Symptoms and aspects on eosinophil activity in allergic rhinitis

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Front cover: *blue anemone hepatica*,
…in memory of my mother.

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II. Ahlström Emanuelsson C, Greiff L, Andersson M, Persson CG, Erjefält JS. Eosinophil degranulation status in allergic rhinitis: observations before and during seasonal allergen exposure. European Respiratory Journal 2004; 24: 750-57.

III. Ahlström Emanuelsson C, Andersson M, Persson CG, Thorsson L, Greiff L. Effects of topical formoterol alone and in combination with budesonide in a pollen season model of allergic rhinitis. Respiratory Medicine 2007; 101: 1106-12.

IV. Uller L, Ahlström Emanuelsson C, Andersson M, Erjefält JS, Greiff L, Persson CG. Early phase resolution of mucosal eosinophilic inflammation in allergic rhinitis. Manuscript.

Abbreviations

CCL5:	CC-chemokine L5 (RANTES)
CCL11:	CC-chemokine L11 (eotaxin)
Cfegs:	Clusters of free eosinophil granules
ECP:	Eosinophil cationic protein
EDN:	Eosinophil derived neurotoxin
EPO:	Eosinophil peroxidase
GM-CSF:	Granulocyte macrophage colony stimulating factor
IL:	Interleukin
IOD:	Integrated optical density
LTB4:	Leukotriene B4
MBP:	Major basic protein
PIF:	Peak inspiratory flow
PMD:	Piecemeal degranulation
PMDi:	Piecemeal degranulation index
SEM:	Standard error of the mean
SD:	Standard deviation
TEM:	Transmission electron microscopy
TNSS:	Total nasal symptom score
VAS:	Visual analogue scale

Introduction

Allergic rhinitis is a major atopic condition. For example, in southern Sweden the prevalence is 15% and increasing by 0.6% per year (Nihlén et al. 2006). There are good treatments available for allergic rhinitis, including antihistamines and topical corticosteroids (Bousquet et al. 2003). Yet, there is a need for new treatment options, particularly for such aiming at new targets and for such associated with reduced side effects.

In allergic rhinitis, treatments may not be compared accurately at seasonal allergen exposure. Onset, intensity, and duration of pollen exposure are unpredictable. This, and a variable sensitivity to allergen between patients, makes studies of crossover design difficult to conduct. Instead, one may resort to controlled allergen challenges, and repeated challenges may be useful in this context (Andersson et al. 2000). This needs to be confirmed and indices of airway inflammation need to be explored in such models.

Eosinophils are generally thought to be pathogenic in allergic disorders. Disappointing results from asthma trials, focusing on a therapeutic approach aiming at the eosinophil component of the disease, have questioned this view (Leckie et al. 2000, Kips et al. 2003). However, a basic prerequisite for a pathogenic role of eosinophils is that they degranulate in diseased tissues. Therefore, it is of interest that degranulation varies between different diseases (Erjefält et al. 2001). Eosinophil degranulation now warrants further exploration in allergic rhinitis focusing on its relation to allergen exposure.

A series of observations suggest the possibility that $ß_2$-agonists are potential treatment options for allergic rhinitis (Svensson et al. 1995a, Proud et al. 1998). Focusing on a potential clinical efficacy of this class of drugs, studies employing allergen-challenges have shown reductions in acutely induced nasal symptoms by high doses of nasal $ß_2$-agonists (Borum & Mygind 1980, Svensson et al. 1995a). However, negative studies are also available (Holt et al. 2000) and effects of $ß_2$-agonists in allergic rhinitis need to be further evaluated.

The knowledge of treatment effects of corticosteroids in allergic rhinitis is based on studies where drugs usually have been given prophylactically as a pre-treatment. However, this approach may fail to reveal significant aspects of corticosteroid actions including differences in corticosteroid sensitivity. There is a

need for studies focusing on corticosteroid interventions at established allergic inflammation and at the resolution phase of inflammation (Uller et al. 2006a). In this context corticosteroid-sensitive features may represent key parts of the inflammatory process that if interfered with separately may produce clinically relevant effects.

In the present thesis, a model employing individualized, repeated allergen challenges has been evaluated in patients with allergic rhinitis. Indices of eosinophil inflammation have been monitored in this model and at seasonal allergen exposure. The possibility to determine eosinophil activity by transmission electron microscopy (TEM) has specifically been addressed. ß$_2$-Agonist and corticosteroid interventions have been investigated, including the effect of corticosteroid treatment during the resolution phase of established allergic inflammation.

Background

Allergic rhinitis

Clinical presentation
Allergic rhinitis can exist alone or in combination with other conditions including asthma. It is characterised by sneezes, rhinorrhea, and nasal blockage and these symptoms appear at allergen exposure. The underlying disease mechanisms comprise a specific allergen-induced inflammation and its consequences to the nasal mucosa. The main treatment options available are antihistamines, corticosteroids, and specific immunotherapy.

Immunological aspects
Allergic inflammation results from exaggerated immune responses to external factors (i.e., allergen) (Howarth 1995). An important part of the response includes the interaction between antigen presenting dendritic cells and Th2-lymphocytes. As a result of this cellular cross talk, specific cytokines are produced, e.g., IL-3, IL-4, IL-5, and GM-CSF. These cytokines regulate the inflammatory response and are involved in IgE synthesis and in eosinophil recruitment and survival.

Mast cell activity
One of the immediate allergic features is the interaction between allergen and specific IgE on the surface of mast cells. This triggers mast cell activation and induces release of histamine, tryptase, and other potent mediators (e.g., leukotrienes, prostaglandins). While histamine is a key mediator of the acute response to allergen, others may have more sustained effects (Pawankar et al. 2003). For experimental purposes, tryptase is an accurate marker of mast cell activity in allergic rhinitis (Castells & Schwartz 1988).

