive skiers < hyperresponsive skiers < mild asthmatics. Tn expression in mild asthmatics was greater than in skiers, with no significant difference evident when comparing mild asthmatics with either hyperresponsive or nonhyperresponsive skiers. No difference appeared in Tn immunoreactivity between hyperresponsive and non-hyperresponsive skiers. Tn immunoreactivity was present in every subject with newly diagnosed asthma, if adequate biopsy material was obtained. The median Tn thickness in these subjects was 6.8 µm (IQr 5.9-8.3), markedly greater than in control subjects (Figure 1).

In skiers, tenascin immunoreactivity thickness did not significantly correlate with any inflammatory cell type. In the group with newly diagnosed asthma, the thickness of the Tn layer correlated positively with most of the inflammatory cell types studied except with neutrophils and mast cells.
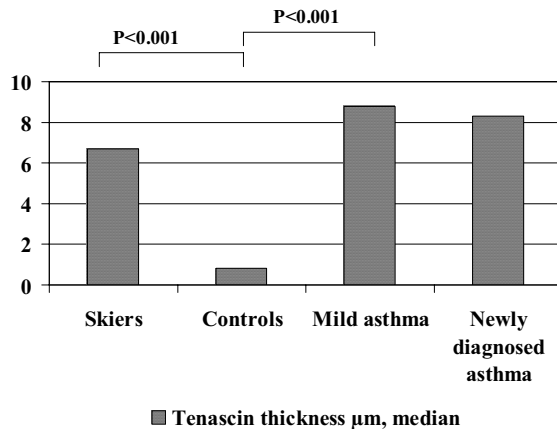
*Figure 1.* Tenascin immunoreactivity band thickness in the bronchial basement membrane (median).

## 4.4 BHR and airway inflammation in bronchial biopsy specimens (I,III)

No difference in T-lymphocyte, macrophage, mast cell, and eosinophil counts emerged between nonhyperresponsive and hyperresponsive skiers (Figure 2), showing that these cells were not responsible for hyperresponsiveness in skiers. Compared to control subjects, both hyperresponsive and nonhyperresponsive skiers had increased lymphocyte (both P<0.001) and macrophage counts (both P<0.001), but eosinophil count was significantly increased only in hyperresponsive skiers (P<0.001). Both hyperresponsive and nonhyperresponsive skiers had lower counts of macrophages (P<0.05), mast cells (P<0.05), and eosinophils (P<0.05) than did subjects with mild asthma. In the skier group, none of the inflammatory cell densities were significantly correlated with PD20FEV1 to methacholine.

For the patients with newly diagnosed asthma, we failed to show any correlation between $PD_{15}FEV_1$ to histamine and any inflammatory cell density in their bronchial

biopsy specimens. However, a significant negative correlation appeared between eosinophil density and $PD_{15}FEV_1$ to histamine in a subgroup of atopic asthmatics (Rho=-0.37, P=0.007), but not in nonatopic subjects (Rho=0.01, P=0.95). When blood eosinophils and serum ECP were measured, neither eosinophils nor serum ECP correlated significantly with $FEV_1$ or $PD_{15}FEV_1$ to histamine.
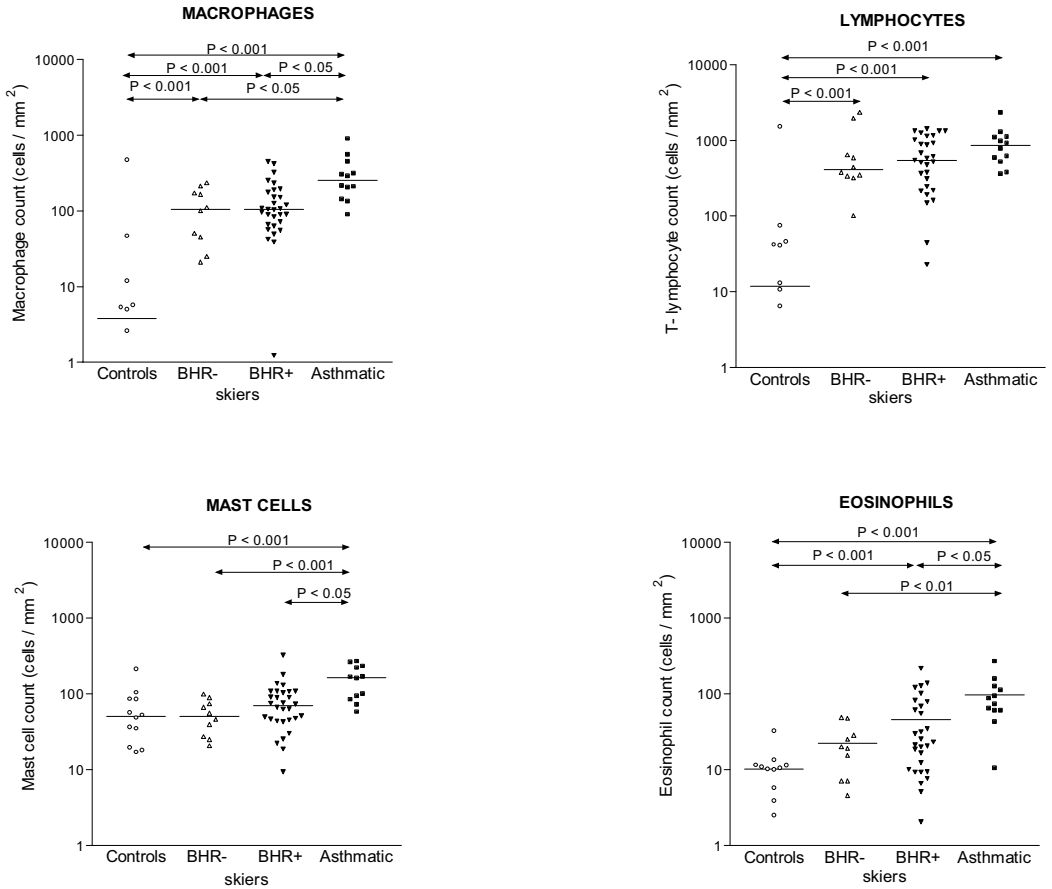
**Figure 2**. Density (cells/mm2) of macrophages, T-lymphocytes, mast cells, and eosinophils in bronchial biopsy specimens from control subjects, skiers with and without BHR, and asthmatic subjects. Horizontal bar=median value. The figure is reprinted from Study I article with permission from Am J Respir Crit Care Med.

## 4.5 Atopy and bronchial biopsy findings

Of the skiers, 16 (38%) were defined as atopic according to the Phadiotop CAP test. However, only five subjects of those with a positive allergy test reported respiratory allergies. Three Phadiotop-negative subjects reported allergic symptoms (unpublished data). The densities of T-lymphocytes, macrophages, and eosinophils did not differ between atopic and nonatopic skiers, but mast cell densities were higher in nonatopic than in atopic skiers ($P<0.01$) (unpublished data). Comparing T-lymphocyte, macrophage, mast cell, and neutrophil densities in the nonatopic skiers and nonatopic mild asthmatics showed no difference, but eosinophil density was greater in nonatopic asthmatics than in nonatopic skiers.

Among the subjects with newly diagnosed asthma, atopy was common: 58 (73%) had positive reactions in the skin prick testing. No significant differences appeared between nonatopic and atopic subjects for the inflammatory cell counts studied (T-lymphocytes, CD8- and CD4-positive lymphocytes, macrophages, eosinophils, neutrophils, or IL4-positive cells).

Tn immunoreactivity thickness was significantly greater in atopic than in nonatopic subjects with newly diagnosed asthma, but with no significant difference between atopic and nonatopic skiers (Figure 3). In atopic subjects, thickness of the Tn layer correlated positively with eosinophils, macrophages, and T-lymphocytes (including CD4- and CD8-positive cells), whereas nonatopic subjects showed a positive correlation only with CD8-positive cells. No correlation appeared with mast cells or neutrophils and tenascin.
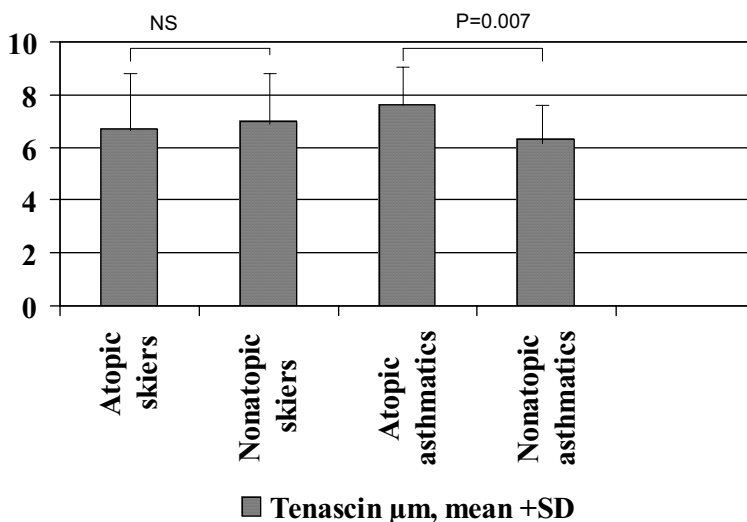
**Figure 3**. *Tenascin immunoreactivity thickness in diagnosed the basement membrane according to atopic status in skiers and in patients with newly asthma.*

## 4.6. Treatment effects

### 4.6.1 The efficacy of inhaled budesonide in airway inflammation and BHR in skiers

The skier treatment study involved 25 elite cross-country skiers: 12 treated with budesonide and 13 with placebo. Mean duration (range) of the treatment period was similar in both groups, in the budesonide group 22.3 (10 to 32) weeks and in the placebo group 22.8 (20 to 27) weeks (P=0.78). One skier in the budesonide group withdrew from the study at 10 weeks because he had experienced a worsening of his asthma-like symptoms. The baseline characteristics between groups were similar except for $PD_{20}FEV_1$ to methacholine which was greater in the budesonide group (Table 6). Compliance was in general good. Only two subjects (placebo group) reported moderate compliance, and the rest reported themselves as showing good compliance.

Treatment with budesonide improved lung function ($FEV_1$) significantly (P=0.005). Responsiveness to methacholine improved in both treatment groups: It decreased in 10 skiers in the placebo group and in 9 skiers in the budesonide group; at the end of the treatment period, BHR had disappeared in 7 skiers in each treatment group. No significant changes were reported in asthmatic symptomsby skiers with good compliance. Asthmatic symptoms worsened in 3 subjects in each treatment group, and improved in 2 subjects in

the placebo group; and in 15 subjects symptoms neither improved nor worsened. The macroscopic inflammatory index decreased in the placebo group but not in the budesonide group.

Assessable paired pre-treatment and post-treatment biopsies were obtained from 11 subjects in the placebo group and 10 in the budesonide group. At baseline, their inflammatory cell densities or Tn immunoreactivity thickness did not significantly differ. There was no evidence of treatment effects. Neither inflammatory cell densities (CD3+ lymphocytes, macrophages, eosinophils, mast cells) nor Tn immunoreactivity thickness (Figure 4) changed significantly within treatment groups (P varied between 0.06-1.0) or between the two treatment groups after the treatment period (Figure 4).

**Table 6**. *Clinical characteristics of study subjects.*

|  | Placebo N=13 | Budesonide N=12 |
|---|---|---|
| Males/females | 9/4 | 10/2 |
| Mean age (mean, range) | 18 (16-20) | 17.9 (16-20) |
| Training hours previous year (mean ±SD) | 468 (91) | 427 (92) |
| Years of competition (mean±SD) | 8.3 (2.0) | 7.5 (2.4) |
| $FEV_1$, % of predicted (mean) | 93.1 | 95.9 |
| $PD_{20}FEV_1$ methacholine (mg) (median, range) | 1.1 (0.5-1.3) | 1.5 * (1.2-1.8) |
| Atopy | 4 | 5 |

*$P<0.05$ compared with placebo group, Mann-Whitney U-test. Table is modified from Study II article.
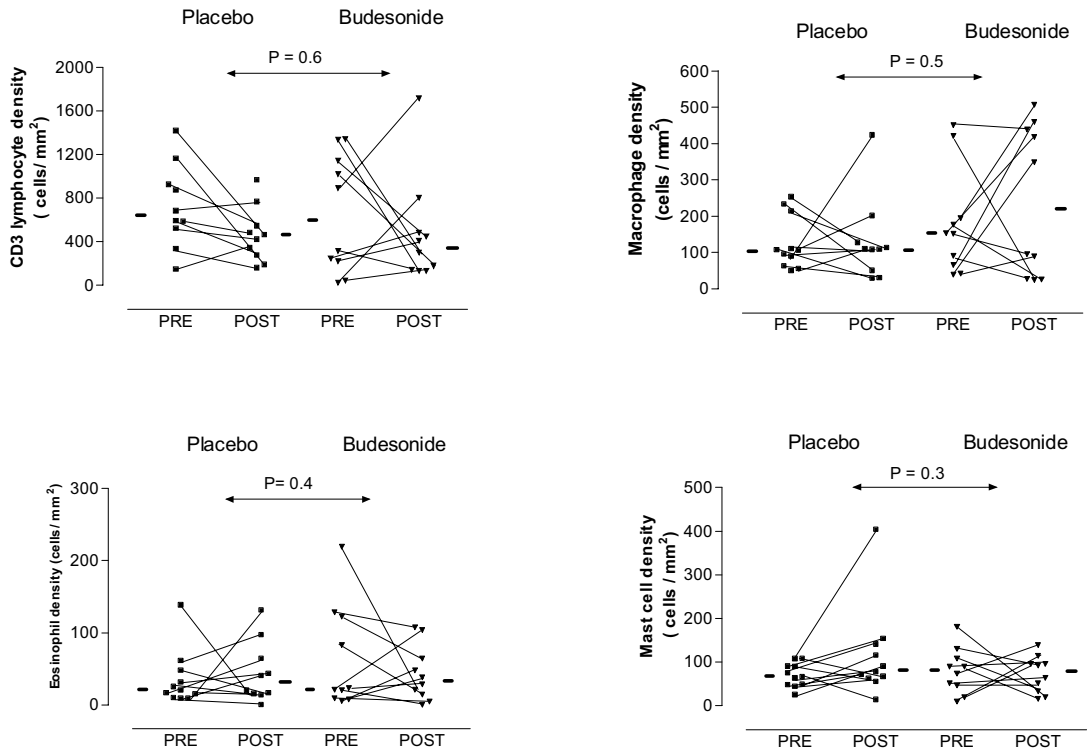
***Figure 4.*** *Inflammatory cell densities (cells/mm2) in bronchial mucosa at baseline and at follow-up. Figure reprinted from Study II article with permission of S. Karger AG, Basel.*

### 4.6.2 The efficacy of salmeterol (SLM), fluticasone propionate (FP), and disodium cromoglycate (DSCG) in airway inflammation and BHR in newly diagnosed asthma (IV)

In the run-in period of the study, 83 patients participated and 80 were eligible for the treatment. One patient was excluded because of an upper respiratory infection at week 13. Another patient was excluded because of an unexpected first epileptic seizure during the treatment period. A total of 78 patients completed the study.

Symptoms and rescue medication use both during the night and day decreased in the SLM, FP, and DSCG groups (P<0.036), but not in the placebo group. Morning PEF was improved with both SLM and FP (by 46 and 75 L/min) (P<0.005) treatments. They also improved lung function measured with FEV1 (by 0.23 L and 0.3 L, respectively) significantly (P<0.005). BHR was improved significantly with SLM, FP, and DSCG, but not with placebo. The doubling doses fromhistamine at the end of the treatment during week 16 were 2.78 for SLM (CI 1.03 to 4.53), 5.22 for FP (CI 3.01 to 7.43), and 1.35 for DSCG (CI 0.50 to 2.20) (all P<0.05). Placebo had no effect on hyperresponsiveness. The doubling dose from placebo was 0.11 DD (CI –0.87 to 1.08). SLM was significantly more effective than placebo (difference 2.67 DD CI 0.02 to 5.32, P=0.048). FP improved BHR significantly more than did DSCG (difference: 3.87 DD CI 1.15 to 6.59, P=0.002) and placebo (difference: 5.11 DD CI 2.39 to 7.83, P<0.001). The increase in doubling dose was achieved at week 8 of treatment and was sustained at week 16 (Figure 5) (unpublished data).
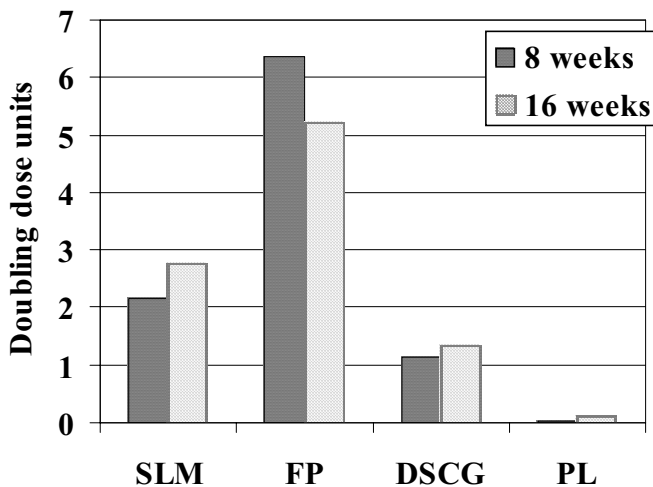


**Figure 5.** *Doubling dose improvement from histamine at weeks 8 and 16 in salmeterol (SLM), fluticasone propionate (FP), disodium cromoglicate (DSCG), and placebo (PL).*

Adequate paired biopsy material was obtained from 65 patients (5 to 6 specimens per bronchoscopy). All subjects tolerated bronchoscopies well, and no complications occurred during or after the bronchoscopy. Some subjects had hemoptysis just after the bronchoscopy, but it disappeared in a few hours. Good-quality biopsy samples were not available from all subjects. Representative samples for assessing inflammatory cell type or Tn (BM area used for quantitation) ranged from 17 to 18 in the SLM group, from 12 to 16 in the FP group, from 18 to 19 in the placebo group, and was 18 in the DSCG group. At baseline, no significant differences appeared in the cell counts, Tn immunoreactivity, BHR to histamine, or lung function between treatment groups.

