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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

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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 hyperresponsiveness (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.

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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
FEV ₁	forced expiratory volume in one second
FP	fluticasone propionate
FVC	forced vital capacity
IgE	immunoglobulin E
IL	interleukin
IQR	interquartile range
mAb	monoclonal antibody
PC ₂₀ FEV ₁	provocative concentration 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 literature

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 visible 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 (TGF β), 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 laminin α 2 and laminin β 2 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, biglycan, 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 et al 1990). Airway eosinophilia is detectable in most asthma patients, but a non-eosinophilic phenotype of asthma is known (Lemi re 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- β) 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 Kroegele 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 oligomeric 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 tumorigenesis 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 β_2 -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 budesonide. 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 collagen 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
Milanesi 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

^aPercentage of epithelium covering BM correlated positively with PC₂₀ methacholine, ^bno difference in the epithelial structures between normal subjects and asthmatics with BHR, ^ca significant negative correlation between eosinophils and PD₁₅FEV₁ to bradykinin, ^d38 subjects were asthmatics, ^ea significant correlation between PC₂₀methacholine and fibrosis found only in normoreactive subjects, ^fa 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: ^amethacholine provocation, ^bhistamine provocation, ^ceucapnic 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 FEV1 (Sterk et al 1993), but in field conditions a fall of 15% in FEV1 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_{CO_2}). (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 are pharyngitis and oral candidiasis, 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 König 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 fluticasone. 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 μ g 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 β_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 β_2 -agonists (O'Byrne and Ädelroth 2006). Recent large trials have raised concern about the risk of deaths caused by long-acting β_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 β_2 -agonists with inhaled corticosteroids reduces the risk for severe asthma exacerbations and hospitalization for asthma.

Combination inhalers of long-acting β_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 β_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 al 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 β_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 β_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 β_2 -agonists only if they show objective documentation of asthma or EIB (Bonini et al 2004). Otherwise the athlete applying for use of a β_2 -agonist should fulfill one of the following criteria: $\geq 12\%$ increase in FEV₁ (of the predicted FEV₁ value) after the administration of an inhaled β_2 -agonist, $\geq 10\%$ fall in FEV₁ in exercise challenge or in EVH, in methacholine challenge PC₂₀ < 4 mg/ml or PD₂₀ ≤ 2 μ mol in a steroid-naïve athlete or PC₂₀ ≤ 6.6 mg mg/ml or PD₂₀ ≤ 13.6 μ mol 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 β_2 -agonist, especially long-acting β_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 β_2 -agonists. Despite β_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 β_2 -agonists in non-asthmatic athletes. He found no evidence that inhaled β_2 -agonists could improve athletic performance (Kinderman 2007). Unlike the inhaled β_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 β_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 β_2 -agonists worsens EIB (Indman and O'Byrne 1996), and therefore cannot be recommended. Daily use of β_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). β_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 cromoglycate 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 β_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 β_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 β_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, Würzburg, 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 (FEV₁) occurred, and the dose causing a 20% fall in (FEV₁) was determined as PD₂₀ FEV₁. Subjects with PD₂₀ FEV₁ ≤ 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 PD₂₀ 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. FEV₁ 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, alopecurus, 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 to 10 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 -70°C 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 (20°C) 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 to 10 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°C for 20 min, then exposed for 30 min to normal rabbit serum (dilution 1:10) and then to primary mAbs overnight at +4°C and processed for the avidin-biotin-peroxidase method in accordance with manufacturer's instructions (Vector Laboratories, Burlingame, CA, USA). Negative controls 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 -20°C 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, West 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 Kruskal-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 Kruskal-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 β_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 with 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 \pm SD)	1.25 μ g ^a \pm 0.44	0.06 mg \pm 0.15 ^b	0.13 mg \pm 0.1 ^b	N.A.
FEV ₁ % of predicted \pm SD	99.2 \pm 2.1	95.7 \pm 3.22	80.9 \pm 11.7	109.1 \pm 4.52
FEV ₁ reversibility in % \pm SD	N.A.	29.34 \pm 17.4	11.2 \pm 8.8	5.39 \pm 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 ^aPD₂₀FEV₁ to methacholine and ^bPD₁₅FEV₁ to histamine. ^c25 of these subjects participated in Study II

Table 5. Inflammatory cell counts as medians (interquartal range) in cells/mm² 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.