Granulocyte activity
Tissue infiltration of activated eosinophils is a hallmark of allergic inflammation (Busse et al. 1994). Increased numbers of eosinophils in the nasal mucosa, and increased levels of eosinophil products including ECP, characterize allergic rhinitis (Linder et al. 1987, Svensson et al. 1990). Acute allergen exposure recruits neutrophils in allergic rhinitis (Freeland et al. 1989, Fransson et al. 2004), but major release of neutrophil mediators may not occur at seasonal allergen exposure (Linder et al. 1987, Wang et al. 1996, Ahlström Emanuelsson et al. unpubl.).

End-organ responses
In allergic rhinitis, end organs of the nasal mucosa, i.e., microvasculature, glands, and nerves, react to the inflammatory activity produced by allergen exposure. The microvasculature responds with vaso-dilatation, increased blood flow, and plasma exudation (e.g., Svensson et al. 1990). Glands respond with increased secretion (Raphael et al. 1991) and nerves with increased signalling (Sarin et al. 2006).

Plasma exudation is reflected by increased levels of plasma proteins on the mucosal surface, including α_2-macroglobulin (mol. wt. 725 kDal) (Svensson et al. 1995b, Greiff et al. 2002). In allergic rhinitis, the levels of plasma proteins in mucosal surface liquids, which can be sampled by nasal lavages, may reflect the intensity of an on-going inflammation (Persson et al. 1998).

Plasma exudation implies a dramatic change to the molecular environment during an inflammatory response (Persson et al. 1998). The extravsation and flux of plasma into the nasal lumen affects the luminal entry of cellular products from the tissue compartment (Meyer et al. 1999). In the present thesis (II, III), this measure was used experimentally to improve the recovery of tissue-derived mediators in luminal samples.

Airway end organs are often hyperresponsive in allergic rhinitis and asthma. The response to cholinergic agonists and sensory nerve stimuli may be increased (Klementsson et al. 1991, Greiff et al. 1995a, Kowalski et al. 1999). Also, the ability of histamine to produce plasma exudation is heightened in on-going allergic rhinitis, i.e., an exudative hyperresponsiveness (Svensson et al. 1995c).

Nasal allergen challenge models

Seasonal allergen exposure
Allergic rhinitis can readily be examined at natural allergen exposure. Hence, key features of allergic inflammation have been revealed in clinical studies, reflecting the accessibility of the nasal airway and the degree of safety by which experimental studies can be undertaken (e.g., II). However, with regard to pharmacological intervention, studies are hampered by the variable onset, intensity, and duration of natural pollen exposure. Therefore, it is difficult to implement crossover studies and parallel-group designs are associated with inferior power to detect treatment specific changes. Accordingly, even large-scale trials have failed to demonstrate dose-dependent effects on total nasal symptoms for such a key class of pharmaceuticals as topical corticosteroids (Bronsky et al. 1997, Stern et al. 1997, Meltzer 1998).

"Peak season" and "a day in the park"

In order to overcome the problem with variable allergen exposure, it has been suggested that only the peak of the pollen season should be evaluated (Bernstein et al. 1997) or that only days with high pollen counts might be used (Stern et al. 1997). A third alternative is a model usually referred to as "a day in the park": Subjects are studied in a defined environment (usually a park) and symptoms are scored for one or two days (Meltzer et al. 1996). However, again, these models are difficult to use when exploring pharmacological effects since they are hard to combine with crossover design interventions and since the study period is short.

Table I. Test models for allergic rhinitis. "Pros" and "Cons" focusing on control of allergen exposure and possibility to carry out studies of crossover design.

	Pros	**Cons**
Acute challenge	Controlled dose. Crossover design.	Does not mimic sustained allergic inflammation.
Repeated challenge	Controlled dose. Crossover design.	Under evaluation as test model for allergic rhinitis.
Pollen chamber	Controlled exposure. Crossover design.	Low throughput. Labour intensive.
Day in the park	"Controlled" exposure. Natural exposure.	Limited study period. Parallel group design.
Seasonal exposure	Natural exposure.	High degree of variability. Parallel group design.

Pollen chamber

Intermediate between natural pollen exposure and the use of allergen challenges (in the laboratory) is the use of a "pollen chamber". This is a unit in which air is circulated and where standardized levels of allergen can be administered and monitored. Under these circumstances, allergen exposure is more natural than at challenges carried out using nasal spray administrations of allergen dissolved in diluent. Furthermore, natural exposure can be mimicked by repeated exposures for set periods of time. Placebo-controlled studies evaluating onset of action, efficacy, and safety of pharmaceuticals can be carried out (Day et al. 1997) and different treatments can be compared (Stübner et al. 2004). The disadvantage is that the model is labour intensive and that the provocation time is relatively limited.

Acute allergen provocation
Reflecting the accessibility of the nasal airway, acute allergen challenge models have frequently been employed to study allergic rhinitis and important information on the immunology, pathophysiology, and pharmacology of allergic inflammation has been generated (Naclerio et al. 1983, Greiff et al. 1995b). Such models permit accurate administration of allergen and high-power crossover designs. However, acute allergen challenges do not produce the full spectrum of allergic airway inflammation. Accordingly, they may be more relevant for studies of acute rather than sustained/chronic allergic inflammation and symptoms.

Repeated allergen provocation
In search for models that would mimic ongoing allergic rhinitis more completely, repeated allergen challenges have been employed. One possibility is to use a low daily dose of allergen for a number of days (about 1% of the dose used in acute challenge experiments) (Roquet et al. (1996). While not producing symptoms, allergic inflammation was induced in this model, reflected by increased nasal lavage fluid levels of ECP. Another possibility is to use a high and fixed daily dose (for seven days) and monitor acute symptoms following challenge. Schmidt et al. (2001) showed that this resulted in nasal symptoms, but no information was given whether or not the challenges produced sustained symptoms.