SLM had no significant effect on any cell type, and cell counts did not differ from those of placebo treatment. Treatment with FP attenuated the eosinophilic inflammation (Figure 6) by reducing EG2-positive cell counts (change –71 cells/mm$^2$, CI -113 to -29, P=0.002). FP also reduced CD3-positive T-lymphocytes, but this change did not reach significance (change –274 cells/mm$^2$, CI –587 to 40, P=0.060). DSCG also attenuated eosinophilic inflammation by reducing eosinophil count significantly (change –54 cells/mm$^2$, CI –127 to 18, P= 0.048). DSCG had no significant effect on CD3-positive lymphocytes (change –182 cells/mm$^2$, CI 408 to 44, P=0.248). No changes occurred in any of the groups for mast cells or neutrophils (Table 7).
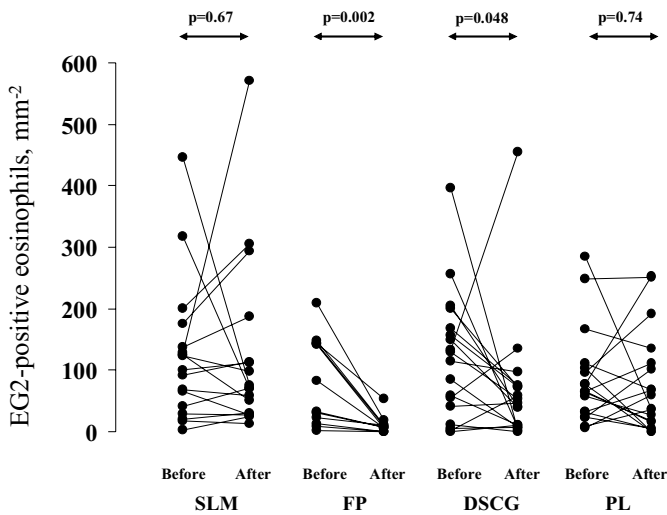


*Figure 6.* *Number of EG2-positive eosinophils in bronchial mucosa at baseline and after a 16-week treatment with salmeterol (SLM), fluticasone propionate (FP), disodium cromoglycate (DCG), and placebo (PL). The figure is reprinted from Study IV article with permission from Elsevier.*

***Table*** *7. Inflammatory cell counts (cells/mm$^2$) in the bronchial biopsy specimens before and after treatments.*

| | Salmeterol | | Fluticasone propionate | | Disodium cromoglygate | | Placebo | |
| | (n=17) | | (n=12) | | (n=18) | | (n= 18) | |
| | *Before* | *After* | *Before* | *After* | *Before* | *After* | *Before* | *After* |
|---|---|---|---|---|---|---|---|---|
| **AA1** | 101.8 | 56.1 | 85.0 | 85.9 | 108.4 | 65.8 | 102.9 | 56.2 |
| | (77.5) | (54.3) | (54.2) | (72.5) | (88.4) | (39.8) | (97.3) | (38.4) |
| **CD3** | 504.5 | 467.2 | 555.2 | 281.7 | 568.5 | 386.2 | 543.9 | 478.2 |
| | (394.0) | (300.7) | (344.2) | (267.2) | (527.5) | (196.4) | (391.8) | (296.8) |
| **EG2** | 123.4 | 125.4 | 81.8 | 11.0[a] | 120.6 | 66.2[b] | 85.9 | 76.8 |
| | (114.6) | (144.4) | (72.0) | (14.6) | (103.4) | (104.1) | (77.4) | (82.4) |
| **ELA** | 23.9 | 32.4 | 54.4 | 67.0 | 37.7 | 61.7 | 43.8 | 43.9 |
| | (16.6) | (25.3) | (64.6) | (46.8) | (31.9) | (55.6) | (40.9 | (33.2) |

*Results are given as mean (with standard deviation). AA1 = mast cells, CD3 = T-lymphocytes, EG2 = activated eosinophils, ELA = neutrophils are presented. [a]P=0.002, [b]P=0.048. The table is modified from the Study IV article.*

The macroscopic inflammatory index did not change significantly during any of the treatments, but showed a tendency towards a decrease in mucosal erythema in the SLM group and a decrease in mucosal edema in the FP group (Figure 7) (unpublished data).

*Figure 7.* Macroscopic evaluation by a bronchoscopist of mucosal oedema and vascularity in bronchoscopy before and after treatment. A five-point scale was used (0 to 4).

**Oedema**



**Erythema**
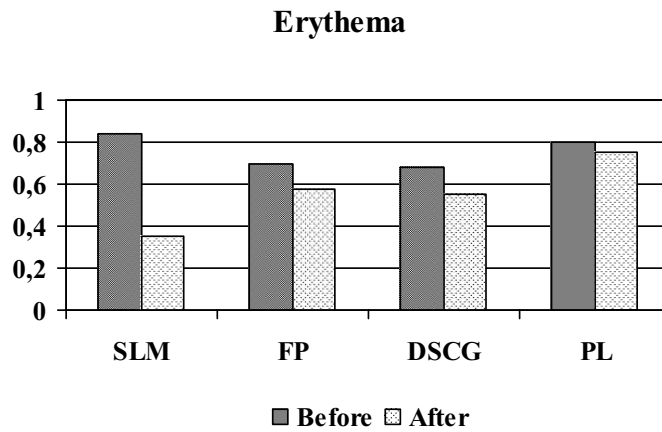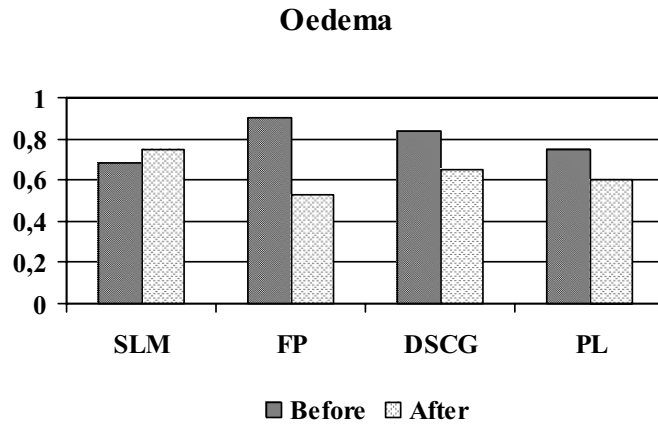
# 5. DISCUSSION

## 5.1 Airway inflammation and remodeling in the bronchial biopsies from elite cross country skiers

In this study we have shown an increased number of inflammatory cells, especially T-lymphocytes and macrophages but also eosinophils and neutrophils, in bronchial biopsy specimens from healthy young ski athletes with no prior diagnosis of asthma. We anticipated that the number of inflammatory cells in the airways of the skiers would be increased, but the magnitude of inflammatory cell densities was unexpected. We cannot for certain exclude, among the skiers, the possibility of undiagnosed asthma. However, none had a prior diagnosis of asthma made by a physician.  As EIB is prevalent among athletics, testing for EIB by field exercise test or with EVH would have resulted in more insights for the study (Rundell et al 2004, Parsons and Mastronarde 2005). Currently, to receive permission for use of anti-asthmatic medication, the competitive athlete must show evidence of reversible airflow obstruction or a 10% decrease in FEV1 in EVH or exercise test. Evidence of nonspecific BHR is insufficient for diagnosis of asthma in elite athletes. However, BHR is prevalent in athletes (Table 2), and asthma and respiratory symptoms are common even in non-asthmatic athletes (Turcotte et al 2003). Studies investigating inflammatory changes in the airways of athletes are small in number.

Helenius et al (1998a) reported increased numbers of eosinophils and neutrophils as well as increased concentrations of EPO and human neutrophil lipocalin (HNL) in induced sputum from asthmatic and nonasthmatic swimmers. They found no correlations between BHR and sputum inflammatory indices. Furthermore, the nonasthmatic swimmers had higher eosinophil counts and higher EPO and HNL in the sputum than did healthy nonathelete controls. Bonsignore et al (2001) reported in healthy marathon runners significantly raised neutrophil counts in induced sputum after a marathon versus baseline. They found higher baseline neutrophil counts than in sedentary controls. Lumme et al (2003), studying ice-hockey players, showed increased numbers of neutrophils and eosinophils in the induced sputum of athletes compared with controls. They found no correlation between BHR and sputum eosinophil counts. These findings are well in keeping with our findings. We also found increased numbers of eosinophils and neutrophils in the bronchial biopsy specimens of skiers. Unfortunately, we did not have enough biopsy material to confirm the difference in neutrophils between controls and skiers. However, the neutrophil count was significantly higher in skiers than in mild asthmatics. This implies that the inflammatory reaction in skiers differ from that of asthma. In contrast to findings in ice-hockey players (Lumme et al 2003) and swimmers (Helenius et al 1998a), we found that eosinophil counts were higher in hyperresponsive skiers. The eosinophil density was significantly lower in skiers than in asthmatics. This also confirms a difference in inflammatory cell profile in skiers compared with asthmatics.

Verges et al (2005), studying airway inflammation and BRH in cross-country skies and triathletes, found significantly higher eosinophil counts in hyperresponsive athletes than in controls or athletes without BHR, which is in accordance with our findings. Interestingly,

in our study, numbers of macrophages and T-lymphocytes were higher than in controls, but did not differ from those of mild asthmatics, which is a unique finding. Making direct comparisons between bronchial biopsy studies and sputum studies is impossible, as sputum induction is unreliable in investigating airway lymphocytes and macrophages compared with bronchoscopy (Fahy et al 1995, Maesterelli et al 1995, Keatings et al 1997).

Like others (Helenius et al 1998a, Lumme et al 2003), we found no correlation between degree of BHR and airway inflammation expressed as inflammatory cell densities. Lymphocytes were observed to form special aggregates in the bronchial biopsies, as reported earlier (Sue-Chu et al 1998). The ski athletes with BHR were more symptomatic than were those without BHR, like athletes in the study by Verges et al (2005). As athletes with BHR had more eosinophils in their sputum or bronchial biopsies, it is possible that the symptoms could at least in part derive from eosinophilic inflammation. However, what is confusing is that we saw distinct inflammatory cell infiltration in the airways of even the asymptomatic and nonhyperresponsive skiers.

Tn expression in the airways of elite athletes has not been studied. We found increased expression of Tn in the subepithelial basement membrane in skiers as well as in patients with mild asthma compared with that of healthy controls. In contrast to findings in atopic asthmatic subjects (Amin et al 2000), we found no difference between atopic and nonatopic skiers. As Tn expression is considered to be a marker of remodeling in asthma (Laitinen et al 1996, Laitinen et al 1997), its presence in the bronchial BM of skiers may reflect injury followed by a repair process in the airways. That Tn expression did not correlate with inflammatory cell counts suggests that there could exist some stimulus other than inflammation causing epithelial injury that induces tenascin re-expression. At least one of these stimuli may be hyperventilation of dry and usually cold air during strenuous training and competitions.

## 5.2 Airway inflammation and remodeling in bronchial biopsies from newly diagnosed asthmatics

We described inflammation in the bronchial mucosa of a large sample of subjects with newly diagnosed asthma. Most of the subjects were steroid-naïve, and all had been on solely rescue β2-agonist treatment for at least 4 weeks before entering the study. One important inclusion criterion was moderate or severe hyperresponsiveness, which made the study group well defined. Comparing biopsy findings between studies is questionable, because the methods quantifying inflammatory cells vary between bronchial biopsy studies (Djukanovic 1996). The numbers of subjects in these studies are limited (usually between 10 to 40) due to the invasiveness of the bronchoscopy.

We found eosinophil counts in subjects with newly diagnosed asthma similar to eosinophil counts in subjects with seasonal asthma and in subjects with chronic asthma, as did a study using the same quantification method (Laitinen et al 1997). This confirms that eosinophils are important effector cells in various types of asthma. In accordance with other studies (Laitinen et al 1996, Altraja et al 1999, Flood-Page et al 2003), we found

distinct tenascin expression in the BM of the bronchial mucosa in every patient. Duration of the disease in these subjects was short (mean duration only 0.33 years), so we can presume that, in the pathogenesis of asthma, tenascin expression is upregulated very early.


## 5.3 Atopy and airway inflammation in newly diagnosed asthma

In the present study we were unable to distinguish atopic asthmatics from nonatopic asthmatics by their bronchial mucosa inflammatory cell densities. This absence of significant differences in inflammatory cell infiltration between patients with atopic and nonatopic asthma was unexpected, since differences in the inflammatory mechanisms have been reported (Humbert et al 1996, Menz et al 1998). Amin et al (2000) found significantly more eosinophils and T-lymphocytes and significantly fewer neutrophils in 13 subjects with atopic asthma than in nine subjects with nonatopic asthma, which is not in accordance with our findings. However, the present study with its markedly greater number of patients failed to show differences in the inflammatory cell counts between atopic and nonatopic asthmatics, leaving the concept of distinct inflammatory cell profiles in these two phenotypes of asthma still vague.

In addition to counting cells in the airway mucosa, it is important to investigate the presence of structural alterations in these asthma phenotypes. In the atopic asthma; airway smooth muscle thickness is clearly increased (Amin et al 2005). The reason for ASM thickening is unclarified, but mast cell-derived cytokines may enhance myofibroblast differentiation into smooth muscle cells at least in allergic asthma (Gizycki et al 1996). We studied the difference in BM composition between atopic and nonatopic asthma. A difference appeared in Tn expression in the bronchial BM according to atopic satus. In patients with atopic asthma, the Tn layer was significantly thicker than in patients with nonatopic, in accordance with an earlier report (Amin et al 2000). In the present study, the thickness of the subepithelial Tn correlated significantly with a wider range of inflammatory cells including eosinophils, macrophages, and T-lymphocytes in the airway mucosa in atopic asthma but not in nonatopic asthma, suggesting that Tn expression in this asthma phenotype may be closely associated with the extent of the inflammatory process. We found no correlation between mast-cell density and Tn thickness; such was found in another study (Amin et al 2000) with a smaller number of subjects. The correlation between eosinophils and Tn thickness in atopic asthma has been corroborated by others (Flood-Page et al 2003). The association of macrophages and T-lymphocytes with Tn that we found remains obscure. Although a problem may exist as to statistical power linked to the smaller number of our nonatopic asthmatics compared to atopic asthmatics, our association between Tn and major inflammatory cells may indicate more uniform structural changes in atopic than in nonatopic asthma.

The mechanisms behind the up-regulation of Tn expression in the airways are mostly unknown. TGF-ß and TNF-α induce Tn expression in cultured bronchial epithelial cells (Linnala et al 1995, Härkönen et al 1995). In human atopic skin, a temporal relationship emerged between the accumulation of eosinophils in the tissue and Tn up-regulation in those fibroblasts which differentiated into myofibroblasts (Phipps et al 2002). In atopic

asthma, allergen challenge has been shown to cause an increase in Tn deposition in the bronchial BM and a simultaneous increase in mucosal eosinophils and neutrophils (Phipps et al 2004, Torrego et al 2007). TGF-ß can be found at increased levels in the BAL fluid of asthmatic patients (Redington et al 1998). TGF-ß, which is produced by inflammatory cells and structural cells in the airways, plays an important role in the immunomodulation and fibrogenesis in asthma (Duvernelle et al 2003).

## 5.4 Effects of treatment in the intervention studies

### 5.4.1 Skiers

Unexpectedly, treatment with budesonide had no effect on airway inflammation or symptom control either in hyperresponsive or in symptomatic skiers. The reason for lack of symptom control may be lack of treatment effect, but the reason may also derive from inadequate symptom recording. Symptoms were assessed based only on the skiers' judgment, expressed after the treatment period. However, no difference in symptoms during treatment appeared between the budesonide and placebo groups. Another weakness of this study was compliance. This was assessed only by asking skiers whether they had used their inhalers. A symptom diary and better compliance assessment would have improved the quality of our study. As we found no significant clinical response, the lack of differences in airway inflammatory changes after treatment between budesonide and placebo groups was unsurprising. Budesonide treatment improves lung function (Haahtela et al 1991) and airway inflammation in asthmatic patients (Laitinen et al 1992). In skiers, budesonide improved lung function significantly, but did not attenuate airway inflammation. The improvement in lung function indirectly indicates that the skiers actually used the study medication. Why budesonide had no similar effect on airway inflammation as it did in asthmatic patients remains obscure. We found a trend towards a Tn immunoreactivity decrease in the budesonide group as evidence of some treatment effect, even though the effect was not as distinct as seen in subjects with seasonal asthma (Laitinen et al 1997). This effect on the BM may explain in part the improvement in $FEV_1$ in the budesonide group.