^adata from 37 skiers, ^bdata from 71 subjects, ^cdata 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) μ m, $P < 0.001$] and in mild asthmatics [8.8 (IQR 7.2-10.8) μ m, $P < 0.001$] than in controls [0.8 (IQR 0-3.1) μ m] 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 μ m (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.

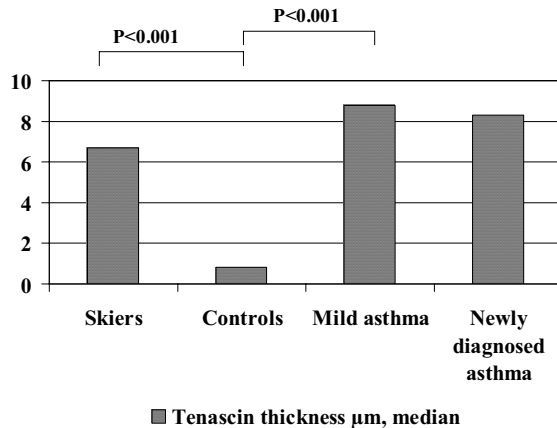


Figure 1. Tenascin immunoreactivity band thickness in the bronchial basement membrane (median).

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 PD₁₅FEV₁ 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.

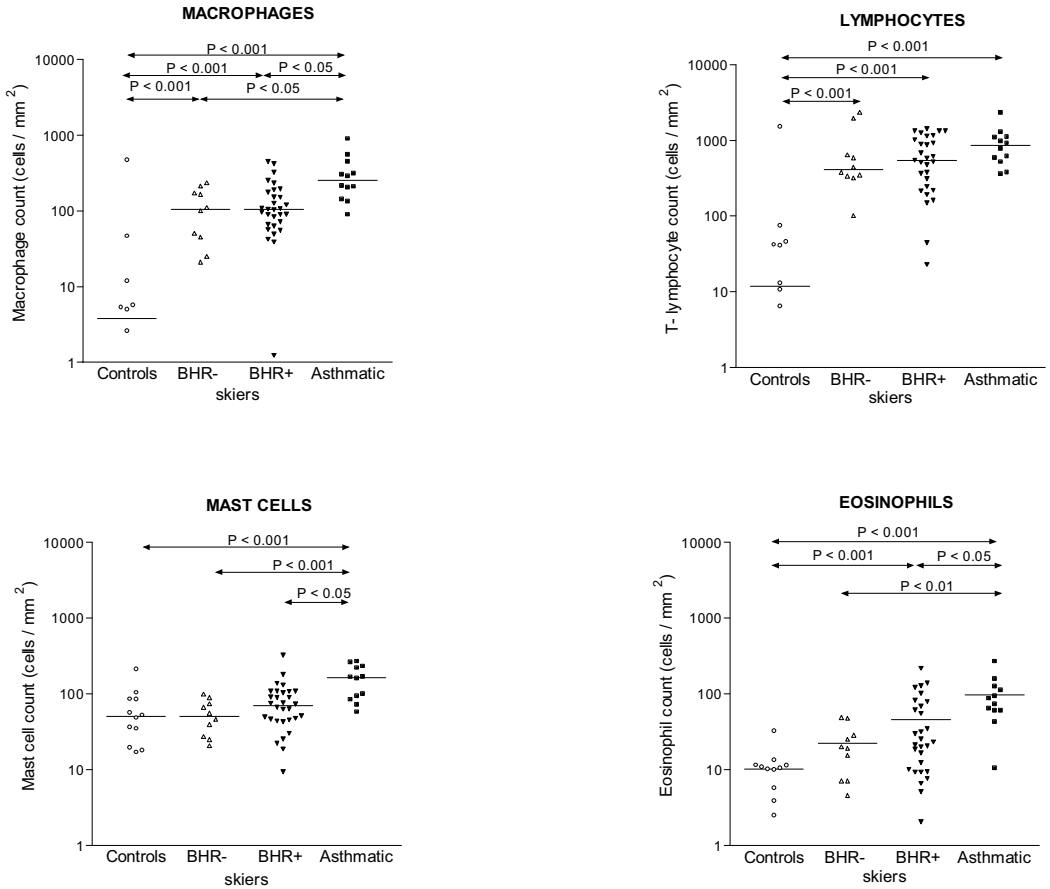