An additional possibility is to employ repeated challenges with individualized symptom-producing yet tolerable doses of allergen. Preliminary observations indicate that such challenge procedures evoke around-the-clock symptoms mimicking those experienced at seasonal pollen exposure (Andersson et al. 2000). The accuracy by which differences in treatment potency may be detected in this model is suggested by a report on dose-dependent, symptom-reducing effects of a topical corticosteroid (Andersson et al. 2000). However, while the model seems promising, it needs to be evaluated further.

Eosinophil activity

Pathophysiological presentation
Tissue accumulation of eosinophils is a characteristic feature of allergic diseases (Reed 1994, Rothenberg 1998). In agreement, increased numbers of eosinophils in biopsies of the nasal mucosa have been demonstrated in patients with allergic rhinitis compared with healthy individuals (Togias et al. 1988). Furthermore, increased numbers of eosinophils have been shown in nasal mucosal surface liquids and in blood in this condition (Klementsson et al. 1991, Kimura et al. 1999).

CC-chemokines

Chemokines are chemotactic proteins grouped into different families depending on positions of four cystein residues (Baysal & Atilgan 2001). Specifically chemotactic for eosinophils are CC-chemokines, and they are likely involved in the pathogenesis of allergic rhinitis and asthma (Holgate et al. 1997, Baraniuk et al. 1997, Lukacs et al. 2001, Pullerits et al. 2000, Greiff et al. 2001, Lloyd 2002). CCL5 and CCL11 are such CC-chemokines.. CCL5 may be of particular interest since it is very sensitive to corticosteroid treatment (Uller et al. 2006a).

Recruitment of eosinophils

Eosinophil recruitment involves: (i) Differentiation and maturation of the eosinophil, (ii) interaction between the eosinophil and endothelial cells, i.e., rolling, adhesion, and transendothelial migration, and (iii) local chemotaxis in the airway tissue (Resnick & Weller 1993). Maturation and release of the eosinophil from the bone marrow into the peripheral circulation is stimulated by cytokines including IL-5 and GM-CSF (Gleich et al. 1993). CCL5 and CCL11 are involved in chemotaxis to the site of inflammation (Collins et al. 1995, Rothenberg 1998).

Eosinophil products

The eosinophil contains specific granules that give it its characteristic appearance with a dense crystaline core surrounded by an outer matrix (Kautz & Demarsh 1954). The secondary granules of eosinophils contain tissue-toxic proteins including ECP, MBP, EPO, and EDN (Egesten & Alumets 1986, Peters et al. 1986). At eosinophil activation, these proteins are released and measurable in nasal lavage fluids (Svensson et al. 1990, Meyer et al. 1999, Marcucci et al. 2001).

Histological eosinophil activation markers

Immunostaining using monoclonal antibodies to eosinophil cationic protein may not distinguish between resting and activated eosinophils (Jahnsen et al. 1995). Instead, to identify and quantify different modes of degranulation of the eosinophil, ultrastructural analysis by TEM has been introduced (Erjefält et al. 1998, Erjefält & Persson 2000). The degree of eosinophil degranulation in allergic rhinitis and its correlation to allergen exposure remains to be examined.

Activation/elimination of eosinophils

Exocytosis, apoptosis, piecemeal degranulation, and cytolysis are eosinophil activation modes (Table II) (Erjefält & Persson 2000). Experimental observations suggest that piecemeal degranulation and cytolysis are the key activation mechanisms in allergic airway disease (Greiff et al. 1998, Erjefält et al. 1998). Elimination of eosinophils from the tissue may include luminal entry of cells and subsequent final clearance by apoptosis and/or mucociliary clearance (Erjefält & Persson 2000, Uller et al. 2001).

Eosinophil apoptosis

Apoptosis is programmed cell death that allows for removal of cells by phagocytosis. It is characterized by presence of electron-dense, condensed chromatin, a preserved plasma membrane, and non-dilated organelles (Majno & Joris 1995). In vitro studies suggest that eosinophilic airway inflammation may be resolved through eosinophil apoptosis (Druilhe et al. 1996, Haslett et al. 1999, Vignola et al. 2000). In agreement, sputum obtained from asthmatic patients following allergen challenge contains apoptotic eosinophils (Foresi et al. 2000). However, biopsy observations have so far failed to show occurrence of eosinophil apoptosis in allergic airway disease (Uller et al. 2004, 2006a, 2006b).

Table II. Modes of eosinophil activation.

Apoptosis	Cytolysis	PMD
Electron dense, condensed chromatin	Chromatolysis	Ragged loss of core material
Preserved plasma membrane	Loss of plasma membrane integrity	Loss or coarsening of granular matrix
Non-dilated cellular organelles	Release of membrane bound granules	More or less empty granule in an intact cell

PMD: Piecemeal degranulation.

Eosinophil cytolysis

Eosinophil cytolysis is characterized by chromatolysis, loss of plasma membrane integrity, and release of membrane-bound specific granules (Persson & Erjefält 1997). Eosinophil cytolysis, which occurs both in vitro and in vivo, can be quantified by counting "clusters of free eosinophil granules" (Cfegs) (Weiler et al. 1996, Greiff et al. 1998, Erjefält et al. 1999). In allergic rhinitis, generation of Cfegs is a significant feature that may represent ultimate activation of nasal mucosal eosinophils (Greiff et al. 1998).

Piecemeal degranulation (PMD)

PMD is characterized by a ragged loss of core material, loss or coarsening of the granular matrix, and more or less empty granules in otherwise intact cells (Fig. 1). PMD can be quantified by TEM by determining the percentage of granules displaying morphological signs of protein release. Each specific granule is evaluated and classified as either intact or activated. Thus, an index reflecting

piecemeal degranulation (PMDi) can be calculated for any given tissue sample (Erjefält et al. 1998, Erjefält & Persson 2000).