In both budesonide and placebo groups BHR spontaneously improved. This may be due to timing this study to the competition season. The training intensity was higher in the autumn just before the beginning of the treatment and lower during the treatment period, which coincided with the competition season. This theory is supported by another study that reported variability in BHR in ski athletes in relation to changes in training intensity (Heir 1994). BHR was highest during the most intensive periods of exercise. Timing of the treatment study could explain the changes in BHR. In asthmatic subjects, budesonide attenuates BHR, but the effect with adose of 800 µg daily is modest (Currie et al 2003). We found no consistent effect of budesonide 400 µg bid on BHR. As the BHR was

spontaneously attenuated in the placebo group as well, changes in BHR most probably were not treatment-related.

No comparable studies examining treatment effects on airway inflammation in athletes have been carried out, probably due to their invasive nature. All in all, studies investigating the treatment effects of antiasthma drugs are few. Helenius and coworkers (2004), did a placebo-controlled study with montelukast, a leukotriene modifier. Their ice-hockey players suffering asthma-like respiratory symptoms showed, after 4 weeks of treatment, no effect on lung function, respiratory symptoms, exhaled nitric oxide, or inflammatory indices in induced sputum. Thus far, no evidence has emerged for the beneficial effects of anti-inflammatory treatment in athletes suffering from asthma-like symptoms. However, studies on anti-asthma medication in athletes are sparse, being mostly concentrated on possible doping-effects. Definite conclusions as to the ineffectiveness of anti-inflammatory asthma medication in athletes with respiratory symptoms thus cannot be drawn.

## 5.4.2 Newly diagnosed asthmatics

This present study compared treatment effects of SLM, FP, and DSCG with placebo in asthmatic subjects with a short duration of the disease. All of the active treatments were clinically effective; symptoms and need for rescue medication diminished. Only SLM and FP improved lung function. The bronchoprotective properties against BHR of SLM, FP, and DSCG have been demonstrated in other studies (Pedersen et al 1993, Nielsen and Dahl 2000, Anderson et al 1994).

We found that FP was the most effective in improving BHR, exerting almost double the DD effect of SLM. SLM has shown no bronchoprotective effect against adenosine-5'-monophosphate (Soler et al 1994), suggesting the bronchoprotection of SLM probably derives from direct action on the smooth muscle. In our study, the bronchoprotective efficacy of SLM may be based merely on the bronchodilation caused by SLM taken in the morning before histamine challenge. The DD effect of DSCG was modest, and probably not clinically significant. In the FP group, $PD_{15}FEV_1$ was normalized at 16 weeks of treatment in six patients, but in no patient in the SLM group. The favorable treatment effect of FP in BHR was not surprising, but the magnitude of DD effect was better than reported earlier with a high dose (1000 µg bid) of FP (Booth et al 1995). However, these results are not well comparable, as the authors reported the attenuation of BHR at doubling dilutions (2.8). One explanation for the good response in BHR in the FP group may be our patients' short duration of asthma. BHR normalizes more frequently in patients with short-duration than with long-duration disease (Boulet et al 2000).

We found no significant differences in the anti-inflammatory efficacy of the treatments, perhaps due to the small numbers of subjects in the treatment groups. A bronchial biopsy study is difficult and expensive to do with a large number of patients. We gathered patients for 6 years to achieve the target number of 80 subjects. The statistical power was calculated to be sufficient for analysis with this number. A within-group decrease in eosinophils occurred in the bronchial mucosa in two treatment groups. FP 250

µg bid had a relatively strong effect, and DSCG 5 mg qid had a moderate but also significant effect against infiltration with EG2-positive eosinophils. Eosinophil reduction with FP has been shown at a high dose (FP 1000 µg bid) and at a moderate dose (250 µg bid) (Booth et al 1994, Olivieri et al 1997). We showed that DSCG, which currently in clinical practice is mostly replaced by inhaled corticosteroids, has a significant anti-eosinophilic effect. Our results for the anti-inflammatory effect of DSCG confirm the results of another study, which was not placebo-controlled (Hoshino et al 1998c). However, the treatment effect of DSCG in our study was less extensive than with FP. The latter information, provided by the present study, may have an impact on the choice between the two medications, since eosinophilia is regarded as the fundamental feature of asthma (Jeffery et al 2000).

In contrast to other findings (Booth 1994, Olivieri 1997) we failed to show significant effects on other inflammatory cells with FP. We saw a tendency toward a decrease in CD3-positive T-lymphocytes, but this change was not statistically significant. In mast cells, macrophages, or neutrophils, no treatment effect occurred. It is possible that our dose of FP was too low. O'Sullivan et al (2002) showed that an FP dose of 500 µg bid was more effective in treating airway inflammation than was 100 µg bid. They found a significant reduction also in T-lymphocytes and macrophages not seen with a lower dose (100 µg bid) of FP. At least part of the attenuation of BHR with FP and DSCG may be explained by their effect on the reversible component of BHR and is thus associated with improvement in airway inflammation (Cockcroft and Davis 2006).

Fear exists of detrimental effects of $\beta_2$-agonists on asthma (Salpeter et al 2006). We demonstrated that treatment with SLM 50 µg bid as monotherapy administered for 16 weeks had neither a proinflammatory nor an anti-eosinophil effect on bronchial mucosal inflammation in symptomatic patients with newly diagnosed asthma with BHR. Although SLM exerted no significant anti-inflammatory effect, it proved to be a potent bronchodilator and symptom-reliever. We found no anti-neutrophilic effect with SLM, although such was evident in another study (Jeffery et al 2002).

The other currently available long-acting $\beta_2$-agonist, formoterol, has had some anti-eosinophilic effect, but only in a subgroup of patients with mild asthma with higher pretreatment eosinophil density in their bronchial mucosa (Wallin et al 1998). Thus far, the results with SLM treatment do not confirm any anti-inflammatory group-effect of long-acting $\beta_2$-agonists. SLM combined with FP for one year has improved lung function, asthma symptoms, and airway eosinophils in induced sputum with no signs of asthma worsening (Koopmans et 2005). Furthermore, adding SLM (50 µg bid) but not adding FP (100 µg bid) to regular inhaled corticosteroid treatment caused a significant reduction in eosinophils in bronchial biopsies (Li et al 1999), but the possible harmful effect of long-acting $\beta_2$-agonists on airway inflammation has not been ruled out. $\beta_2$-agonists may exert some proinflammatory action. A combination of SLM and FP treatment led to increased collagen and fibronectin deposition in the bronchial BM in rat model of allergen-induced airway inflammation (Vanacker et al 2002).

An in vitro study demonstrated that the $\beta_2$-agonists isoproterenol and SLM blocked corticosteroid-induced eosinophil apoptosis (Nielson and Hadjokas 1998). Regular treatment with the short-acting $\beta_2$-agonist terbutaline raised sputum eosinophil counts in

patients with mild or moderate asthma even when combined with corticosteroid treatment (Aldridge et al 1999). McIvor et al (1998) showed that SLM attenuates asthma symptoms and delays the recognition of asthma exacerbation, while at the same time eosinophilic inflammation increases. The present study was too short in duration to find possible treatment-caused asthma exacerbations. Loss of bronchoprotection against EIA or allergen challenge may be a problem with chronic use of $\beta_2$-agonists, especially in asthmatics exercising regularly (Ramage et al 1994, Nelson 1995).

We found no significant effects on the bronchial BM in any treatment arm. However, the FP and DSCG groups showed a tendency towards decreased Tn expression in the BM. A positive treatment effect in BM Tn has been shown with budesonide 400 µg bid in patients with seasonal asthma (Laitinen et al 1997). Possibly a longer treatment period or higher dose of FP could have induced significant changes in Tn, but the budesonide dose used by Laitinen (1997) is comparable with the dose of 250 µg FP bid in the present study. For the Altraja group (1999), treatment with nedocromil sodium had no effect on Tn immunoreactivity in the BM, but treatment with the short-acting $\beta_2$-agonist albuterol reduced Tn expression. Our long-acting $\beta_2$-agonist SLM had no influence on Tn expression, and similarly Roberts and coworkers (1999) found no effects of SLM on airway inflammatory cells in BAL or bronchial biopsy specimens, or in the depth of BM collagen. A combination of FP and SLM (100/50 µg bid) had no effect on overall thickness of BM (Jarjour et al 2006). Thus evidence exists that SLM would have an effect on airway remodeling even when added to FP. In a recent report, treatment with Anti-IL-5 was effective in reducing Tn immunoreactivity thickness and density, and at the same time reducing TGF-ß-positive eosinophils (Flood-Page et al 2003). This confirms that TGF-ß is an important factor in Tn regulation. However, more studies are needed to clarify treatment effects on the ECM of BM.


## 5.5 Bronchial biopsy findings and bronchial hyperresponsiveness

In the ski athletes we were unable to find any correlation between degree of BHR and inflammatory cells or Tn in the bronchial BM. However, our skiers with BHR had a slightly higher density of eosinophils in their bronchial mucosa. In the study of newly diagnosed asthma, we found a significant correlation between degree of BHR and inflammation only with eosinophil density in atopic asthmatics but no such correlation in nonatopic asthmatics. The most consistent correlations between BHR and inflammatory cell counts have been shown with eosinophil accumulation in the airways and the degree of bronchial hyperresponsiveness measured with nonspecific stimuli like inhaled methacholine (Woolley et al 1994, Chetta et al 1996, Gibson et al 2000). This association of inflammatory cell counts and bronchial hyperresponsiveness has, however, remained unclear (O'Byrne 1995, Power et al 1993). Crimi and coworkers (1998) failed to show any correlation between number of eosinophils in BAL or in bronchial biopsy specimens and $PD_{20}FEV_1$ to methacholine, suggesting that other factors are mainly responsible for airway hyperresponsiveness. Biopsy studies correlating inflammatory changes in BHR as

measured by direct stimuli like histamine and methacholine have usually included rather small numbers of subjects; measurement of inflammatory parameters differ greatly between studies, making it difficult to draw firm conclusions. The present study comprised a large number of patients compared with earlier numbers, but still we could not confirm the association between eosinophilic infiltration and BHR.

In the general population, asymptomatic BHR is fairly common (19 to 62%) (Jansen et al 1997) compared with the prevalence of asthma: 4 to 8% in Finland (Pallasaho et al 2005). Asymptomatic BHR may, however, still be a risk factor for development of symptomatic asthma. Laprise and Boulet (1997), following 90 subjects with asymptomatic BHR, found that the major risk factors for development of symptomatic asthma in this population were genetic predisposition and, in sensitized subjects, exposure to allergen. In one large-population study, BHR was an independent risk factor for asthma but also a risk factor for chronic obstructive lung disease irrespective of atopy (Brutsche et al 2006). In our study of nonasthmatic athletes, BHR was more common among atopic than among nonatopic skiers.

Remodeling in the BM has been shown to correlate with BHR. We failed to show any correlation between Tn layer thickness and BHR, either in the skiers or in asthmatic subjects with a recent diagnosis. Recently, Kariyawasam and coworkers (2007) studying the effects of allergen challenge on BHR and inflammatory cells and BM content, found, however, that allergen challenge caused an increase in BHR evident at 24 hours and at 7 days. They measured bronchial BM Tn and procollagen III expression and inflammatory cell densities at the same time-points and found that Tn expression and inflammatory cell densities returned to baseline at day 7, but procollagen III expression persisted. Their results suggest that neither inflammatory nor Tn is important in persistent BHR, but collagen expression in the BM may be.

The introduction of indirect measurements of BHR gives more possibilities to study the variable component of BHR in asthma and to relate BHR to reversible airway inflammation (Cockcroft and Davis 2006). Indirect challenges include physical stimuli such as exercise and hyperventilation or chemical stimuli such as adenosine monophospate (AMP) or mannitol. Bronchial provocation with AMP may be more sensitive in reflecting airway inflammation (van den Berge et al 2001). The provocative concentration of AMP causing a 20% fall in FEV1 correlated negatively with sputum eosinophils as a surrogate marker of airway inflammation, whereas methacholine did not correlate with markers of inflammation (Prosperini et al 2002). Furthermore, BHR to mannitol correlates with airway inflammation as assessed by exhaled NO and eosinophils in induced sputum (Pjorsbjerg et al 2007).

## 5.6 Possible explanations for airway inflammatory changes in cross-country skiers

Why nonasthmatic skiers show inflammation in their airways is unknown. These inflammatory changes in the airway mucosa may result from repeated airway irritation from inhaling large amounts of dry, cold air during heavy exercise like training and

competition. As temperature decreases, the humidity of the ambient air decreases substantially (Houghton 1985), causing a decrease in humidity of the inspired air. In skiers, BHR, asthma, and asthma-like symptoms persist even during summertime and after the end of their competitive career (Larsson et al 1993, Verges 2004). In swimmers, cessation of high-level training has caused attenuation of BHR and a decrease in sputum eosinophils along with improvement in asthma symptoms. In some subjects, asthma even disappeared (Helenius et al 2002). Swimmers who continued active swimming for 5 years showed an increase in BHR and sputum eosinophils. Endurance training raises minute ventilation substantially.

Skiing is a cold-weather sport, and much training and all competition takes place in a cold environment. In dogs, repeated hyperventilation induces BHR and production of inflammatory cells (eosinophils, neutrophils and macrophages) and leukotrienes (Davis and Freed 2001). Alaskan racing sled dogs work in a very cold environment and therefore have been considered a model of "ski asthma" (Davis et al 2002). Bronchoscopic examination of these dogs showed macroscopic inflammation and increased counts of inflammatory cells compared with those in sedentary dogs. In another animal experiment, Davis and coworkers (2005) found that in horses, exercise in subfreezing temperature led to increased concentration of the TH2-type cytokines IL4, IL5, and IL10 in the BAL fluid compared with leves during exercise in warm air. In humans, marathon racing raised the plasma levels of the cytokines IL-10, IL8, and IL-1 receptor antagonist (Nieman et al 2001). IL-8 is a strong neutrophil chemotactic cytokine and may indicate neutrophil activation during heavy exercise. In healthy subjects, cooling of the facial skin causes bronchoconstriction even while they are simultaneously breathing warm air (Koskela and Tukiainen 1995). Skiers have their faces and whole bodies exposed to cold air, and bronchoconstriction due to this exposure is possible, but not yet studied. Continuous work in a cold environment for a long period (12 months) leads to increased respiratory symptoms and BHR (Jammes et al 2002). These findings strengthen the theory that exposure to cold air during hyperpnoea caused by strenuous physical exercise is mainly responsible for the airway inflammation and BHR occurring in skiers.

Strenuous and prolonged endurance training leads to a transient decrease in the immune defense, especially against respiratory viruses (Nieman 2000). Endurance athletes like cross-country skiers are therefore at increased risk for respiratory tract infections. In skiers, upper respiratory tract infections increase in BHR transiently when the subjects continue training during the infection (Heir et al 1995). In sedentary controls, BHR does not increase during the infection. Thus the increased frequency of respiratory tract infections could also contribute to the BHR seen in our skiers.

Although strenuous exercise especially in cold air may be harmful for the airways of athletes, it may have beneficial effects on nonathletic persons. In the European Community Respiratory Health Survey II, frequency and duration of physical exercise were inversely related to BHR (Shaaban et al 2007). A 25-year follow-up of Finnish men reinforces these findings. Pulmonary function decline was slowest in men who were in the highest tertile in physically activity (Pelkonen et al 2003). The benefits of exercise and physical training have been shown in nonelite long-distance runners. Scichilone and coworkers (2005) showed that training attenuated BHR in nonelite long-distance runners.

The runners in that study had better lung function than did sedentary controls. According to a Finnish questionnaire study, recreational skiing did not lead to increased prevalence of respiratory symptoms or obstructive pulmonary disease in subjects living in a cold environment (Kotaniemi et al 2003). Indirect evidence arises from animal studies that low and moderate aerobic exercise may reduce airway inflammation and remodeling (Vieira et al 2007).

# 6. PRACTICAL CONSIDERATIONS AND FUTURE CHALLENGES

Asthma, BHR, and respiratory symptoms are common among elite athletes, and more attention should be paid to studying the underlying reasons and possible treatments. At the moment, asthmatic athletes are compelled to prove their asthmatic condition again and again with objective lung function measurements in order to earn permission to use their appropriate asthma medication during important international games like the Olympics (Carlsen et al 2008). This sometimes leads to halting the anti-inflammatory medication in order to show reversible airway obstruction or BHR, and this may lead to worsening of their asthma. Regulations for the use of anti-asthma medication from the Medical Commission of the International Olympic Committee have raised concern about asthma management in athletes (Weiler 2003, Bonini et al 2004, Dickinson et al 2005). However, the diagnostic change in $FEV_1$ required in an exercise challenge, is smaller in athletes (10%) than in nonathletic persons (15% according to ATS guidelines) (Carlsen et al 2008). Sports-induced respiratory disorders and chronic bronchial asthma in athletes differ. Those athletes with chronic asthma should be treated according to international asthma guidelines. Their treatment should be continuous, without interruption of their anti-inflammatory treatment. No interruption in their treatment should take place only to detect variable airflow obstruction for doping regulatory reasons.