Figure 2. Density (cells/mm²) of macrophages, T-lymphocytes, mast cells, and eosinophils in bronchial biopsy specimens from control subjects, skiers with and without BHR, and asthmatic subjects. Horizontal bar=median value. The figure is reprinted from Study I article with permission from Am J Respir 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.

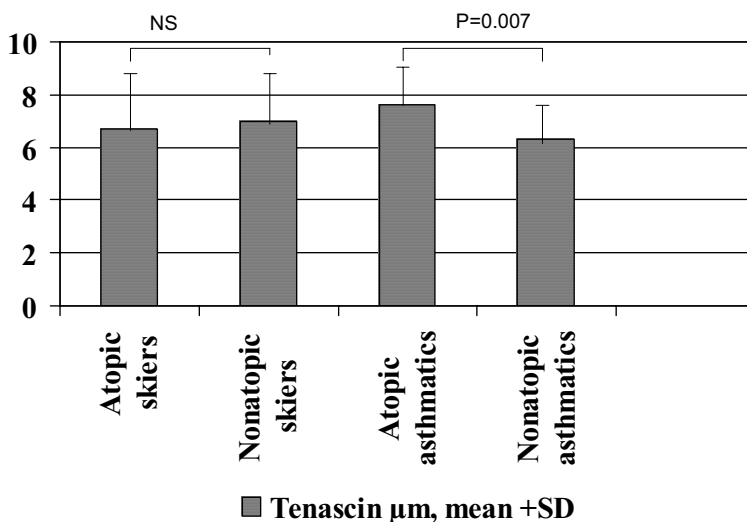


Figure 3. Tenascin immunoreactivity thickness in diagnosed the basement membrane according to atopic status in skiers and in patients with newly asthma.

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 symptoms by 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.