Current aspects

When evaluating the eosinophil as treatment target in allergic airway inflammation it may be important to consider the use of TEM. This technique, in combination with relevant immunohistochemistry, has the potential to allow for accurate monitoring of eosinophil activity. This may be of particular importance since eosinophil degranulation has been shown to vary markedly between different eosinophilic conditions without any clear correlation to total tissue eosinophil numbers (Erjefält et al. 2001).

When Study II was conducted, no information was available on how the eosinophil degranulation status of eosinophils (focusing on PMD) was affected by sustained allergen exposure. Furthermore, it was unknown to what degree eosinophil degranulation correlated to other indices of eosinophil activity, such as eosinophil numbers and nasal mucosal surface liquids levels of ECP. Consequently, these aspects were the focus of Study II.

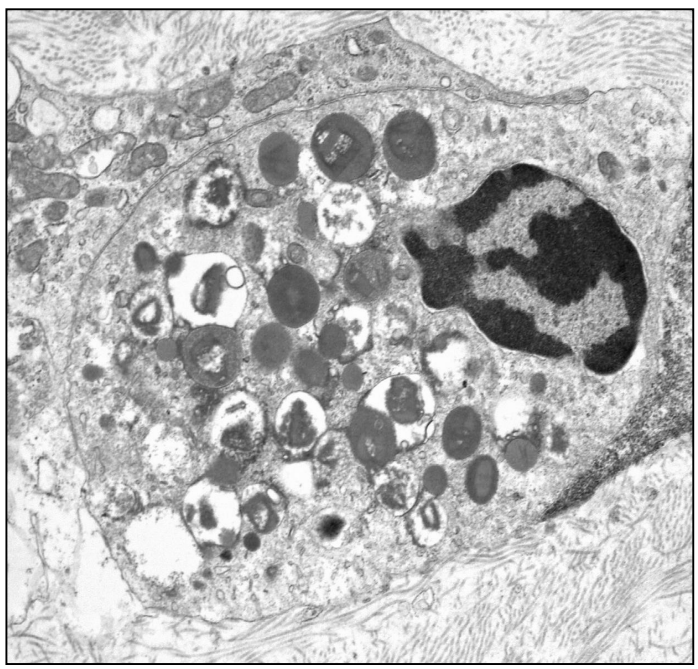

Fig. 1. A TEM micrograph demonstrating a single eosinophil undergoing typical piecemeal degranulation (II), displayed as coarsening of the granule content.

ß$_2$-Agonists and allergic rhinitis

Experimental observations

ß$_2$-Agonists exert effects that may be characterized as anti-inflammatory. Original observations with terbutaline (Persson et al. 1986), and later formoterol (Erjefält et al. 1991, Tokuyama et al. 1991, Baluk & McDonald 1994), demonstrated that these pharmaceuticals inhibited plasma exudation produced by inflammatory stimuli. Later, studies involving the human nasal airway showed similar results (Svensson et al. 1995a, Proud et al. 1998, Parameswaran et al. 2006). Furthermore, experiments in vitro involving short and long acting ß$_2$-agonists demonstrated that these pharmaceuticals were effective in reducing mast cell histamine release (Nials et al. 1994, Chong et al. 1998). Together, these observations suggest that ß$_2$-agonists may exert an anti-inflammatory action in allergic rhinitis.

Clinical efficacy

In asthma, ß$_2$-agonists have very potent effects, reflecting their bronchodilator capacity (Barnes et al. 2002). Focusing on a potential clinical efficacy of these pharmaceuticals in allergic inflammation, studies employing allergen-challenges have demonstrated reductions in acutely induced nasal symptoms by high doses of the ß$_2$-agonists fenoterol and terbutaline (Borum & Mygind 1980, Svensson et al. 1995a). However, negative studies are also available (Svensson 1982, Holt et al. 2000). For example, in patients with allergic rhinitis examined at seasonal allergen exposure, topical formoterol once daily for one week was reported to fail to affect symptoms of allergic rhinitis compared with placebo (Holt et al. 2000). Further studies are warranted in this field.

Current aspects

Whereas ß$_2$-agonists may have no or only marginal effects on symptoms of allergic rhinitis, little is known about whether or not they add to the efficacy of anti-allergy drugs. In this context, ß$_2$-agonists may increase the expression of corticosteroid receptors (Eickelberg et al. 1999). Conversely, it has been reported that corticosteroids increase the expression of ß$_2$-receptors in the nasal mucosa (Baraniuk et al. 1997) and restore down-regulated ß$_2$-receptors seen in patients on regular treatment with ß$_2$-agonists (Mak et al. 1995). Accordingly, it may be hypothesized that a ß$_2$-agonist in combination with an intranasal corticosteroid may increase the potency of the corticosteroid and possibly improve the clinical efficacy (Barnes 2002).

Corticosteroids and allergic inflammation

Clinical effects

A topical corticosteroid is the most effective treatment for allergic rhinitis and asthma available and it is now first line treatment for adults with allergic rhinitis (Bousquet et al. 2003, Mullol et al. 2008). Its effect reflects actions on a range of cellular levels, which overall have a broad suppressive effect on inflammatory processes. In contrast, innate defence mechanisms, including LTB4 generation, neutrophil actions, microvascular responses, and epithelial restitution are less affected (Freeland et al. 1989, Greiff et al. 1997, Erjefält et al. 1995). This profile is beneficial when corticosteroids are used in the treatment of allergic rhinitis and asthma. Furthermore, they are usually given topically, which reduces systemic side effects to very low levels.

Gene transcription

Corticosteroids interfere with gene expression and protein synthesis through binding to the glucocorticoid receptor, resulting in altered gene transcription. Consequently, production of many pro-inflammatory cytokines is down regulated and production of anti-inflammatory molecules is up regulated (Barnes & Adcock 1993, Schleimer & Bochner 2004). For example, production of Th2 cytokines and CC-chemokines associated with allergic inflammation is typically reduced by corticosteroids. In agreement, topical corticosteroid treatment of the human nasal airway reduces the mucosal output of IL-5, GM-CSF, CCL5, and CCL11 in allergic rhinitis (Weido et al. 1996, Linden et al. 2000, Greiff et al. 2001, Erin et al. 2005). Parallel studies in animal models are readily available (e.g., Shen et al. 2002, Eum et al. 2003). Notably, corticosteroid intervention has often been given well in advance of allergen exposure.