The present study showed marked inflammation in the airways of nonasthmatic cross-country skiers, inflammatory changes not predicted by lung function tests and induced by the sport. Little is known about the course of the inflammatory reaction in the airways of skiers during the years of their active racing career and afterwards. Longitudinal studies are necessary to reveal what happens to the respiratory symptoms and lung function of skiers over the course of time. Changes in respiratory symptoms and BHR may be irreversible in skiers (Larsson et al 1993) as opposed to swimmers (Helenius et al 2002), in whom airway inflammation and BHR was attenuated or even abolished after the end of the active sports career. Noninvasive methods are less complex than bronchoscopy, and biopsy can clarify the extent of airway inflammation among endurance athletes. Induced sputum is one feasible method for assessing extent of inflammation (Helenius et al 2002, Lumme et al 2003). Exhaled nitric oxide (NO) has been considered an indirect marker of airway inflammation even in mild asthma (Ekroos et al 2002). It could serve as a useful surrogate marker for airway inflammation in endurance athletes (Verges et al 2005), and needs further studies.

Very few studies investigate anti-asthma medication in athletes suffering from respiratory symptoms and airway inflammation induced by their sport. Studies with a more detailed protocol are essential before rejection of inhaled corticosteroids or antileukotrienes as useless medication in treating athletes' airway disorders. It would be intriguing to study effects of cromones in athletes: The present study of asthmatics showed the antieosinophilic effect of DSCG. Both DSCG and nedocromil sodium inhibit hyperosmolarity-induced bronchoconstriction and EIB including hyperpnoea-induced bronchoconstriction (Anderson et al 1996). The use of a heat-exchanger mask may be beneficial during exercise in cold air and thus also in cold-weather sports athletes. This

mask warms and humidifies the inspired air. In asthmatic subjects, a heat-exchanger mask can inhibit cold-induced bronchoconstriction at least as well as could the inhaled β2-agonist albuterol Beuther and Martin 2006). Effects of heat exchanger-masks should be explored in skiers.

Duration of asthma before diagnosis seems to influence BHR. The shorter the time from the onset of disease, the better the possibilities are to attenuate BHR (Haahtela et al 1994, Grönke et al 2002). It is possible to tailor asthma treatment using BHR assessment as a tool to adjust the inhaled corticosteroid dose (Sont et al 1999). This kind of tailoring, aimed at reducing in BHR, was associated with fewer asthma exacerbations and better lung function than with the reference strategy. The authors found that the strategy to adjust the treatment to attenuate BHR was associated with a decrease in BM thickness. Thus aiming treatment to reduce BHR may affect remodeling in the airways. The present study revealed a tendency for FP to reduce the thickness of Tn in the BM in subjects with newly diagnosed asthma. Simultaneously, BHR decreased significantly. However, using BHR measurements in the follow-up of asthma is time-consuming and cannot be applied in the asthma management plan of every asthmatic. Further investigations are necessary to determine the usefulness of BHR in routine practice. We showed that DSCG caused a modest decrease in BHR as well as a decrease in eosinophilic airway inflammation. The anti-inflammatory effects of DSCG deserve more attention and further study.

# 7. CONCLUSIONS

Marked inflammatory changes occurred in the bronchial mucosa of elite ski athletes, both in hyperresponsive and nonhyperresponsive subjects. In skiers, airway inflammation was less extensive than in mild asthma, but was distinctly different from that of non-athletic control subjects. The inflammatory cell pattern was different from that in asthmatic subject; it showed a distinct neutrophilic cell infiltration but less extensive infiltration of eosinophils, mast cells, and macrophages in bronchial biopsy specimens.

Inflammatory changes in the bronchial biopsy specimens from skiers were unrelated to lung function or symptoms. Inflammatory changes were apparent even in asymptomatic skiers.

In the skiers, tenascin expression was increased in the bronchial basement membrane as a sign of remodeling, possibly reflecting bronchial epithelial injury. Enhanced tenascin expression appeared in patients with mild persistent asthma as well as newly diagnosed asthma.

Bronchial hyperresponsiveness showed no correlation with inflammatory cell densities or tenascin expression in the bronchial mucosa.

Airway remodeling in atopic asthma differs from that in nonatopic asthma, in which the injury-repair phenomenon seems to be less active because tenascin expression is lower.

Budesonide failed to improve airway inflammation or tenascin expression in the basement membrane in skiers. Bronchial hyperresponsiveness in ski athletes varied in both the budesonide and placebo groups, possibly due to training intensity, and was unrelated to airway inflammation, which persisted.

Salmeterol treatment had no antieosinophilic effect on the airway mucosa in patients with newly diagnosed asthma. Treatment with fluticasone propionate or disodium cromoglicate attenuated the eosinophilic inflammation in the airway mucosa in subjects with these patients. None of these treatments reduced basement membrane tenascin expression.

These findings suggest that inflammation and BHR in skiers and asthmatic subjects are of differing origins. Inflammatory cell pattern and response to anti-inflammatory treatment differed. Treatment with inhaled corticosteroid reduced BHR and improved lung function and respiratory symptoms in asthmatic subjects, but not in the ski athletes. In ski athletes, strenuous training combined with inhalation of large amounts of cold, dry air irritates

the airways and may cause these distinct inflammatory changes in their lower airways.

# ACKNOWLEDGEMENTS

their assistance during study bronchoscopies. I am most grateful to Marja Aarnio and Sirpa Huhtaniitty for their help in many practical matters.

I thank all study subjects for taking part in these studies – their cooperation was indispensable.

I thank Carol Norris, PhD, for expert language revision.

Many thanks go to my colleagues at the former Lung department at Laakso hospital, Drs Antti Ahonen, Anneli Ettilä, Auli Hakulinen, Ritva Koskinen, Anne Maunumäki, Timo Mäntylä, Liisa Nuorteva, Päivi Rantanen, Pekka Saarelainen, Paul Sandström, and Mikko Vuorio, for teaching me so much about pulmonary medicine during the beginning of my career – and for a friendly atmosphere at work.

I have worked at the Helsinki University Central Hospital, Division of Pulmonary Medicine first during my specialization in pulmonary medicine in the 1990ies, and as ward physician since the year 2000. We have had a great team spirit in our clinic, and I have enjoyed working with you all: Aija, Annamari, Annette, Adel, Antti, Heikki, Hilkka, Hille, Irmeli, Juho, Klaus, Laura, Maija, Milla, Paula P, Paula R, Ulla, and many others.

I am most grateful to all my friends for being there for me all these years – your support and friendship has been invaluable. Special thanks to you, Birgit, Erja, and Marja, for being my closest friends, and for your invaluable help during this work. Many thanks go to the "sewing circle" ladies Anu, Sari, and Tiina for good company and interesting talks. Warm thanks go to Pentti Selin for taking the photograph of the cover of this thesis.

Exercise has always been an important part of my life. I thank the ladies at the Lady Aerobic Club and instructor Adele Salonen for the refreshing Wednesday evenings since 1996.

I also want to thank my lovely cocker spaniels Nöpö (moved to dogs' heaven in 2006), Tessa, and Topi for giving me so much joy during these years.

Part of this thesis was written in Rauma, a beautiful old town on the west coast of Finland. I thank Seija and Tauno, Tiia, Tommi, and Minna for their hospitality and kindness during my visits to Rauma.

I am deeply grateful to my parents Lea and Pasi Julkunen for their love, encouragement, and support. I am very much indebted to them for taking care of my son and my dogs many times during this work. I am very proud of my son Risto, who grew up from schoolboy to a young student of technology during the years of this work.

Finally, my warmest thanks go to Kai, for your love, patience, and support during the writing process of this thesis.

Helsinki, May 2008

# References

Ackerman V, Marini M, Vittori E, Bellini A, Vassali G, Mattoli S. Detection of cytokines and their cell sources in bronchial biopsy specimens from asthmatic patients. Relationship to atopic status, symptoms, and level of airway hyperresponsiveness. Chest 1994;105:687-96

Alam R, Stafford S, Forsythe P, Harrison R, Faubion D, Lett-Brown MA, Grant JA. RANTES is a chemotactic and activating factor for human eosinophils. J Immunol 1993;150:3442-8

Alaranta A, Alaranta H, Heliövaara M, Airaksinen M, Helenius I. Ample use of physician-prescribed medication in Finnish athletes. Int J Sports Med 2006;27:919-25

Aldridge RE, Hancox RJ, Taylor DR, Cowan JO, Winn MC, Frampton CM, Town GI. Effects of terbutaline and budesonide on sputum cells and bronchial hyperresponsiveness. Am J Respri Crit Care Med 1999;161:1459-64

Altraja A, Laitinen A, Virtanen I, Kampe M, Simonsson BG, Karlsson SE, Håkansson L, Venge P, Sillastu H, Laitinen LA. Expression of laminins in the airways in various types of asthmatic patients: a morphometric study. Am J Respir Cell Mol Biol 1996a;15:482-8

Altraja A, Laitinen A, Meriste S, Marran S, Martson T, Sillastu H, Laitinen LA. Effect of regular nedocromil sodium or albuterol on bronchial inflammation in chronic asthma. J Allergy Clin Immunol 1996b;98:S58-64

Altraja A, Laitinen A, Meriste S, Marran S, Martson T, Sillastu H, Laitinen LA. Regular albuterol or nedocromil sodium-effects on airway subepithelial tenascin in asthma. Respir Med 1999;93:445-53

American Thoracic Society, Medical Section of the American Lung Association. Lung function testing: selection of reference values and interpretative strategies. Am Rev Respir Dis 1991;144: 1202-1218

Amin K, Ludviksdottir D, Janson C, Nettelbladt O, Björnsson E, Roomans GM, Boman G, Seveus L, Venge P. Inflammation and structural changes in the airways of patients with atopic and nonatopic asthma. Am J Respir Crit Care Med 2000; 162:2295-2301

Amin K, Ekberg-Jansson A, Lofdahl CG, Venge P. Relationship between inflammatory cells and structural changes in the lungs of asymptomatic and never smokers: a biopsy study. Thorax 2003;58:135-42

Amin K, Janson C, Boman G, Venge P. The extracellular deposition of mast cell products is increased in hypertrophic airways smooth muscles in allergic asthma but not in nonallergic asthma Allergy 2005;60:1241-7

Amishima M, Munakata M, Nasuhara Y, Sato A, Takahashi T, Homma Y. Kawakami Y. Expression of epidermal growth factor and epidermal growth factor receptor immunoreactivity in the asthmatic human airway. Am J Respir Crit Care Med 1998;167:1907-12

Anderson SD, du Toit JI, Rodwell LT, Jenkins CR. Acute effects of sodium cromoglycate on airway narrowing induced by 4.5 percent saline aerosol. Outcome before and during treatment with aerosol corticosteroids in patients with asthma Chest 1994;105:673-80

Anderson SD, Rodwell LT, Daviskas E, Spring JF, du Toit J. The protective effect of nedocromil sodium and other drugs on airway narrowing provoked by hyperosmolar stimuli: A role for the airway epithelium? J Allergy Clin Immunol 1996;98:124-34

Anderson SD, Daviskas E. Pathophysiology of exercise-induced asthma: role of respiratory water loss. In Weiler J, ed. Allergic and respiratory disease in sports medicine. New York: Marcel Dekker; 1997 p. 87-114

Anderson S, Holzer K. Exercise-induced asthma: is it the right diagnosis in elite athletes. J Allergy Clin Immunol 2000;419-28

Anderson SD, Argyros GJ, Magnussen H, Holzer K. Provocation by eucapnic voluntary hyperpnoea to identify exercise induced bronchoconstriction. Br J Sports Med 2001;35:344-7

Anderson S, Fich K, Perry CP, Sue-Chus M, Crapo R, McKenzie D, Magnussen H. Responses to bronchial challenge submitted for approval to use inhaled beta2-agonists before an event at the 2002 Winter Olympics. J Allergy Clin Immunol 2003;111:45-50

Andersson F, Kjellman M, Forsberg G. Moller C, Arheden L. Comparison of the cost-effectiveness of budesonide and sodium cromglycate in the management of childhood asthma in everyday clinical practice. Ann Allergy Asthma Immunol 2001;86:537-44

Azzawi M, Bradley B, Jeffery PK, Frew AJ, Wardlaw AJ, Knowles G. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. Am Rev Respir Dis 1990;142:1407-13

Backer V, Ulrik CS. Bronchial responsiveness to exercise in a random sample of 494 children and adolescents from Copenhagen. Clin Exp allergy 1992;22:741-7

Balza E, Siri A, Caocci F, Linnala A, Virtanen I, Zardi L. Production and characterization of monoclonal antibodies spesific for different epitopes of human tenascin. FEBS Lett. 1993;332:39-43

Bardagi S, Aguno A, Conzallez CA, Romero PV. Prevalence of exercise-induced airway narrowing in schoolchildren from a Mediterranean town. Am Rev Respir Dis 1993;147:1112-5

Barnes PJ, Holgate ST, Laitinen LA, Pauwels R. Asthma mechanisms, determinants of severity and treatment: the role of nedocromil sodium. Report of a workshop held in Whistler, British Columbia, Canada, 18-19 May 1995. Clin Exp Allergy 1995;25:771-87

Barnes PJ, Adcock IM. Transcription factors and asthma. Eur Respir J 1998;12:221-34

Barnes PJ. Corticosteroids: the drugs to beat. Eur J Pharmacol 2006;533:2-14

Barnes PJ. Scientific rationale for using a single inhaler for asthma control. Eur Respir J 2007;29:587-95

Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, Fitzgerald M, Gibson P, Ohta K, O'Byrne P, Pedersen SE, Pizzichini E, Sullivan SD, Wenzel SE, Zar HJ. Global strategy for asthma management and prevention: GINA executive summary. Eur Respir J 2008;31:143-78

Beasley R, Roche WR, Roberts JA, Holgate ST. Cellular events in the bronchi in mild asthma and after bronchial provocation. Am Rev Respir Dis 1989;139:806-17

Becket PA, Howarth PH. Pharmacotherapy and airway remodelling in asthma? Thorax 2003;58:163-74

Beato M, Herrlich P, Schutz G. Steroid hormone receptors: many actors in search of a plot. Cell 1995;83:851-7

Becket PA, Howarth PH. Pharmacotherapy and airway remodelling in asthma? Thorax 2003;58:163-74

Begueret H, Berger P, Vernejoux J-M, Dubuisson L, Marthan R, Tunon-de-Lare JM. Inflammation of bronchial smooth muscle in allergic asthma. Thorax 2007;62:8-15

Bentley AM, Menz G, Sortz CHR, Robinson DS, Bradley B, Jeffery PK, Durhan AR, Kay AB. Identification of T lymphocytes, macrophages, and activated eosinophils in the bronchial mucosa in intrinsic asthma. Relationship to symptoms and bronchial hyperresponsiveness. Am Rev Respir Dis 1992;146:500-6

Bergeron C, Tulik MK, Hamid Q. Tools used to measure airway remodelling in research. Eur Respir J 2007;29:379-89

Beuther DA, Martin RJ. Efficacy of a heat exchanger mask in cold exercise-induced asthma. Chest 2006;129:1188-93

Bissonnette EY, Befus AD. Anti-inflammatory effect of β2-agonists: inhibition of TNF-α release from human mast cells. J Allergy Clin Immunol 1997;100:825-31

Bleecker E R, McFadden ER, Boushey HA, Edell ES, Eschenbacher WL, Godard PP, Goldstein RA, Holgate ST, Hunninghake GW, Hurd SS, Laitinen A, Lichtenstein, Prograis L, Rankin JA, Sri Ram J, Reed CE, Reynolds HY, Wood E. Workshop summary and guidelines: Investigative use of bronchoscopy, lavage and bronchial biopsies in asthma and other airway disease. J Allergy Clin Immunol. 1991;88:808-814

Bonini S, Brusasco V, Carlsen K-H, Dlegano L, Giacco SD, Haahtela T, Rasi G, van Cauwnberge PB. Diagnosis of asthma and permitted use of inhaled ß2-agonists in athletes. Allergy 2004;59:33-6

Bonini S, Bonini M, Bousquet J, Brusasco V, Canonica GW, Carlsen KH, Corbetta L et al. Rhinitis and asthma in athletes: an ARIA document in collaboration with GA2LEN. Allergy 2006;61:681-92

Bonsignore MR, Morici G, Riccobono L, Insalaco G, Bonanno A, Profita M, Paterno A, Vassalle C, Mirabella A, Vignola M. Airway inflammation in nonasthmatic amateur runners. Am J Physiol Lung Cell Mol Physiol 2001;281:L668-76

Bonsignore MR, Chimenti L, Paternò A, Vultaggio M, Bonanno A, Bellia V, Bonsignore G, Morici G. Longitudial changes and apoptosis in induced sputum cells in long-distance runners (abstract). Proc Am Thorac Soc 2006;3:A485

Booth H, Richmond I, Ward C, Gardiner PV, Harkawat R, Walters EH. Effect of high dose inhaled fluticasone propionate on airway inflammation in asthma, Am J Respir Crit Care Med 1995;152:45-52

Boschetto P, Rogers DF, Fabbri LM, Barnes PJ. Corticosteroid inhibition of airway microvascular leakage. Am Rev Respir Dis 1991;143:605-9

Boulet LP, Laviolette M, Turcotte H, Cartier A, Dugas M, Malo JL, Boutet M. Bronchial subepithelial fibrosis correlates with airway responsiveness to methacholine. Chest 1997;112:45-52