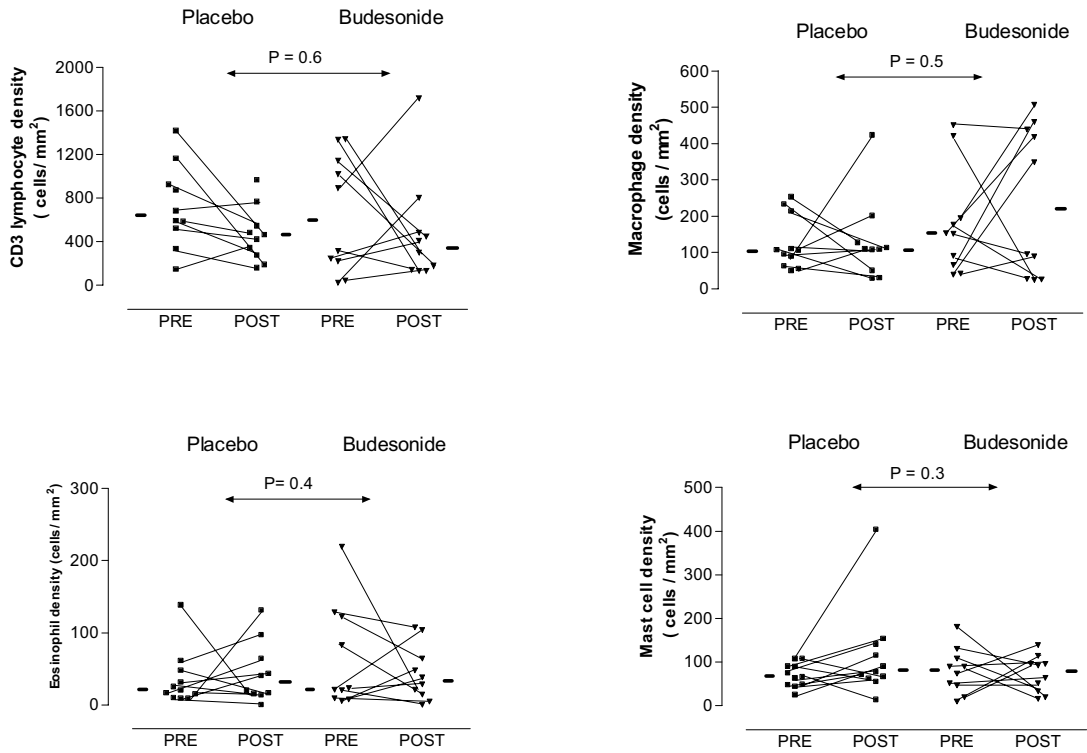


Figure 4. Inflammatory cell densities (cells/mm²) in bronchial mucosa at baseline and at follow-up. Figure reprinted from Study II article with permission of S. Karger AG, Basel.

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 from histamine 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).

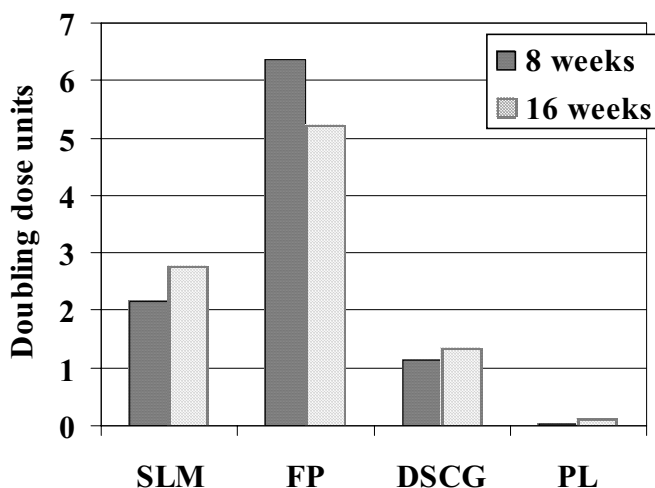


Figure 5. Doubling dose improvement from histamine at weeks 8 and 16 in salmeterol (SLM), fluticasone propionate (FP), disodium cromoglycate (DSCG), and placebo (PL).

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/mm², CI -113 to -29 , $P=0.002$). FP also reduced CD3-positive T-lymphocytes, but this change did not reach significance (change -274 cells/mm², CI -587 to 40 , $P=0.060$). DSCG also attenuated eosinophilic inflammation by reducing eosinophil count significantly (change -54 cells/mm², CI -127 to 18 , $P=0.048$). DSCG had no significant effect on CD3-positive lymphocytes (change -182 cells/mm², CI 408 to 44 , $P=0.248$). No changes occurred in any of the groups for mast cells or neutrophils (Table 7).