Effects on eosinophils

Eosinophil production in the bone marrow as well as recruitment of eosinophils from the blood to the airway tissue is inhibited by corticosteroid treatment (Gauvreau et al. 2000, Shen et al. 2002). Furthermore, corticosteroids attenuate allergen-induced activation of eosinophils in allergic rhinitis and asthma (Klementsson et al. 1991, Gauvreau et al. 1996). Many cytokines, e.g., IL-5 and GM-CSF, inhibit eosinophil apoptosis in vitro (Tai et al. 1991). Since corticosteroid treatment decreases the levels of IL5 and GM-CSF in allergic inflammation (Linden et al. 2000, Walsh et al. 2003), it may theoretically induce eosinophil apoptosis. However, convincing demonstrations of eosinophil apoptosis in diseased tissues are lacking and whether corticosteroid induced eosinophil apoptosis actually occurs in allergic rhinitis is unknown.

End-organ responses and responsiveness

Corticosteroids reduce end-organ responses associated with allergic inflammation. For example, the plasma exudation response is attenuated (Pipkorn et al. 1987, Svensson et al. 1994), which likely is secondary to actions on inflammatory cells rather than a direct microvascular anti-permeability effect (Greiff et al. 1997). The increased responsiveness to topical challenges, which characterises allergic rhinitis and asthma, is also sensitive to corticosteroid treatment. In previous studies, the exudative responsiveness, i.e., the ability of the mucosa to respond to histamine with plasma exudation, has been studied in patients and demonstrated to be particularly corticosteroid sensitive (Meyer et al. 2003). In the present Study II and III, this aspect of allergic inflammation was monitored.

Current aspects

While studies on allergic animals and humans demonstrate very broad anti-inflammatory effects of corticosteroids, these observations have largely been generated in situations where the treatment has been given prior to allergen exposure, i.e., prophylactic treatment. In contrast, recent observations in animals suggest that corticosteroids given to airways with established allergic inflammation may not exhibit the same wide range of effects (Uller et al. 2006a). Thus, in a study designed to examine this type of action of corticosteroids, prophylactic treatment inhibited allergen challenge-induced up-regulation of CCL5 and CCL11 along with several other CC-chemokines whereas the same dose administered when allergic inflammation was established only affected one chemokine, i.e., CCL5 (Uller et al. 2006a). Importantly, this effect was associated with resolution of allergic inflammation. The finding suggests that an anti-CCL5 action is involved in the therapeutic effect of corticosteroids and that CCL5 may be viewed as a specific treatment target. However, it is not known whether or not early corticosteroid-induced resolution of allergic airway inflammation involves a specific reduction of CCL5 in human airways.

Clinical methods and methodological considerations

Allergen titration procedure

A key feature of the present allergen challenge model was the use of individually selected doses of birch and grass pollen allergen (I, III, IV). These were chosen based on the results of a careful titration procedure. Increasing doses of allergen, i.e., 100, 300, 1.000, and 3.000 SQ units per nasal cavity (Aquagen, ALK-Abelló, Hørsholm, Denmark), were administered at ten-minute intervals using a spray-device delivering 100 µL per actuation. The scheme was followed until the subject responded with at least five sneezes or recorded a symptom score of two or more on a scale from zero to three for either nasal secretion or blockage (below). The dose that produced this effect was chosen for the allergen challenge series and was given once daily for seven days. In the present studies (I, III, IV), repeated administrations of the chosen dose produced significant yet tolerable symptoms.

Symptom registration

Nasal symptoms were registered using a graded scale. In study I, III and, IV, morning and evening registration reflected the preceding twelve hours and in study II each registration reflected the last 24 hours. The symptoms blocked nose, runny nose, and the most prominent of itchy nose or sneezes were each scored on a 4-graded scale: 0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms, 3 = severe symptoms. In the titration experiments (carried out to determine individual allergen sensitivity) and for recordings ten minutes following allergen challenge, the number of sneezes were translated into a score: 0 = 0 sneezes, 1 = 1-4 sneezes, 2 = 5-9 sneezes, 3 = 10 or more sneezes. The recordings were added to a total nasal symptom score (TNSS) ranging from 0 to 9, for post challenge, morning, and evening recordings, respectively.

An alternative to the present graded score would have been to use a visual analogue scale (VAS). The advantage of a VAS is that the variable is continuous and that the methodology is validated (Bousquet et al. 2007). However, graded scales have also been used frequently, and it was the method used by Andersson et al. (2000) in the work that preceded the present series of studies. In Study I and III, as well as in later work (Korsgren et al. 2007, Widegren et al. 2009), the graded scale (as outlined above), and the three days' evaluation period (I, III), has resulted in stable recordings and interpretable results. Notably, the levels of symptoms

reached post challenge as well as in the morning and evening have been very stable between studies.

Nasal PIF

Nasal peak inspiratory flow (PIF) was measured using a flow meter (Clement-Clark Int., Harlow, UK) equipped with a facial mask. At each occasion, the highest of three measurements was registered. An alternative to nasal PIF measurements would have been to use rhinomanometry (Ciprandi et al. 2005). This technique gives reliable readings that correspond very well with nasal obstruction, but it is labour intensive and has to be carried out at the clinic. In contrast, the present nasal PIF meter can be managed by the test subjects themselves and can be used at home, such as for the morning and evening recordings in the present studies (I, III). The nasal PIF technique is well validated (Holmström et al. 1990, Hellgren et al. 1997), and in the present studies changes in nasal PIF correlated well with changes in nasal blockage (data not shown).