Boulet LP, Turcotte H, Laviolette M, Naud F, MC Bernier, Martel S, Chakir J. Airway hyperresponsiveness, inflammation, and subepithelial collagen deposition in recently diagnosed versus longstanding asthma. Influence of inhaled steroids. Am J Respir Crit Care Med 2000;162:1308-13

Bourdin A, Neveu D, Vachier I, Paganin F, Godard P, Chanez P. Specificity of basement membrane thickening in severe asthma. J Allergy Clin Immunol 2007;119:1367-74

Bousquet J, Chanez P, Lacoste JY, Barneon G, Ghavanian N, Enander I, Venge P, Ahlstedt S, Simony-Lafontaine J, Godard P, Michell FB. Eosinophilic inflammation in asthma. N Engl J Med 1990;323:1033-9

Bradding P, Walls AF, Holgate ST. The role of the mast cell in the pathophysiology of asthma. J Allergy Clin Immunol 2006:117;1277-84

Bradley BL, Azzawi M, Jacobson M, Assoufi B, Collins JV, Irani AM, Schwarz LB, Durhan SR, Jeffery PK, Kay AB. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. J Allergy Clin Immunol 1991;88:661-74

Brannan JD, Gulliksson M, Anderson SD, Chew N, Seale JP, Kumlin M. Inhibition of mast cell PGD2 release protects against mannitol-induced airway narrowing. Eur Respir J 2006;27:944-50

Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. N Eng J Med 2002;346:1699-705

Brusasco V, Crimi E, Pellegrino R. Airway hyperresponsiveness in asthma. Not just a matter of airway inflammation. Thorax 1998;53:992-8

Brutsche MH, Downs SH, Schindler C, Gerbase MW, Schwartz J, Frey M, Russi EW, Ackerman-Liebrich U, Leuenberger P. Bronchial hyperresponsiveness and the development on asthma and COPD in asymptomatic individuals: SAPALDIA Cohort Study. Thorax 2006;61:671-7

Burke CM, Sreenan S, Pathmakanthan S, Patterson J, Schmekel B, Poulter LW. Relative effect of inhaled corticosteroids on immunopathology and physiology in asthma: a controlled study. Thorax 1996;61:003-9

Busse WW, Wanner A, Adams K, Reynolds HY, Castro M, Chowdhury B, Kraft M, Levine RJ, Peters SP, Sullivan EJ. Investigative bronchoprovocation and bronchoscopy in airway diseases. Am J Respir Crit Care 2005;172:807-16

Busse WW, Sosman JM. Isoproterenol inhibition of isolated neutrophil function. J Allergy Clin Immunol 1984;73:404-10

Butchers PR, Vardey CJ, Johnson M. Salmeterol: a potent and long-acting inhibitor of inflammatory mediator release from human lung. Br J Pharmacol 1991;104:672-6

Calhoun WJ, Hinton KL, Kratzenberg JJ. The effect of salmeterol on markers of airway inflammation following segmental allergen challenge. Am J Respir Crit Care Med 2001;163

Carlsen KH, Anderson SD, Bjermer L, Bonini S, Brusasco V, Canonica W, Cummiskey J, Delgado L, Del Ciacco SR, Drobnic F, Haahtela T, Larsson K, Palange P, Popov T, van Causewnberge P. Treatment of exercise-induced asthma, respiratory and allergic disorders in sports and the relationship to doping: Part II of the report from the Joint Task Force of European Respiratory Society (ERS) and European Academy of Allergy and Clinical Immunology (ECAACI) in cooperation with GA2LEN. Allergy 2008;63:492-505

Carroll N, Elliot J, Morton A, James A. Structure of large and small airways in nonfatal and fatal asthma. Am Rev Respir Dis 1993;147:405-10

Carroll NG, Mutavdzic S, James AL. Distribution and degranulation of airway mast cells in normal and asthmatic subjects. Eur Respir J 2002a;19:879-85

Carroll NG, Mutavdzic S, James AL. Increased mast cells and neutrophils in submucosal mucous glands and mucus plugging in patients with asthma. Thorax 2002b;57:677-82

Chetta A, Foresi A, Del Donno M, Consigli GF, Bertorelli G, Pesci A, Barbee RA, Olivieri D. Bronchial responsiveness to distilled water and methacholine and its relationship to inflammation and remodeling of the airways in asthma. Am J Respir Crit Care Med 1996;153:910-7

Chetta A, Foresi A, Del Donno M, Bertorelli G, Pesci A, Olivieri D. Airways remodeling is a distinctive feature of asthma and is related to severity of disease. Chest 1997;111:852-7

Chetta A, Zanini A, Foresi A, Del Donno M, Castagnaro A, D'Ippolito R, Baraldo S, Testi R, Saetta M, Olivieri D. Vascular component of airway remodeling in asthma is reduced by high dose of fluticasone. Am J Respir Crit Care Med 2003;167:751-7

Chimenti L, Morici G, Paternò A, Bonanno A, Siena L, Licciardi A, Veca M, Guccione W, Macaluso F, Bonsignore G, Bonsignore MG. Endurance training damages small airway epithelium in mice. Am J Respir Crit Care Med 2007;175:442-9

Cho S, Seo JY, Choi DC, Yoon HJ, Cho YJ, Min KU, Lee GK, Seo JW, Kim YY. Pathological changes according to the severity of asthma. Clin Exp Allergy 1996;26:1210-9

Clark TJ. Effect of beclomethasone dipropionate delivered by aerosol in patients with asthma. Lancet 1972;1:1361-4

Cohen MD, Ciocca V, Panettieri RA Jr. TGFβ 1 modulates human airway smooth-muscle cell proliferation induced by mitogens. Am J Respir Cell Mol Biol 1997;16:85-90

Cockroft DW, Murdock KY, Berscheid BA. Relationship between atopy and bronchial hyperresponsiveness to histamine in a random population. Ann Allergy 1984;53:26-9

Cockcroft DW, Davis BE. Mechanisms of airway hyperresponsiveness. J Allergy Clin Immunol 2006;118:551-9

Cokugras H, Akcakaya N, Seckin I, Camcioglu Y, Sarimurat N, Aksoy F. Ultrastructural examination of bronchial biopsy specimens from children with moderate asthma. Thorax 2001;56:25-9

Corrigan CJ, Hamid Q, North J, Barkans J, Moqbel R, Durham S, Gemou-Engesaeth V, Kay AB. Peripheral blood CD4 but not CD8 T-lymphocytes in patients with exacerbation of asthma transcribe and translate messenger RNA encoding cytokines with prolong eosinophil survival in the context of a Th2-type pattern: effect of glucocorticoid therapy. Am J Cell Mol Biol 1995;12:567-78

Crimi E, Balbo A, Milanese M, Miadonna A, Rossi GA, Brusasco V. Airway inflammation and occurrence of delayed bronchoconstriction in exercise-induced asthma. Am Rev Respir Dis 1992;146:507-12

Crimi E, Spanevello A, Neri M, Ind PW, Rossi GA, Brusasco V. Dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma. Am J Respir Crit Care Med 1998;157:4-9

Currie GP, Lipworth BJ. Bronchoprotective effects of leukotriene receptor antagonist in asthma: a meta-analysis. Chest 2002;122:146-50

Currie GP, Fowler SJ, Lipworth BJ. Dose response of inhaled corticosteroids on bronchial hyperresponsiveness: a meta-analysis. Ann Allergy Asthma Immunol 2003;90:197-8

Dahl R. Systemic side effects of inhaled corticosteroids in patients with asthma. Respir Med 2006;100:1307-17

Dahlén SE, Hedqvist P, Hammarström S, Samuelsson B. Leukotrienes are potent constrictors of human bronchi. Nature 1980;288:484-6

Dahlén SE. Treatment of asthma with antileukotrienes: first line or last resort therapy. Eur J Pharmacol 2006;533:40-56

Davis MS, Freed AN. Repeated hyperventilation causes peripheral airways inflammation, hyperreactivity, and impaired bronchodilation in dogs. Am J Respir Crit Care Med 2001;164:785-9

Davis MS, McKiernan B, McCullough S, Nelson S,Jr, Mandsager RE, Willard M, Dorsey K. Racing Alaskan sled dogs as a model of "ski asthma". Am J Respir Crit Care Med 2002 Sep 15;166:878-82

Davis MS, Malayer JR, Vandeventer L, Royer CM, McKenzie EC, Williamson KK. Cold weather exercise and airway cytokine expression. J Appl Physiol 2005;98:2132-6

Daviskas E, Gonda E, Sanderson SD. Mathematical modeling of heat and water transport in human respiratory tract. J Appl Physiol 1990;69:362-72

Deal EC, McFadden ER Jr, Ingram RH Jr, Jaeger JJ. Hyperpnea and heat influx: initial reaction sequence in exercise-induced asthma. J Appl Physiol 1979a;46:476-83

Deal EC, McFadden ER Jr, Ingram RH Jr, Strauss RH, Jaeger JJ. Role of respiratory exchange on production of exercise-induced asthma. J Appl Physiol 1979b;46:467-75

Devalia JL, Sapsford RJ, Rusznak C, Toumbis MJ, Davies RJ. The effects of salmeterol and salmutamol on ciliary beat frequency of cultured human bronchial epithelial cells, in virto. Pulm Pharmacol 1992;5:257-63

Diamant Z, van der Molen T. Treating asthma: is there a place for leukotriene receptor antagonists. Respir Med 2005;99:655-62

Diamant Z, Boot JD, Virchow JC. Summing up 100 years of asthma. Respir Med 2007;101:378-88

Dickinson JW, Whyte GP, McConnel AK, Harries MG. Impact of change in the IOC-Mc asthma criteria: a Brithish perspective. Thorax 2005;60:629-32

Djukanović R, Wilson JW, Britten KM, Wilson SJ, Walls AF, Roche WR, Howarth PH, Holgate ST. Quantification of mast cells and eosinophils in the bronchial mucosa of symptomatic atopic asthmatics and healthy control subjects using immunohistochemistry. Am Rev Respir Dis 1990;142:863-71

Djukanović R, Wilson JW, Britten KM, Wilson SJ, Walls AF, Roche WR, Howarth PH, Holgate ST. Effect of an inhaled corticosteroid on airway inflammation and symptoms in asthma. Am Rev Respir Dis 1992;145:559-74

Djukanović R. Bronchoscopy as research tool for the study of asthma pathogenesis and effects of antiasthma drugs. J Allergy Clin Immunol 1996;98:S41-5

Druilhe A, Wallaert B, Tsicopoulos A, Lapa e Silva JR, Tillie-Leblond I, Tonnel AB, et al. Apoptosis, proliferation, and expression of Bcl-2, Fas, and Fas ligand in bronchial biopsies from asthmatics. Am J Respir Cell Mol Biol 1998;19:747-57

Dunnill MS. The pathology of asthma with special reference to changes in the bronchial mucosa. J Clin Pathol 1960;13:27-33

Durcan N, Costello RW, McLean WG, Blusztajn J, Madziar B, Fenech AG, Hall IP, Gleich GJ, McGarvey L, Walsh M-T. Eosinophil-mediated cholinergic nerve remodeling. Am J Respir Cell Mol Biol 2006;34:775-86

Durham SR, Till SJ, Corrigan CJ. T lymphocytes in asthma: Bronchial versus peripheral responses. J Allergy Clin Immunol 2000;106:S221-6

Duvernelle C, Freund V, Frossard N. Transforming growth factor-beta and ist role in asthma. Pulm Pharmacol Ther 2003;16:181-96

Edwards AM, Howell JBL. The chromones: history, chemistry and clinical development. A tribute to the work of Dr R.E.C Altounyan. Clin Exp Allergy 2000;30:756-74

Ekroos H, Karjalainen J, Sarna S, Laitinen LA, Sovijärvi AR. Short-term variablity of exhaled nitric oxide in young male patients with asthma and in health subjects. Respir Med 2002;96:895-900

Empey DW, Laitinen LA, Jacobs L, Gold WM, Nadel JA. Mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infections. Am Rev Respir Dis 1976;113:131-9

Erjefält I, Persson CG. Pharmacologic control of plasma exudation into tracheobronchial airways. Am Rev Respir Dis 1991;143:1008-14

Erjefält JS, Sundler F, Persson CGA. Epithelial barrier formation by airway basal cells. Thorax 1997;52:213-7

Fahy JV, Liu J, Wong H, Boushey HA. Comparison of samples collected by sputum induction and bronchoscopy from asthmatic and healthy subjects. Am J Respir Crit Care Med 1995;152:53-8

Fedorov IA, Wilson SJ, Davies DE, Holgate ST. Epithelial stress and structural remodelling in childhood asthma. Thorax 2005;60:389-94

Ferrari M, Balestreri F, Baratieri S, Biasin C, Oldani V, Lo Cascio V. Evidence of the rapid protective effect of formoterol dry-powder inhalation against exercise-induced bronchospasm in athletes with asthma. Respiration 2000;67:5003-13

Fiocchi A, Riva E, Santini I, Bernardo L, Sala M, Mirri GP. Effect of nedocromil sodium on bronchial hyperreactivity in children with nonatopic asthma. Ann Allergy Asthma Immunol 1997;79:503-6

Flood-Page P, Menzies-Gow A, Phiss S, Ying S, Wangoo A, Ludwig MS, Barnes N, Robinson D, Kay BA. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. J Clin Invest 2003;112:1029-36

Foos GF. Bronchial hyperresponsiveness: too complex to be useful? Curr Opin Pharmacol 2003; 3:233-8

Foresi A, Bertorelli G, Pesci A, Chetta A, Olivieri D. Inflammatory markers in bronchoalveolar lavage and in bronchial biopsy in asthma during remission. Chest 1990;98:528-35

Frossard N, Freund V, Advenier C. Nerve growth factor and its receptors in asthma and inflammation. Eur J Pharmacol 2004;500:453-65

Garcia-Zepeda EA, Rothenberg RT, Ownbey RT, Celestin J, Leder P, Luster AD. Human eotaxin is a specific chemoattractant for eosinophil cells and provides a new mechanism to explain tissue eosinophilia. Nat Med 1996;2:449-56

Gauvreau GM, Ronnen GM, Watson RM, O'Byrne PM. Exercise-induced bronchoconstriction does not cause eosinophilic airway inflammation or airway hyperresponsiveness in subjects with asthma. Am J Respir Crit Care Med 2000;162:1302-7

Gerritsen J, Köeter GH, Postma DS, Knol K. Prognosis of asthma from childhood to adulthood. Am Rev Respir Dis 1989;140:1325-30

Gibson PG, Mattoli S, Sears MR, Dolovitch J, Hargreave FE. Increased peak flow variability in children with asymptomatic hyperresponsiveness. Eur Respir J 1995;1731-5

Gibson PG, Saltos N, Borgas T. Airway mast cells and eosinophils correlate with clinical severity and airway hyperresponsiveness in corticosteroid-treated asthma. J Allergy Clin Immunol 2000;105:752-9.

Gibson PG, Saltos N, Fakes K. Acute anti-inflammatory effects of inhaled budesonide in asthma. A randomized controlled trial. Am J Respir Crit Care Med 2001;163:32-36

Gizycki MJ, Adelroth E, Rogers AV, O'Byrne PM, Jeffery PK. Myofibroblast involvement in the allergen-induced late response in mild atopic asthma. Am J Respir Cell Mol Biol 1997;16:664-73

Gleich GJ, Flavahan NA, Fujisawa T, Vanhoutte PM. The eosinophil as a mediator of damage to respiratory epithelium: a model for bronchial hyperreactivity. J Allergy Clin Immunol 1988; 81:776-81

Godfrey S, Konig P. Inhibition of exercise-induced asthma by different pharmacological pathways. Thorax 1976;31:137-43

Goldsmith RL, Bigger JT Jr, Steinman RC and Fleiss JL. Comparison of 24-hour parasympathetic activity in endurance-trained athletes. Am Coll Cardiol 1992;20:552-8

Greening AP, Ind PW, Northfield M, Shaw G. Added salmeterol versus higher-dose corticosteroid in asthma patients with symptoms on existing inhaled corticosteroids. Lancet 1994;344:219-224

Grönke L, Kanniess F, Holz R, Jörres A, Magnussen H. The relationship between airway hyperresponsiveness, markers of inflammation and lung function depends on the duration of the asthmatic disease. Clin Exp Allergy 2002;32:57-63

Haahtela T, Järvinen M, Kava T, Kiviranta K, Koskinen S, Lehtonen K, Nikander K, Person T, Reinikainen K, Selroos O, Sovijärvi A, Stenius-Aarniala B, Svahn T, Tammivaara R, Laitinen LA. Comparison of a β2-agonist, terbutaline, with an inhaled corticosteroid, budesonide, in newly detected asthma. N Engl J Med 1991;321:388-92

Haahtela T, Järvinen M, Kava T, Kiviranta K, Koskinen S, Lehtonen K, Nikander K, Person T, Selroos O, Sovijärvi A, Stenius-Aarniala B, Svahn T, Tammivaara R,

Laitinen LA. Effects of reducing or discontinuing inhaled budesonide in patients with mild asthma. N Engl J Med 1994;331:700-5

Hahn C, Islamian AP, Renz H, Nockher WA. Airway epithelial cells produce neurotrophins and promote the survival of eosinophils during allergic inflammation. J Allergy Clin Immunol 2006;117:787-94

Hallstrand TS, Moody MW, Wurfel MM, Schwartz LB, Henerson WR Jr, Aitken ML. Inflammatory basis of exercise-induced bronchonostriction. Am J Repir Crit Care Med 2005;172:679-86

Hamid Q, Tulic MK, Liu MC, Moqbel R. Inflammatory cells in asthma: Mechanisms and implications for therapy. J Allergy Clin Immunol 2003;111:S5-17

Hancox RJ, Subbarao P, kamada D, Watson RM, Hargreave FE, Inman MD. Beta2-agonist tolerance and exercise-induced bronchospasm. Am J Repir Crit Care Med 2002;165:1068-70

Heinke S. Szucs G, Norris A, Droogmans G, Nilius B. Inhibition of volume-activated chloride currenta in endothelial cells by cromones. Br J Pharmacol 1995;115:1393-8

Heir T. Longitudinal variations in bronchial responsiveness in cross-country skiers and control subjects. Scand J Med Sci Sports 1994;4:134-9

Heir T, Oseid S. Self reported asthma and exercise-induced symptoms in high-level competitive cross-country skiers. Scand J Med Sci Sport 1994; 4:124-7

Heir T, Aanestad G, Carlsen KH, Larsen S. Respiratory tract infection and bronchial responsiveness in elite athletes and sedentary control subjects. Scand J Med Sci Sports 1995;5:94-99

Helenius IJ, Tikkanen HO and Haahtela T. Exercise-induced bronchospasm at low temperature in elite athletes. Thorax 1996;51:628-9

Helenius IJ, Rytilä P, Metso T et al Respiratory symptoms, bronchial responsiveness and cellular characteristics of induced sputum in elite swimmers. Allergy 1998a;53:346-52

Helenius IJ, Tikkanen HO, Sarna S and Haahtela T. Asthma and increased bronchial responsiveness in elite athletes: atopy and sports as risk factors. J Allergy Clin Immunol 1998b:101:646-52.