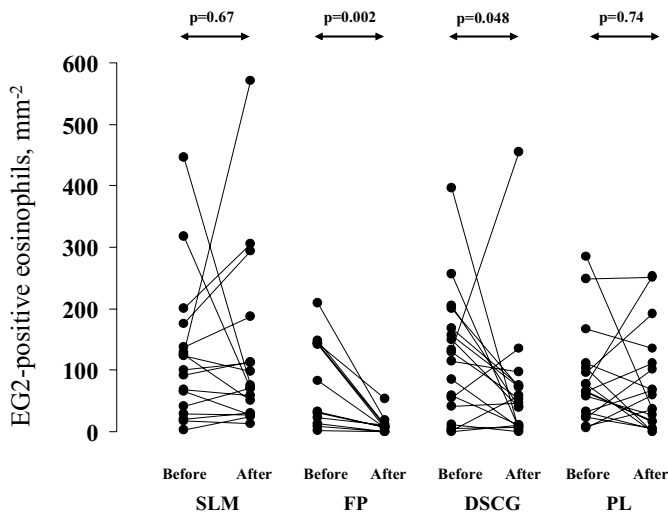


Figure 6. Number of EG2-positive eosinophils in bronchial mucosa at baseline and after a 16-week treatment with salmeterol (SLM), fluticasone propionate (FP), disodium cromoglycate (DCG), and placebo (PL). The figure is reprinted from Study IV article with permission from Elsevier.

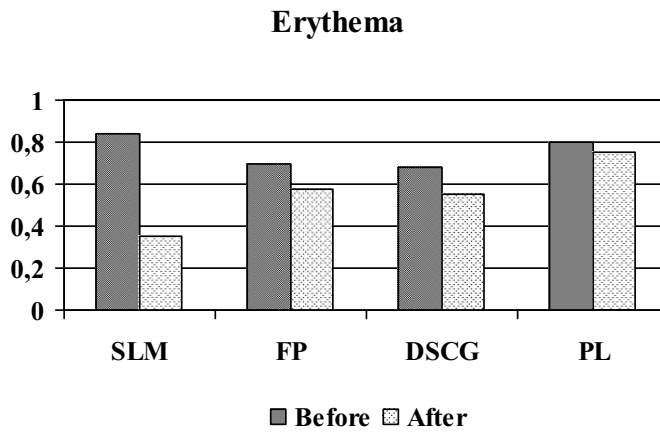
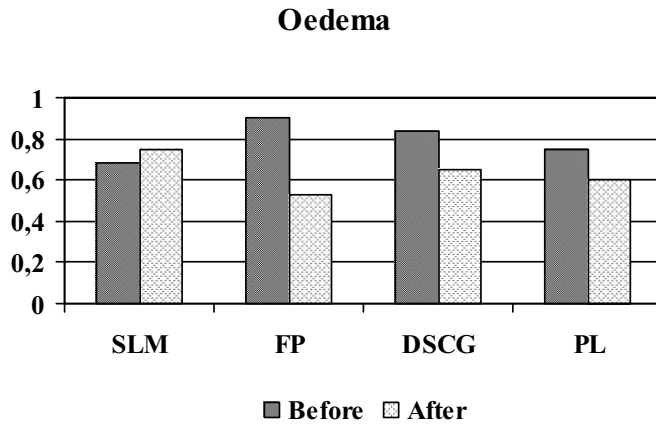
Table 7. Inflammatory cell counts (cells/mm²) in the bronchial biopsy specimens before and after treatments.

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

Results are given as mean (with standard deviation). AA1 = mast cells, CD3 = T-lymphocytes, EG2 = activated eosinophils, ELA = neutrophils are presented. ^aP=0.002, ^bP=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).



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 Mastrorarde 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 nonathlete 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 skiers 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 β 2-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 status. 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- β 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- β can be found at increased levels in the BAL fluid of asthmatic patients (Redington et al 1998). TGF- β , 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 FEV₁ 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 a dose of 800 μ g daily is modest (Currie et al 2003). We found no consistent effect of budesonide 400 μ g 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 β_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 β_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 β_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 al 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 β_2 -agonists on airway inflammation has not been ruled out. β_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 β_2 -agonists isoproterenol and SLM blocked corticosteroid-induced eosinophil apoptosis (Nielson and Hadjokas 1998). Regular treatment with the short-acting β_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 β_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 β_2 -agonist albuterol reduced Tn expression. Our long-acting β_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- β -positive eosinophils (Flood-Page et al 2003). This confirms that TGF- β 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 PD₂₀FEV₁ 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 monophosphate (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 levels 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 physical 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 FEV₁ 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 β_2 -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.

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