Nasal lavage

In Studies II-IV, a pool device was used to lavage the nasal mucosa: A compressible plastic container equipped with a nasal adapter (Greiff et al. 1990). The adapter was inserted into one of the nostrils and the container was compressed while the subject was leaning forward in a 60° flexed neck position. The nasal pool fluid (i.e., saline) was then instilled in one of the nasal cavities and was kept there for a certain time. When the pressure on the device was released the fluid returned into the container. The lavage fluids were centrifuged at 325g for 10 min at 4°C and samples were obtained from supernatant and frozen awaiting analysis.

Alternative techniques to obtain nasal mucosal surface liquids would have been filter papers, low volume lavages, head back lavages etc. (Naclerio et al. 1983, Erin et al. 2005, Message et al. 2008). The advantage with the present technique is that the lavage fluid reaches a large surface are, it can be used to bring defined concentrations of solutes in contact with the mucosa, and it can maintain the fluid in contact with the mucosa for an extended period of time. Furthermore, it is easy to operate and can be managed by the study subjects themselves. A disadvantage is that a high volume is used (usually 15 mL) and that cytokines, mediators etc. present in low concentrations may escape detection. In Study III this was reflected by a need to concentrate the lavage fluids in order to monitor tryptase.

At some occasions (II, III), the pool device was used to carry out lavages with histamine (40 and 400 μg/mL), reflecting its versatility. The rationale was that histamine produces stable plasma exudation responses and that this process, through its flux bulk plasma with specific binding proteins (Peterson & Venge 1987), might rinse the extracellular space and facilitate luminal entry of

inflammatory mediators (Persson et al. 1998). Previous studies suggest that such "induced exudation" or "lamina propria lavage" may be useful in monitoring allergic airway inflammation (Persson et al. 1998). In Study III, this was suggested by the observation that corticosteroid-induced reductions in ECP and tryptase were more evident in histamine lavages than in saline lavages.

Nasal biopsies

Biopsies were obtained from the inferior turbinates. Topical anaesthesia and mucosal decongestion was achieved using tetracain (20 mg/mL) and adrenalin (0.1 mg/mL) delivered first by a spray device and thereafter by a cotton swab. In addition, ten minutes later, a mixture of carbocain (10 mg/mL) and adrenalin (5 mg/mL) was injected into the turbinate.

Using a cutting forceps with a 3 mm drilled out punch, biopsies were taken about 5 mm from the turbinate's anterior margin. In Study II, one of the two biopsies was immediately placed in PBS buffer for later TEM analysis. The other was placed in Stephanini's fixative overnight at 4°C and processed for EPO and ECP immunohistochemistry. In Study IV, the biopsies were directly frozen in TissueTek mounting medium and stored at 80°C for later cryosectioning and histological analysis.

In the present studies (II, IV), nasal biopsies were obtained in well-defined experimental situations. A common but inferior approach would have been to use material obtained during surgical procedures. The biopsy technique was adopted from Fokkens et al. (1988), and was designed to cut out pieces of the mucosa rather than tearing the mucosa, thus minimizing the risk of mechanical artefacts.

In Study II, despite the biopsy technique used, occasional biopsies were of inferior quality, particularly in the group intended for TEM. Biopsies displaying mechanical artefacts were excluded from further analysis. Similarly, biopsies without eosinophils were, for obvious reasons, not included in the TEM analysis of eosinophil degranulation status. Biopsy exclusion and analysis was done in a blinded fashion and according to pre-determined exclusion criteria.

Aims, designs and selected results

Study I

Establishing a model of seasonal allergic rhinitis and demonstrating dose-response to a topical glucocorticosteroid. Ann Allergy Asthma Immunol 2002; 89: 159-65.

Aim

To validate an allergen challenge model in allergic rhinitis in terms of its ability to discriminate between effects on nasal symptoms of different doses of a topical corticosteroid.

Design

Thirty-eight patients with allergic rhinitis to birch or grass pollen allergen received treatment with budesonide (64 and 256 µg) and mometasone furoate (200 µg) for ten days in a placebo-controlled, double blinded, randomized, and crossover design (Table III). The washout periods were at least two weeks.

Table III. Study scheme. The scheme indicates one of four treatment/challenge periods.

	Study day									
	-2	-1	0	1	2	3	4	5	6	7
Treatment	X	X	X	X	X	X	X	X	X	X
Allergen				X	X	X	X	X	X	X
Evaluation period								X	X	X

After three days' treatment, individualized nasal challenges with birch or grass pollen allergen were administered once daily for seven days. Nasal symptoms were scored and nasal PIF recorded every morning and evening as well as ten

minutes post challenge. Observations during the last three days of the challenge series were used to calculate mean TNSS and mean nasal PIF for morning, evening, and post challenge observations.

Results

Thirty-six patients attended at least three of four treatment periods and were considered evaluable. Around-the-clock nasal rhinitis symptoms were produced during the evaluation period (Fig. 2). During the washout periods, symptoms returned to baseline levels.

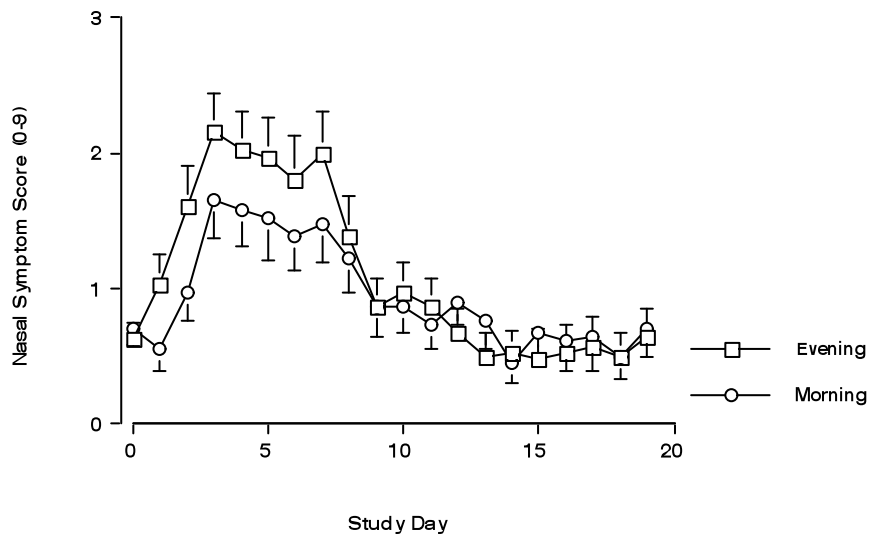

Fig. 2. Evening and morning symptoms in the placebo group (mean±SEM).