Helenius I, Haahtela T. Allergy and asthma in elite summer sports athletes. J Allergy Clin Immunol 2000;106:444-52

Helenius I, Rytila P, Sarna S, Lumme A, Helenius M, Remes V, et al. Effect of continuing or finishing high-level sports on airway inflammation, bronchial hyperresponsiveness, and asthma: a 5-year prospective follow-up study of 42 highly trained swimmers. J Allergy Clin Immunol 2002;109:962-8.

Helenius I, Lumme A, Öunap J, Obase Y, Rytilä P, Sarna S, Alaranta A, Remes V, Haahtela T. No effect of montelukast on asthma-like symptoms in elite ice hockey players. Allergy 2004;59:39-44

Helenius I, Lumme A, Haahtela T. Asthma, airway inflammation and treatment in elite athletes. Sports Med 2005;35:565-74

Hoag JE, McFadden ER Jr. Long-term effect of cromolyn sodium on nonspecific bronchial hyperresponsiveness: a review. Ann Allergy 1991;66:53-63

Holgate ST, Dahlén SE. From slow reacting substance to leukotrienes: a testimony of scientific endeavour an achievement. In: Holgate ST and SE Dahlén (ed.) SRS-A to leukotrienes. The drawning of a new treatment, Balckwell Science, London, 1997:1-23

Holgate ST. Epithelium dysfunction in asthma. J Allergy Clin Immunol 2007;120:1233-1244

Holzer K, Anderson SD, Doulass J. Exercise in elite summer athletes: challenges for diagnosis. J Allergy Clin Immunol 2002;110:374-80

Holzer K, Brukner P. Screening of athletes for exercise-induced bronchoconstriction. Clin J Sport Med 2004;14:134-8

Hopp RJ, Townley RG, Biven RE, Bewtra AK, Nair NM. The presence of airway reactivity before the development of asthma. Am Rev Respir Dis 1990;141:2-8

Hoshino M, Nakamura Y. The effect of inhaled sodium cromoglycate on cellular infiltration into the bronchial mucosa and the expression of adhesion molecules in asthmatics. Eur Respir J 1997;10:858-65

Hoshino M, Nakamura Y, Sim JJ, Yamashiro Y, Uchida K, Hosaka K, Isogai S. Inhaled corticosteroid reduced lamina reticularis of the basement membrane by modulation of insulin-like growth factor (IGF)-1 expression in bronchial asthma. Clin Exp Allergy 1998;28:568-77

Hoshino M, Nakamura Y, Sim J, Shimojo J, Isogai S. Bronchial subepithelial fibrosis and expression of matrix metalloproteinase-9 in asthmatic airway inflammation. J Allergy Clin Immunol 1998b;102:783-8

Hoshino M, Nakamura Y, Sim JJ, Tomioka H. A comparative study of the effects of ketotifen, disodium cromoglycate, and beclomethasone dipropionate on bronchial mucosa and asthma symptoms in patients with atopic asthma. Respir Med 1998c;92:942-50

Houghton DD (Ed) . Handbook on applied meteorology. Wiley-Interscience, New Your, USA, 1985:84

Hsia HC, Schwarzbauer JE. Meet the tenascins: multifunctional and mysterious. J Biol Chem 2005;280:26641-4

Huang J, Olivenstein R, Taha R, Hamid Q, Ludwig M. Enhanced proteoglycan deposition in the airway wall of atopic asthmatics. Am J Respir Crit Care Med 1999;160:725-9

Humbert M, Durham SR, Ying S, Kimmit P, Barkans J, Assoufi B, Pfister R, Menz G, Robinson DS, Kay BA, Corrigan CJ. IL-4 and IL-5 mRNA and Protein in bronchial biopsies from patients with atopic and nonatopic asthma: evidence against "nonatopic" asthma being a distinct immunopathologic entity. Am J Respir Crit Care Med 1996;154:1497-1504

Härkönen E, Virtanen I, Linnala A, Laitinen LE, Kinnula VL. Modulation of fibronectin and tenascin production in human bronchial epithelial cells by inflammatory cytokines in vitro. Am J Respir Cell Mol Biol 1995;13:109-115

Indman MD, O'Byrne PM. The effect of regular inhaled albuterol on exercise-induced bronchoconstriction. Am J Respir Crit Care Med 1996;153:65-9

Ingber DE, Dike L, Hansen L, Karp S, Liley H, Maniotis A, McNamee H, Mooney D, Plopper G, Sims J, et al. Cellular tensegrity: exploring how mechanical changes in the cytoskeleton regulate cell growth, migration, and tissue pattern during morphogenesis. Int Rev Cytol 1994;150:173-224

Ioachim E, Charchanti A, Briasoulis E, Karavasilis V, Tsanou H, Arvanitis DL, Agnantis NJ, Pavlidis N. Immunohistochemical expression of extracellular matrix components tenascin, fibronectin, collagen type IV and laminin in breast cancer : their prognostic value and role in tumour invasion and progression. Eur J Cancer 2002;38:2362-70

Jacobsen EA, Taranova AG, Lee NA, Lee JJ. Eosinophils: Singularly destructive effector cells or purveyors of immunoregulation. J Allergy Clin Immunol 2007;119:1313-20

Jansen DF, Times W, Kraan J et al (A)symptomatic bronchial hyperresponsiveness and asthma. Respir Med 1997;91:121-34

Jammes Y, Delvolgo-Gori MJ, Badier M, Guillot C, Gazazian G, Parlenti L. One-year occupational exposure to a cold environment alters lung function. Arch Environ Health 2002;57:360-5

Jarjour NN, Calhoun WJ. Exercise-induced asthma is not associated with mast cell activation or airway inflammation. J Allergy Clin Immunol 1992;89:60-8

Jarjour NN, Wilson SJ, Koenig SM, Laviolette M, Moore WC, Davis WB, Doherty DE, Hamid Q, Israel E, Kavuru MS, Ramsdell JW, Tashkin DP, Reilly DS, Yancey SW, Edwards LD, Stauffer JL, Dorinsky PM, Djukanovic R. Control of airway inflammation maintained at a lower steroid dose with 100/50 µg of fluticasone propionate/salmeterol. J Allergy Clin Immunol 2006;118:44-52

Jeffery PK, Wardlaw AJ, Nelson FC, Collins JV, Kay BA. Bronchial biopsies in asthma. An ultrastuctural, quantitative study and correlation with hyperreactivity. Am Rev Respir Dis 1989;140:1745-53

Jeffery PK, Godfrey RW, Adelroth E, Nelson F, Rogers A, Johansson SA. Effects of treatment on airway inflammation and thickening of basement membrane reticular collagen in asthma. A quantitative light and electron microscopic study. Am Rev Respir Dis 1992;145:890-9

Jeffery PK, Laitinen A, Venge P. Biopsy markers of airway inflammation and remodelling. Respir Med 2000;94:S9-S15

Jeffery PK, Venge P, Gizycki MJ, Egerod I, Dahl R, Faurschou P. Effects of salmeterol on mucosal inflammation in asthma: a placebo-controlled study. Eur Respir J 2002;20:1378-85

John M, Lim S, Seybold J, Jose P, Robichaud A, O'Connor B, Barnes PJ, Chung KF. Inhaled corticosteroids increase interleukin-10 but reduce macrophage inflammatory protein-1-alpha, granulocyte-macrophage colony-stimulating factor, and interferon-gamma release from alveolar macrophages in asthma. Am J Respir Crit Care Med 1998;157:256-62

Johnson BD, Saupe KW, Dempsey JA. Mechanical constraints on exercise hyperpnea in endurance athletes. J Appl Physiol 1992;73:874-86

Jones PL, Jones FS: Tenascin-C in development and disease:gene regulation and cell function. Matrix Biology 2000; 19:581-596

Juniper EF, Kline PA, Vanzieleghem MA, Ramsdale EH, O'Byrne PM, Hargreave FE. Effect of long-term treatment with an inhaled corticosteroid (budesonide) on airway hyperresponsiveness and clinical asthma in nonsteroid-dependent asthmatics. Am Rev Respir Dis 1990;142:832-6

Juuti A, Nordling S, Louhimo J, Lundin J, Haglund C. Tenascin C expression is upregulated in pancreatic cancer and correlates with differentiation. J Clin Pathol 2004;57:1151-5

Kaarteenaho-Wiik R, Kinnula V, Herva R, Pääkkö P, Pöllänen R, Soini Y. Distribution and mRNA expression of tenascin-C in developing human lung. Am J Respir Cell Mol Biol 2001;25;341-6

Kaarteenaho-Wiik R, Sademies O, Paakko P, Risteli J, Soini Y. Extracellular matrix proteins and myofibroblasts in granulomas of sarcoidosis, atypical mycobacteriosis, and and tuberculosis of the lung. Human Pathol 2007;38;147-53

Kamath AV, Pavord ID, Ruparelia PR, Chilvers ER. Is the neutrophil the key effector cell in severe asthma? Thorax 2005;60:529-30

Kariyawasam HH, Aizen M, Barkans J, Robinson DS, Kay BA. Remodeling and airway hyperresponsiveness but not cellular inflammation persist after allergen challenge in asthma. Am J Respir Crit Care Med 2007;175:896-904

Karjalainen J. Exercise response in 404 young men with astma: no evidence for a late asthmatic reaction. Thorax 1991;46:100-4

Keatings VM, Evans DJ, O'Connor BJ, Barnes PJ. Cellular profiles in asthmatic airways: a comparison of induced sputum, bronchial washing, and bronchoalveolar lavage fluid. Thorax 1997;20:372-4

Kim ES, Kim SH, Kim KW, Park JW, Kim YS, Sohn MH, Kim K-E. Basement membrane thickening and clinical features of children with asthma. Allergy 2007;62:635-40

Kinderman W. Do inhaled beta2-agonists have an ergogenic potential in non-asthmatic competitive athletes. Sports Med 2007;37:95-102

Kolnaar BGM, Folgering H, van den Hoogen HJM, van Weel C. Asymptomatic bronchial hyperresponsiveness in adolescents and adults. Eur Respir J 1997;10:44-50

Koopmans JG, Lutter R, Jansen HM, van der Zee. Adding salmeterol to an inhaled corticosteroid: long term effects on bronchial inflammation in asthma. Thorax 2006;61:306-12

Koskela H, Tukiainen H. Facial cooling, but not nasal breathing of cold air, induces bronchoconstriction: a study in asthmatic and healty subjects. Eur Respir J 1995;8:2088-93

Kotaniemi JT, Latvala J, Lundbäck B, Sovijärvi A, Hassi J, Larsson K. Does living in a cold climate or recreational skiing increase the risk for obstructive respiratory diseases or symptoms? J Circumpolar Health 2003;62:142-57

Kukafka DS, Lang DM, Porter R et al. Exercse-induced bronchospasm in high school athletes vs a free running testi: incidence and epidemiology. Chest 1998;114:1612-22

Lacoste JY, Bousquet J, Chanez P, Van Vyve T, Simony-Lafontaine J, Lequeu N, Vic P, Enander I, Godard P, Michel FB. Eosinophilic and neutrophilic inflammation in asthma, chronic bronchitis, and chronic obstructive pulmonary disease. J Allergy Clin Immunol 1993;92:537-48

Laitinen A, Altraja A, Kämpe M, Linden M, Virtanen I, Laitinen LA. Tenascin is increased in airway basement membrane of asthmatics and decreased by an inhaled steroid. Am J Respir Crit Care Med 1997;156:951-8

Laitinen A, Lindqvist A, Halme M, Altraja A, Laitinen LA. Leukotriene E(4)-induced persistent eosinophilia and airway obstruction are reversed by zafirlukast in patients with asthma. J Allergy Clin Immunol 2005;15:259-65

Laitinen LA, Kokkola K. Bronchial reactivity in pulmonary tuberculosis. Scand J Resp Dis 1974;Suppl 89:201-5

Laitinen LA, Kava T. Bronchial reactivity following uncomplicated influenza A infection in healthy subjects and in asthmatic patients. Eur J Respir Dis 1980;10:51-8

Laitinen LA, Heino M, Laitinen A, Kava T, Haahtela T. Damage of the airway epithelium and bronchial reactivity in patients with asthma. Am Rev Respir Dis 1985;131:599-606

Laitinen LA, Laitinen A, Haahtela T. A comparative study of the effects of an inhaled corticosteroid, budesonide, and a β2-agonist, terbutaline on airway inflammation in newly diagnosed asthma: A randomized, double-blind, parallel-group controlled trial. J Allergy Clin Immunol 1992;90:32-42

Laitinen LA, Laitinen A, Haahtela T. Airway mucosal inflammation even in patients with newly diagnosed asthma. Am Rew Repir Dis 1993a;147:697-704

Laitinen LA, Laitinen A, Haahtela T, Vilkka V, Spur BW, Lee TH. Leukotriene E4 and granulocytic infiltration into asthmatic airways. Lancet 1993b;341:989-90

Laitinen LA, Laitinen A, Altraja A, Virtanen I, Kämpe M, Simonsson BG, Karlsson SE, Håkansson L, Venge P. Bronchial biopsy findings in intermittent or "early" asthma. J Allergy Clin Immunol 1996;98:S3-6

Langdeau JB, Turcotte H, Bowie DM et al Airway hyperresponsiveness in elite athletes. Am J Respir Crit Care Med 2000;161:1479-84

Langdeau JB, Boulet LB. Prevalence and mechanisms of development of asthma and airway hyperresponsiveness in athletes. Sports Med 2001;31:601-16

Laprise C, Boulet LP. Asymptomatic airway hyperresponsiveness: A three-year follow-up. Am J Respir Crit Care Med 1997;156:403-9

Laprise C, Laviolette M, Boutet M, Boulet LP. Asymptomatic airway hyperresponsiveness: relationships with airway inflammation and remodelling. Eur Respir J 1999;14:63-72

Larsson K, Ohlsén P, Larsson L, Malmberg P, Rydström P-O, Ulriksen H. High prevalence of asthma in cross-country skiers. BMJ 1993; 307:1326-9

Larsson L, Hemmingsson P, Boethius G. Self-reported obstructive airway symptoms are common in young cross-country skiers. Scand J Med Sci Sports 1994;4:124-7

Lazarus SC, Boushey HA, Fahy JV, Chinchilli VM, Lemanske RF Jr, Sorkness CA, Kraft M, Fish JE, Peters SP, Craig T, Drazen JM, Ford JG, Israel E, Martin RJ, Mauger EA, Nachman SA, Spahn JD, Szefler SJ. Asthma Clinical Research Network for the National Heart, Lung, and Blood Institute. Long-acting beta2-agonist monotherapy vs continued therapy with inhaled corticosteroids in patients with persistent asthma: a randomized controlled trial. JAMA 2001;285:2583-93

Leff JA, Busse WW, Pearlman D, et al Montelukast, a leukotriene antagonist, for the treatment of mild asthma and exercise-induced bronchoconstriction. N Engl J Med 1998;339:147-52

Lemanske RF Jr, Sorkness CA, Mauger EA, Lazarus SC, Boushey HA, Fahy JV, Drazen JM, Chinchilli VM, Craig T, Fish JE, Ford JG, Israel E, Kraft M, Martin RJ, Nachman SA, Peters SP, Spahn JD, Szefler SJ. Asthma Clinical Research Network for the National Heart, Lung, and Blood Institute. Inhaled corticosteroid reduction and elimination in patients with persistent asthma receiving salmeterol: a randomized controlled trial. JAMA 2001 285:2594-603

Lemiére C, Ernst P, Olivenstein R, Yamauchi Y, Govindaraju K, Ludwig MS, Martin JG, Hamid Q. Airway inflammation assessed by invasive and non-invasive means in severe asthma: Eosinophilic and noneosinophilic phenotype. J Allergy Clin Immunol 2006;118:1033-9

Leuppi JD, Kuhn M, Comminot C, Reinhart WH. High prevalence of bronchial hyperresponsiveness and asthma in ice hockey players. Eur Respir J 1998;12:13-16

Li X, Ward C, Thien F, Bish R, Bamford T, Bao X, et al. An antiinflammatory effect of salmeterol, a long-acting beta(2) agonist, assessed in airway biopsies and bronchoalveolar lavage in asthma. Am J Respir Crit Care Med 1999;160:1493-9

Liesker JJW, ten Hacken NHT, Rutgers SR, Zeinstra-Smith M, Postma DS, Timens W. Mast cell numbers in airway smooth muscle and PC20AMP in asthma and COPD. Respir Med 2007;101:882-7

Linnala A, Kinnula V, Laitinen LA, Lehto V-P, Virtanen I. Transforming growth factor-regulates the expression of fibronectin and tenascin in BEAS 2B human bronchial epithelial cells. Am J Respir Cell Mol Biol 1995;13:578-85

Lozewicz S, Wells C, Gomez E, Ferguson H, Richman P, Devalia J, Davies RJ. Morphological integrity of the bronchial epithelium in mild asthma. Thorax. 1990;45:12-15

Lumme A, Haahtela T, Ounap J, Rytilä P, Obase Y, Helenius M, Remes I, Helenius I. Airway inflammation, bronchial hyperresponsiveness and asthma in elite ice hockey players. Eur Respir J 2003;22:113-7

Lundgren R, Söderberg M, Hörstedt P, Stenling R. Morphological studies of bronchial mucosal biopsies from asthmatics before and after ten years of treatmen with inhaled steroids. Eur Repir J 1988;1;883-9

Mackie EJ, Halfter W, Liverani D. Induction of tenascin in healing wound. J Cell Biol 1988;107:2757-67

Mackie EJ, Tucker RP. The tenascin-C knockout revisited. J Cell Sci 1999;112:3847-53

Maesterlli P, Saetta M, Stefano AD, Calcagni PG, Turato G, Ruggierei MP, Roggeri A, Mapp CE, Fabbri LM. Comparison of leukocyte counts in sputum, bronchial biopsies, and broncoalvelolar lavage. Am J Respir Crit Care Med 1995;152:1926-31

Magnussen H, Richter K, Taube C. Are chronic obstructive pulmonary disease (COPD) and asthma different diseases? Clin Exp Allergy 1998;28(suppl5):187-94;203-5

Mannix ET, Farber MO, Palange P, Galassetti P, Manfredi F. Exercise-induced asthma in figure skaters. Chest 1996;109:312-5

Mannix ET, Manfredi F, Farber MO. A comparison of two challenge tests for identifying exercise-induced bronchospasm in figure skaters. Chest. 1999 Mar;115:649-53.

Mannix ET, Roberts MA, Dukes HJ, Magnes CJ, Farber MO. Airways hyperresponsiveness in high school athletes. J Asthma 2004;41:567-74

Manolitsas ND, Wang J, Devalia JL, Trigg CJ, McAulay AE, Davies RJ. Regular albuterol, nedocromil sodium, and bronchial inflammation in asthma. Am J Respir Crit Care Med 1995;15:1925-30

Marom Z. Stelhamer JH, Bach M, Morton DR, Kaliner M. Slow-reacting substances, leukotrienes C4 and D4, increase the release of mucus from human airways in vitro. Am Rev Respir Dis 1982;126:449-51

Martin RJ, Cicutto LC, Smith HR, Ballard RD, Szefler SJ. Airways inflammation in nocturnal asthma. Am Rev Respir Dis 1991;143:351-7

Martineau L, Horan MA, Rothwell NJ, Little RA. Salbutamol, a ß2-adrenoceptor agonist, increases skeletal muscle strength in young men. Clin Sci 1992;83:615-21

McFadden Jr ER. Hypothesis: exercise-induced asthma as vascular phenomenon. Lancet 1990;335:880-3

McIvor RA, Pizzichini E, Turner MO, Hussack P, Hargreave FE, Sears MR. Potential masking effects of salmeterol on airway inflammation in asthma. Am J Respir Crit Care Med 1998;158:924-30

McKenzie DC, McLuckie SL,Stirling DR. The protective effects of continuous and interval exercise in athletes with exerciese-induced asthma. Med Sci Sports Exerc 1994;26:951-6

McParland BE, Macklem PT, Pare PD. Airway wall remodeling: friend or foe? J Appl Physiol 2003;95:426-34

Menz G, Ying S, Durham SR, Corrigan CJ, Robinson D, Hamid Q, Pfister R, Humbert M, Kay BA.. Molcecular concepts of IgE-initited inflammation in atopic and nonatopic asthma. Allergy 1998;53:15-21

Merker H-J. Morphology of the basement membrane. Microsc Res Tech 1994;28:95-124

Milanese M, Crimi E, Scordamaglia A, Riccio A, Pellegrino R, Canonica GW, Brusasco V. On the functional consequenses of bronchial basement membrane thickening. J Appl Physiol 2001;91:1035-40

Motojima S, Frigas E, Loegering DA, Gleich GJ. Toxicity of eosinophil cationic proteins for guinea pig tracheal epithelium in vitro. Am Rev Respir Dis 1989;139:801-805

Munoz NM, Vita A, Neely SP, McAllister K, Spaethe SM, White SM. Leff AR. Beta adrenergic modulation of formyl-methionine-leucine-phenylalanine-stimulated secretion of eosinophil peroxidase and leukotriene C4. J Pharmacol Exp Ther 1994;268:1339-43

Möller GM, Overbeek SE, van Helden-Meeuwsen CG, Hoogsteden HC, Bogaard JM. Eosinophils in the bronchial mucosa in relation to methacholine dose-response curves in atopic asthma. J Appl Physiol 1999;86:1352-6

Mönkäre S, Haahtela T, Ikonen M, Laitinen LA. Bronchial hyperreactivity to inhaled histamine in patients with farmer's lung. Lung 1981;159:145-51

Nakahara H, Gabazza EC, Fujimoto H, Nishii Y, D'alessandro-Gabazza CN, Bruno NE, Takagi T, Hayashi T, Maruyama J, Maruyama K, Imanaka-Yoshida K, Suzuki K, Yoshida T, Adachi Y, Taguchi O. Deficiency of tenascin C attenuates allergen-induced bronchial asthma in the mouse. Eur J Immunol 2006;36:3334-45

Nakamura Y, Hoshino M, Sim JJ, Ishii K, Hosaka K, Sakamoto T. Effect of the Leukotriene antagonist pranlukast on cellular infiltration in the bronchial mucosa of patients with asthma. Thorax 1998;53:835-41

Nelson H. Drug Therapy: (beta)-Adrenergic bronchodilators N Engl J Med 1995;333:499-506

Nelson HS, Weiss ST, Bleecker ER, Yancey SW, Dorinsky PM. The Salmeterol Multicenter Asthma Research Trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol. Chest 2006;129:15-26

Nielsen LP, Dahl R. Therapeutic ratio of inhaled corticosteroids in adult asthma. A dose-range comparison between fluticasone propionate and budesonide, measuring their effect on bronchial hyperresponsiveness and adrenal cortex function. Am J Respir Crit Care Med 2000;162:2053-7

Nielson CP, Hadjokas NE. Beta-adrenoreceptor antagonist block corticosteroid inhibition in eosinophils. Am J Respir Crit Care Med 1998;157:184-91

Nieman DC. Is infection risk linked to exercise workload? Med Sci Sports Exerc 2000;32:406-11

Nieman DC, Henson DA. Smith LL. Utter AC, Vinci DM, Davis JM, Kaminsky DE, Shute M. Cytokine changes after a marathon race. J Appl Physiol 2001;91:109-14

Nieminen M. Unimodal distribution of bronchial hyperresponsiveness to methacholine in asthmatic patients. Chest 1992; 102:1537-43

O'Byrne PM. What is asthma? An update on the mechanisms. J Invest Allergol Immunol 1995;5:6-11

O'Byrne PM, Ädelroth E. β2 Déjá vu. Chest 2006;129:3-4

Ohashi Y, Motojima S, Fukuda T, Makino S. Airway hyperresponsiveness, increased intracellular spaces of bronchial epithelium, and increased infiltration of eosinophils and lymphocytes in bronchial mucosa in asthma. Am Rev Respir Dis 1992;145:1469-76

Olivieri D, Chetta A, Del Donno M, Bertorelli G, Casalini A, Pesci A, Testi R, Goresi A. Effect of short-term treatment with low-dose inhaled fluticasone propionate on airway inflammation and remodeling in mild asthma: a placebo-controlled study. Am J Respir Crit Care Med 1997;155:1864-71

Ollerenshaw SL, Woolcock AJ. Characteristics of the inflammation in biopsies from large airways of subjects with asthma and subjects with chronic airflow limitation. Am Rev Respir Dis 1992;145:922-7

Oppenheimer J, Nelson HS. Safety of long-acting β-agonists in asthma: a review. Curr Opin Pulm Med 2008;14:64-9

Ordoñez C, Ferrando R, Hyde DM, Wong HH, Fahy JV. Epithelial desquamation in asthma. - artifact or pathology? Am J Respir Crit Care Med 2000a;162:2324-9

Ordoñez CL, Shaughnessy TE, Matthay MA, Fahy JV. Increased neuthrophil numbers and IL-8 levels in airway secretions in acute severe asthma: Clinical and biological significance. Am J Respir Crit Care Med 2000b;161:1185-90

Orend G, Chiquet-Ehrismann R. Tenascin-C induced signaling in cancer. Cancer Lett 2006;244:143-63

Orsida BE, Li X, Hickey B, Thien F, Wilson JW, Walters EH. Vascularity in asthmatic airways: relation to inhaled steroid dose. Thorax 1999;5:289-95

O'Sullivan S, Cormican L, Murphy M, Poulter LW, Conor M. Effects of varying clinical doses of fluticasone propionate on the physiology and bronchial wall immunopathology in mild-to-moderate asthma. Chest 2002;122:1966-72

O'Sullivan S, Akveld M, Burke CM, Poulter LW. Effect of the addition of montelukast to inhaled fluticasone propionate on airway inflammation. Am J Respir Crit Care Med 2003;167:745-50

O'Sullivan S, Cormican L, Burke CM, Poulter LW. Fluticasone induces T cell apoptosis in the bronchial wall of mild to moderate asthmatics. Thorax 2004;59:657-61

Pallasaho P, Meren M, Raukas-Kivioja A, Rönmark E. Different labeling of obstructive airway diseases in Estonia, Finland, and Sweden. Eur J Epidemiol 2005;20:975-83

Park H-S, Jung K-S, Hwang S-C, Nahm D-H, Yim H-E. Neutrophil infiltration and release of IL-8 in airway mucosa from subjects with grain dust-induced occupational asthma. Clin Exp Allergy 1998;28:724-30

Parsons JP, Mastrodarne JG. Exercise-induced bronchoconstriction in athletes. Chest 2005;128:3966-74

Pascual RM, Peters SP. Airway remodelling contributes to the progressive loss of lung function in asthma: An overview. J Allergy Clin Immunol 2005;116:477-86

Pauwels RA, Löfdahl CG, Postma DS, Tattersfield AE, O'Byrne P, Barnes PJ, Ullman A.Effect of inhaled formoterol and budesonide on exacerbations of asthma. N Engl J Med 1997;337:1405-11

Payne DNR, Rogers AV, Ädelroth E, Bandi V, Guntupalli KK, Bush A, Jeffery PK. Early thickening of the reticular basement membrane in children with difficult asthma. Am J Respir Crit Care Med 2003;167:78-83

Pedersen B, Dahl R, Larsen BB, Venpe P. The effect of salmeterol on the early- and late-phase reaction to bronchial allergen and postchallenge variation in bronchial reactivity, blood eosinophils, serum eosinophil cationic protein, and serum eosinophil protein X

Pelkonen M, Notkola IL, Lakka T, Tukiainen H, Kivinen P, Nissinen A. Delaying decline in pulmonary function with physical activity. A 25-year follow-up. Am J Respir Crit Care Med 2003;168:494-9

Pepe C, Foley S, Shannon J, Lemiere C, Olivenstein R, Ernst P, Ludwig MS, Martin JG, Hamid Q. Differences in airway remodeling between subjects with severe and moderate asthma. J Allergy Clin Immunol 2005;116:544-9

Pesci A, Foresi A, Bertorelli G, Chetta A, Olivieri D. Histochemical characteristics and degranulation of mast cells in epithelium and lamina propria of bronchial biopsies from asthmatic and normal subjects. Am Rev Respir Dis 1993;147:684-9

Phipps S, Ying S, Wangoo A, Ong Y-E, Levi-Schaffer F, Kay AB. The relationship between allergen-induced tissue eosinophilia and markers of repair and remodeling in human atopic skin. J Immunol 2002;169:4604-12

Phipps S, Benyahia F, Ou TT, Barkans J, Robinson DS, Kay BA. Acute allergen-induced airway remodeling in atopic asthma. Am J Respir Cell Mol Biol 2004;31:62632

Pjorsbjerg C, Brannan JD, Anderson SD, Backer V. Relationship between airway responsiveness to mannitol and to methacholine and markers of airway inflammation, peak flow variability and quality of life in asthma patients. Clin Exp Allergy 2007;38:43-50

Pohjantähti H, Laitinen J, Parkkari J. Exercise-induced bronchospasm among healthy elite cross-country skiers and non-athletic students. Scand J Med Science Sports 2005;15:324-8

Pohunek P, Warner JO, Turziková J, Kurdman J, Roche WR. Markers of eosinophilic inflammation and tissue re-modeling in children before clinically diagnosed bronchial asthma. Pediatr Allergy Immunol 2005;16:43-51

Polosa R. Critical appraisal of antileukotriene use in asthma managment. Curr Opin Pulm Med 2007;13:24-30

Poppius H, Muittari A, Kreus KE, Korhonen O. Exercise asthma and disodium crmoglycate. BMJ 1970;4:337-9

Poston RN, Chanez P, Lacoste JY, Litchfield T, Lee TH, Bousquet J. Immunohistochemical characterization of the cellular infiltration in asthmatic bronchi. Am Rev Respir Dis 1992;145:918-21

Potts JE. Adverse respiratory health effects of competitive swimming: the prevalence of symptoms, illnesses, and bronchial responsiveness to methacholine and exercise (dissertation).Vancouver, University of British Columbia 1994

Potts J. Factors associated with respiratory problems in swimmers. Sports Med 1996;21:256-61

Power C, Sreenan S, Hurson B, burke C. Poulter LW. Distribution of immunocompetent cells in the bronchial wall of clinically healthy subjects showing bronchial hyperresponsiveness. Thorax 1993;48;1125-9

Probstmeier R, Stichel CC, Muller HW, Asou H, Pesheva P. Chondroitin sulfates expressed on oligodendrocyte-derived tenascin-R are involved in neural cell recognition. Functional implications during CNS development and regeneration. J Neurosci Res. 2000;60:21-36

Prosperini G, Rajakulasingatam K, Cacciola RR, Spizucca L, Rorke S, Holgate ST, Di Maria GU, Polosa R. Changes in sputum counts and airway hyperresponsiveness after budesonide: Monitoring anti-inflammatory response on the basis of surrogate markers of airway inflammation. J Allergy Clin Immunol 2002;110:855-61

Pääkkö P, Kaarteenaho-Wiik R, Pöllänen R, Soini Y. Tenascin mRNA exterssion at the foci of recent injury in usual interstitial pneumonia. Am J Respir Crit Care Med 2000;161:967-72

Qiu Y, Zhu J, Bandi V, Guntupalli KK, Jeffery PK. Bronchial mucosal inflammation and upregulation of CXC chemoattractants and receptors in severe exacerbations of asthma. Thorax. 2007;62:475-82

Ramage L, Lipworth BJ, Ingram CG, Cree IA, Dhillon DP. Reduced protection against exercise induced bronchoconstriction after chronic dosing with salmeterol. Repir Med 1994;88:363-8

Ramos-Barbon D, Presley JF. Hamid QA. Fixman ED. Martin JG. Antigen-specific CD4+ T cells drive airway smooth muscle remodeling in experimental asthma. J Clin Invest 2005;115:1580-9

Ramsdale EH, Morris MM, Roberts RS Hargreave FE. Asymptomatic bronchial hyperresponsiviness in rhinitis. J Allergy Clin Immunol 1985;75:573-7

Redington AE, Roche WR, Holgate ST, Howarth PH. Co-localization of immunoreactive transforming growth factor-beta 1 and decorin in bronchial biopsies from asthmatic and normal subjects. J Pathol 1998;186:410-5

Rhoden KJ, Meldrum LA, Barnes PJ. Inhibition of cholinergic neurotransmission in human airways by beta sub 2-adrenoreceptors. J Appl Physiol 1988;65:700-5

Rinkema LE, Bemis KG, Fleisch JH Production and antagonism of cutaneous vascular permeability in the guinea pig in response to histamine, leukotrienes and A23187. J Pharmacol Exp Ther 1984;230-550-7

Roberts JA, Bradding P, Britten KM, Walls AF, Wilson S, Gratziou C, Holgate ST, Howarth PH. The long-acting β2-agonist salmeterol xinafoate: effects on airway inflammation in asthma. Eur Respir J 1999;14:275-82

Roche WR, Beasley R, Williams JH, Holgate ST.Subepithelial fibrosis in the bronchi of asthmatics. Lancet 1989;1:520-4

Roisman GL, Danel CJ, Lacronique JG, Alhenc-Gelas F, Dusser DJ. Decreased expression of angiotensin-converting enzyme in the airway epithelium of asthmatic subjects is associated with eosinophil inflammation. J Allergy Clin Immunol 1999;104:402-10.