All treatments reduced symptoms and improved nasal PIF compared with placebo. The reduction in evening symptoms was significantly greater with budesonide 256 µg than with budesonide 64 µg (Fig. 3). Furthermore, the improvement in morning and post challenge nasal PIF was significantly greater with the higher dose of budesonide compared with the lower dose.

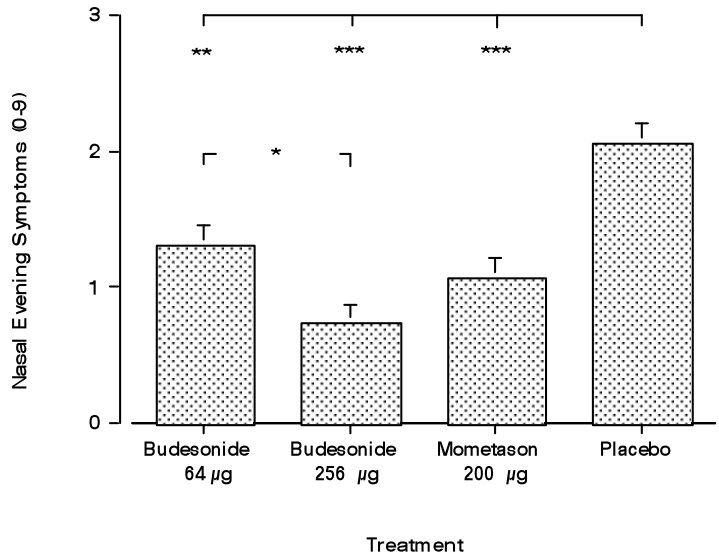

Fig. 3. Example of results showing dose-dependent reductions in mean evening TNSS during the three days' evaluation period (mean±SEM). (* Denotes $p<0.05$, ** denotes $p<0.01$, *** denotes $p<0.001$.)

Conclusions

The model was repeatable with no detectable carryover effects of the allergen challenge series. Using the model, it was possible to detect dose-dependent effects of a topical corticosteroid on total nasal symptoms of allergic rhinitis. Nasal PIF was successfully included as an objective measure. We suggest that the model is useful for comparing treatments of allergic rhinitis and be helpful in dose-finding studies for new topical corticosteroids (Ahlström Emanuelsson et al. 2004, Korsgren et al. 2007).

Study II

Eosinophil degranulation status in allergic rhinitis: observations before and during seasonal allergen exposure. Eur Respir J 2004; 24: 750-7.

Aim

To determine eosinophil activities (modes and degree of degranulation) in allergic rhinitis as presented before and during seasonal allergen exposure.

Design

Twenty-three patients with allergic rhinitis to birch pollen allergen were recruited. The history was verified by a skin-prick test. Nasal symptoms were recorded during a birch pollen season (March 18 to May 6). Nasal biopsies were obtained before and late during the season and analyzed for extracellular ECP (immuno-fluorescence microscopy), numbers of eosinophils (bright field microscopy), and degree of eosinophil degranulation (TEM). Saline nasal lavages with and without histamine (0.4 mg/mL) were performed before and three times during the season. ECP and α_2-macroglobulin were analysed as indices of eosinophil activity and plasma exudation, respectively.

Results

At allergen exposure, symptoms of allergic rhinitis were expectedly increased (Fig. 4). In parallel, there was an increase in numbers of tissue eosinophils (Fig. 5) and nasal lavage fluid levels of α_2-macroglobulin and ECP (data not shown).

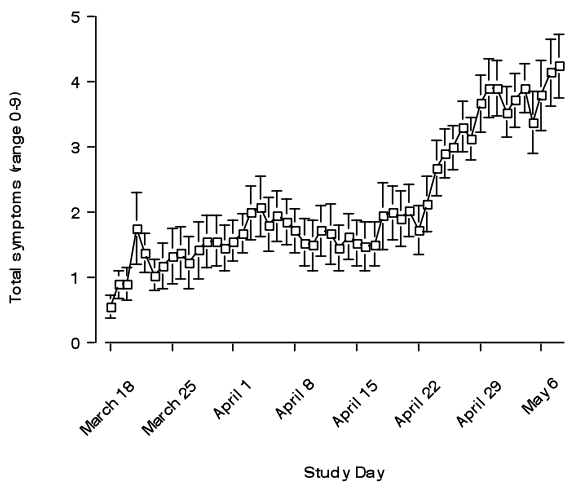

Fig. 4. Nasal symptoms of allergic rhinitis during the study period (mean±SEM). Moderate symptoms were observed when the second biopsy was obtained.

Prior to the pollen season, eosinophils were observed in 56% of the biopsies. Moreover, the biopsies featured signs of mild to moderate degranulation at this observation point, but levels of ECP were low in saline as well as histamine lavages. At seasonal allergen exposure, eosinophil numbers and eosinophil PMDi were markedly increased (Fig. 5).