Rupp NT, Guill MF and Brudno DS. Unrecognized exercise-induced bronchospasm in adolescent athletes. Am J Dis Child. 1992;146:941-4

Rundell KW, Mayers LB, Wilber RL, Szmedra L, Schmitz HR. Self-reported symptoms and exercise-induced asthma in the elite athlete. Med Sci Sports Exerc 2001;33:208-13

Rundell KW, Anderson SD, Spiering BA, Judelson DA. Field exercise vs laboratory eucapnic voluntary hyperventilation to identify airway hyperresponsiveness in elite cold weather athletes. Chest 2004;125:909-15

Rusko H. Training for cross country skiing. In Rusko H (editor). Cross Country Skiing. Blackwell Science Ltd 2003:62-100

Ryan G, Latimer KM, DolovichJ Hargreave FE. Bronchial responsiveness to histamine: Relationship ti diurnal variation fo peak flow rate, improvement after bronchodilator, and airway calibre. Thorax 1982;37:423-9

Saga Y, Yagi T, Ikawa Y, Sakakura T, Aizawa S. Mice develop normally without tenascin. Genes Dev 1992;6:1821-31

Salpeter SR. Buckely NS, Ormiston TM, Salpeter EE. Meta-analysis: effect of long-acting β-agonist on severe asthma exacervations and asthma-related deaths. Ann Intern Med 2006;144:904-12

Sapienza S, Du T, Eidelman DH, Wang NS, Martin JG. Structural changes in the airways of

sensitized brown Norway rats after antigen challenge. Am Rev Respir Dis 1991;144:423-7

Schoene RB, Giboney K, Schimmel C et al. Spirometry and airway reactivity in elithe track and field athletes. Clin J Sport Med 1997;7:257-61

Scichilone N, Morici G, Marchese R, Bonanno A, Profita M, Togias A, Bonsignore MR. Reduced airway responsiveness in nonelite runners. Med Sci Sports Exerc 2005;37:2019-25

Sears MR, Taylor DR, Print CG, Lake DC, Li QQ, Flannery EM, et al Regular inhaled beta-agonist treatment in bronchial asthma 1990. Lancet 1990;36:1391-6

Shaaban R, Leynaert B, Soussan D, Atno JM. Chinn S, de Marco R, Garcia-Aymerich J, Heinrich J, Janson C, Jarvis D, Sunyer J, Svanes C, Wjist M, Burney PG, Neukirjch F, Zureik M. Physical activity and bronchial hyperresponsiveness: European Community Respiratory Health Survey II. Thorax 2007;62:403-10

Shiba K, Kasahara K, Nakajima H, Adachi M. Structural changes of the airway wall impair respiratory function, even in mild asthma. Chest 2002;122:1622-6

Simons FE, Gerstner TV, Cheang MS. Tolerance to the bronchoprotective effect of salmeterol in adolescents with exercise-induced asthma using concurrent inhaled glucocorticoid treatment. Pediatrics 1997;99:655-9

Simons F, Estelle R. A comparison of beclomethasone, salmeterol, and placebo in children with asthma. N Engl J Med 1997;337:1659-65

Soler M, Joos L, Bolliger CT, Elsasser S, Perruchoud AP. Bronchoprotection by salmeterol: cell stabilization or functional antagonism? Comparative effects on histamine- and AMP-induced bronchoconstriction. Eur Respir J 1994;7:1973-7

Sont JK, Han J, van Krieken JM, Evertse CE, Hooijer R, Willems LN, Luuk NA, Sterk PJ. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. Thorax 1996;51:496-502.

Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway

hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. Am J Respir Crit Care Med 1999;159:1043-51

Sovijärvi A, Malmberg P,Reinikainen K et al A rapid dosimetric method with controlled tidal breathing for histamine challenge. Repeatability and distribution of bronchial reactivity in a clinical material. Chest 1993;104:164-70

Sovijärvi ARA, Haahtela T, Ekroos HJ, Lindqvist A, Saarinen A, Poussa T, Laitinen LA. Sustained reduction in bronchial hyperresponsiveness with inhaled fluticasone propionate within three days in mild asthma: time course after onset and cessation of treatment. Thorax 2003;58:500-4

Sparrow D, O'Connor G, Colton T, Barry CL. Weiss ST. The relationship of nonspesific bronchial hyperresponsiveness to the occurrence of respiratory symptoms and decreased levels of pulmonary function. Am Rev Respir Dis 1987;135:1255-60

Sterk PJ, Fabbri LM, Quanjaer PH. Airway hyperresponsiveness: Standardised challenge testing with pharmacological, physical and sensitising stimuli in adults. Eur Repir J 1993;6-SUPPL 16:53-83

Storms WW. Exercise-induced asthma: diagnosis and treatment for the recreational or elite athlete. Med Sci Psorts Exerc 1999;31:S33-8

Stromme SB, boushel R, Ekblom B, Huikuri H, Tulpppo MP, Jones JL. Cardiovascular and respiratory aspects of exercise – endurance training. In Textbook of sports medicine, Kjaer M, Krogsgaard M, Magnusson P, Engebretsen L, Roos H, Takala T (editors), Blackwell Science, USA, 2003

Sue-Chu M, Larsson L, Bjermer L. Prevalence of asthma in young cross-country skiers in central Scandinavia: differences between Norway and Sweden. Respir Med 1996; 90:99-105

Sue-Chu M, Karjalainen EM, Altraja A, Laitinen A, Laitinen LA, Naess AB, Larsson L, Bjermer L. Lymphoid aggregates in endobronchial biopsies from young elite cross-country skiers. Am J Respir Crit Care Med 1998;158:597-601

Sur S, Crotty TB, Kephart GM, Hyma BA, Colby TV, Reed CE, Hunt LW, Gleich GJ, Sudden-onset fatal asthma. A distinct entity with few eosinophils and relatively more neutrophils in the airway submucosa? Am Rev Respir Dis 1993;148:713-9

Söderberg M, Hellström S, Sandström T, Lundgren R, Bergh A. Structural characterization of bronchial biopsies from healthy volunteers: a light and electron microscopical study. Eur Respir J 1990;3:261-6

Tashkin DP, Altose MD, Bleecker ER, Connett JE, Kanner RE, Lee WW, Wise R. The lung health study: airway responsiveness to inhaled methacholine in smokers with mild to moderate airflow limitation. The Lung Health Study Research Group. Am Rev Respir Dis 1992;145:301-10

Taylor DR. Sears MR. Herbison GP. Flannery EM. Print CG. Lake DC. Yates DM. Lucas MK. Li Q. Regular inhaled beta agonist in asthma: effects on exacerbations and lung function. Thorax 1993;48:134-8

Tikkanen H, Helenius I. Asthma in runners. BMJ 1994; 309:108

van den Toorn LM, Overbeek SE, de Jongste JC, Leman K, Hoogsteden HC, Prins JB. Airway inflammation is present during clinical remission of atopic asthma. Am J Respir Crit Care Med 2001;164:2107-13

Torrego A, Hew M, Oates T, Sukkar M, Chung KF. Expression and activation of TGF-β isoforms in acute allergen-induced remodelling in asthma. Thorax 2007;62:307-13

Trautman A, Schid-Grendelmeier P, Krüger K, Crameri R, Akdis M, Akkaya A, Bröcker EB, Blaser K, Akdis CA. T cells and eosinophils cooperate in the induction of bronchial epithelial cell apoptosis in ashma. J Allergy Clin Immunol 2002;109:329-37

Trigg CJ, Manolitsas ND, Wang J, Calderón MA, McAulay AM, Jordan SE, Herdman MJ, Jhalli N, Duddle JM, Hamilton SA, Devalia JL, Davies RJ. Placebo-controlled

immunopathologic study of four months of inhaled corticosteroids in asthma. Am J Respir Crit Care Med 1994;150:17-11

Turcotte H, Langdeau JB, Thibault G, Boulet LP. Prevalence or respiratory symptoms in an athlete population. Respir Med 2003;97:955-63

Van Den Berge M, Kerstens HAM, Meijer RJ, De Reus DM, Koeter GH, Kauffman HF, Postma DS. Corticosteroid-induced improvement in the PC20 of adenosine monophosphate is more closely associated with reduction in airway inflammation than improvement in th PC20 of methacholine. Am J Respir Crit Care Med 2001;164:1127-32

van Schayk CO, Bijl-Hofland ID, Cloosterman SGM, Folgering HTM, van der Elshout FJJ, Van Weel C. Potential masking effect on dyspnoea perception by short- and long-acting β2-agonists in asthma. Eur Respir J 2002;19:240-5

Van Schoor J, Pauwels R, Joos G. Indirect bronchial hyper-responsiveness: the coming age of a specific group of bronchial challenges. Clin Exp Allergy 2005;35:250-61

Van Vyve T, Chanez P, Lacoste JY, Bousquet J, Michel FB, Godard P. Assesment of airway inflammation in asthmatic patients by visual endoscopic scoring systems. Eur Repir J 1003;6:1116-21

Vanacke NJ, Palmans ELS, Pauwels RA, Kips JC. Effect of combining salmeterol and fluticasone on the progression of airway remodeling. Am J Respir Crit Care Med 2002;166:1128-34

Varpela E, Laitinen LA, Keskinen H, Korhola O. Asthma, allergy and bronchial hyperreactivity to histamine in patients with bronchiectases. Clin Allergy 1978;8:273-80

Venaille TJ, Mendis AH, Phillips MJ, Thompson PJ, Robinson BW. Role of neutrophils in mediating human epithelial cell detachment from native basement membrane. J Allergy Clin Immunol 1995.

Venge P, Dahl R, Fredens K, Peterson CGB. Epithelial injury by human eosinophils. Am Rev Respir Dis 1988;138:S54-7

Venge P, Henriksen J, Dahl R. Eosinophils in exercise-induced asthma. J Allergy Clin Immunol 1991;88:699-704

Verges S, Flore P, Rousseau Blanchi MP, Wuyam B. A 10-year follow-up study of pulmonary function in symptomatic elite cross-country skiers – athletes and bronchial dysfunction. Scand J Med Sci Sports 2004;14:381-7

Verges S, Devouassoux G, Flore P, Rossini E, Fior-Gozlan M, Levy P, Wuyan B. Bronchial hyperresponsiveness, airway inflammation, and airflow limitation in endurance athletes. Chest 2005;127:1935-41

Vieira RP, Claudino RC, Duarte ACS, Santos ABG, Perini A, Raria Neto HCC, Mauad T, Martins MA, Dolhnikoff M, Carvalho CRF. Aerobic exercise decreases chronic allergic lung inflammation and airway remodeling in mice. Am J Respir Crit CareMed 2007;176:871-7

Vignola AM, Chanez P, Chiappara G, Merendino A, Zinnanti E, Bousquet J, Bellia V, Bonsignore G. Release of transforming growth factor-beta (TGF-beta) and fibronectin by alveolar macrophages in airway diseases. Clinl Exp Immunol 1996;106:114-9

Vignola AM, Chanez P, Chiappara G, Merendino A, Pace E, Rizzo A, la Rocca AM, Bellia V, Bonsignore G, Bousquet J. Transforming growth factor-beta expression in mucosal biopsies in asthma and chronic bronchitis. Am J Respir Crit Care Med 1997;156:591-9

Vignola AM, Chiappara G, Gagliardo R, Gjomarkaj M, Merendino A, Siena L, Bousquet J, Bonsignore G. Apoptosis and airway inflammation in asthma. Apoptosis 2000;5:473-85

Vignola AM, Chiappara G, Siena L, Bruno A, Gagliardo R, Merendino AM, Polla BS, Arrigo AP, Bonsignore G, Bousquet J, Chanez P. Proliferation and activation of bronchial epithelial cells in corticosteroid-dependent asthma. J Allergy Clin Immunol 2001;108:738-46.

Viljanen AA, Halttunen PK, Kreus KE, Viljanen BC. Spirometric studies in non-smoking, healthy adults. Scand J Clin Lab Invest 1982;42(suppl 159):5-20

Villaran C, O'Neill SJ, Helbling A, van Noord JA, Lee TH, Chuchalin AG, Langley SJ, Gunawardena KA, Suscovic S, Laurenzi M, Jasan JV, Menten J, Leff JA. Montelukast versus salmeterol in patients with asthma and exercise-induced bronchoconstriction. J Allergy Clin Immunol 1999;104:547-53

Virtanen I, Laitinen A, Tani T, Pääkkö P, Laitinen LA, Burgeson RE, Letho V-P. Differential expression of laminins and their integrin receptors in the developing and adult human lung. Am J Respir Cell Mol Biol 1996;15:184-96

Voy R. The U.S Olympic committee experience with exercise-induced bronchospasm, 1984. Med Sci Sports Exerc 1986;18:328-30

Waalkans HJ, van Essen-Zandvliet EEM, Gerritsen J, Duiverman EJ, Keerbijn KF, Knol K  The effect of an inhaled corticosteroid (budesonide) on exercise-induced asthma in children. Eur Respir J 1993;6:652-6

Wallin A, Sandström T, Söderberg M,Howarth P, Lundbäck B, Della-Cioppa G, Wilson S, Judd M, Djukanovic R, Holgate ST, Lindberg A, Larssen L. The effects of regular inhaled formoterol, budesonide, and placebo on mucosal inflammation and clinical indices in mild asthma. Am J Respir Crit Care Med 1999;159:7-86

Wallin A, Sue-Chu M, Bjermer L, Ward J, Sandstrom T, Lindberg A, et al. Effect of inhaled fluticasone with and without salmeterol on airway inflammation in asthma. J Allergy Clin Immunol 2003;112:72-8.

Wang JH, Trigg CJ, Devalia JL, Jordan S, Davies RJ. Effect of inhaled beclomethasone dipropionate on expression of proinflammatory cytokines and activated eosinophils in the bronchial epithelium of patients with mild asthma. J Allergy Clin Immunol 1994;94:1025-34

Ward C, Pais M, Bish R, Reid D, Feltis B, Johns D, Walters EH. Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. Thorax 2002;57:309-16

Wardlaw AJ. Molecular basis for selective eosinophil trafficking  in asthma: a multistep paradigm. J Allergy Clin Immunol 1999;104:917-26

Warner JA, Kroegel C. Pulmonary immune cells in health and disease: mast cells and basophils. Eur Respir J 1994;7:1326-41

Weiler JM, Metger WJ, Donnelly AL et al. Prevalence of bronchial hyperresponsiveness in highly trained athletes. Chest 1986;90(1):23-8

Weiler JM, Layton T, Hunt M. Asthma in United States Olympic athletes who participated in the 1996 Summer Games. J Allergy Clin Immunol 1998;102:722-6

Weiler JM, Bonini S, Coifman R, Graig T, Delgado L, Capão-Felipe M, Passalai D, Randolph C, Storms W. American Academy of Allergy, Asthma & Immunology Work Group Report: Exercise-induced asthma. J Allergy Clin Immunol 2001;119:1349-58

Weiler JM. Why must Olympic athletes prove that they have asthma to be permitted to take inhaled β2-agonists. J Allergy Clin Immunol 2003;111:36-7

Weiler JM, Bonini S, Coifman R, Graig T, Delgado L, Capão-Filipe M, Passali D, Randolph C, Storms W. American Academy of Allergi, Asthma & Immunology Work Group Report: exercise-induced asthma. J Allergy Clin Immunol 2007;119:1349-58

Wenzel SE, Westocott JY, Larsen GL. Bronchoalveolar lavage fluid mediator levels 5 minutes after allergen challenge in atopic subjects with asthma: relationship to the development of late asthmatic response. J Allergy Clin Immunol 1991;87:540-8

Wenzel SE, Szefler SJ, Leung DY, Sloan SI, Rex MD, Martin RJ. Bronchoscopic evaluation of severe asthma. Persistent inflammation associated with high dose glucocorticoids. Am J Respir Crit Care Med 1997;156:737-43.

Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. Am J Respir Crit Care Med 1999;160:1001-8

Wheatly JR, Amis TC, Engel LA. Oronasal partitioning of ventilation during exercise. J Appl Physiol 1991;71:546-51

Woolley KL, Adelroth E, Woolley MJ, Ellis R, Jordana M, O'Byrne PM. Granulocyte-macrophage colony-stimulating factor, eosinophils and eosinophil cationic protein in subjects with and without mild, stable, atopic asthma. Eur Respir J 1994;7:1576-84

Zetterström O, Buhl R, Mellem H, Parpina M, Hedman J, O'Neill S, Ekström T. Improved asthma control with budesonide/formoterol in a single inhaler, compared with budesonide alone. Eur Respir J 2001;18:262-268

Zhao Y. Tenascin is expressed in the mesenchyme of the embryonic lung and down-regulated by dexamethasone in early organogenesis. Biochem Biophys Res Commun 1999;263:597-602

www.ginasthma.com, accessed in April 2008

www.wada-ama.org/rtecontent/document/asthma_TUEC accessed December 2007

www.wada-ama.org, accessed in December 2007