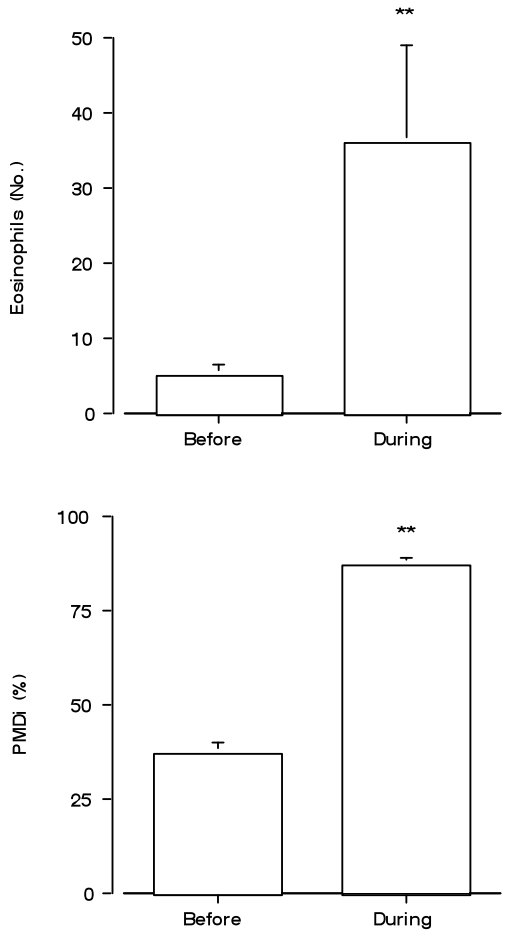

Fig. 5. Piecemeal degranulation index (PMDi) as determined by TEM analysis (lower panel) and eosinophil numbers (upper panel) in nasal mucosal biopsies obtained before and during the pollen season (mean±SEM). Eosinophil numbers refer to numbers per 0.1 mm^2. (** Denotes $p<0.01$, c.f. observation before season.)

Histamine produced plasma exudation, reflected as increased nasal lavage fluid levels of α_2-macroglobulin. This process likely moved tissue ECP into the airway lumen Accordingly, a positive correlation was observed between the levels of ECP and α_2-macroglobulin in these lavages as well as between the levels of ECP and eosinophils degranulation as assessed by TEM (Table IV).

Table IV. Result of the Spearman correlation test. Note that the PMDi correlated to ECP in histamine lavages. (* Denotes $p<0.05$.)

	ECP in saline lavage	ECP in histamine lavage
PMDi	0.479	0.629*
Eosinophil numbers	0.122	0.637*

Conclusions

Low-grade eosinophil piecemeal degranulation occurs in the nasal mucosa already outside the pollen season. However, the degree of degranulation is markedly increased at seasonal allergen exposure. The combination of elevated eosinophil numbers and increased degranulation contributes to the observed raise in extracellular cytotoxic granule proteins (ECP) during the pollen season. In support, eosinophil numbers and the degree of degranulation correlate with levels of ECP in histamine lavages. Arguably, ECP in such lavages may be a useful activity marker of tissue eosinophil activity.

Study III

Effects of topical formoterol alone and in combination with budesonide in a pollen season model of allergic rhinitis. Respir Med 2007; 101: 1106-12.

Aim

To examine whether or not a topical ß$_2$-agonist (formoterol), alone or in combination with a corticosteroid, affects symptoms and signs of allergic rhinitis.

Design

Forty patients with allergic rhinitis to birch or grass pollen were recruited. Prior to the pollen season, these subjects received treatment with formoterol (9 µg), budesonide (64 µg), and formoterol in combination with budesonide (in the same doses) in a placebo-controlled, double blinded, randomized, and crossover design (Table V). Treatments were given as one spray actuation and one inhalation per nostril in the morning.

Table V. Study scheme. The scheme indicates one of four treatment/challenge periods. Note that the nasal lavage was carried out 24 hours after the final allergen challenge.

	Study day														
	-6	-5	-4	-3	-2	-1	0	1	2	3	4	5	6	7	8
Treatment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Allergen								X	X	X	X	X	X	X	
Evaluation*												X	X	X	
Nasal lavages															X

*Regarding symptoms.

The study comprised four 15-days' treatment periods separated by at least two weeks. After seven days' treatment, individualized allergen challenges were given once daily for seven days (while the treatment continued). Nasal symptoms and nasal PIF were recorded ten and 20 minutes after allergen challenge as well as every morning and evening. Means of recordings from the last three days of each challenge period were used in the analysis. Nasal lavages with and without histamine were carried out at the end of each treatment period. Lavage fluid levels

of α_2-macroglobulin, ECP, and tryptase were measured reflecting plasma exudation, eosinophil activity, and mast cell activity, respectively.

Results

Budesonide reduced nasal symptoms of allergic rhinitis and improved nasal PIF in the morning and evening as well as post allergen challenge. Symptoms and nasal PIF were not affected by formoterol. Furthermore, formoterol did not add to the symptom reducing effects of budesonide (Table VI).

Table VI. Mean TNSS and mean nasal PIF recorded in the evening, in the morning and 20 minutes post challenge during three days' evaluation period (mean±SD).

	B + F	Budesonide	Formoterol	Placebo
Evening TNSS	0.69±0.85***	0.72±0.87***	1.80±1.62	1.97±1.88
Morning TNSS	0.70±0.84***	0.85±0.82***	1.59±1.42	1.74±1.58
Evening nPIF	179±55***	176±58***	159±60	154±58
Morning nPIF	166±52**	165±55	152±55	152±56
Post ch. TNSS	1.76±1.39***	1.89±1.27***	3.20±1.62	3.46±1.57
Post ch. PIF	131±56***	128±63***	107±52	105±51

TNSS: Total nasal symptom score. PIF: Peak inspiratory flow. B: Budesonide. F: Formoterol. Ch: challenge. (** Denotes $p<0.01$, *** denotes $p<0.001$, c.f. placebo.)

α_2-Macroglobulin, ECP, and tryptase in nasal lavage fluids were reduced by budesonide (Fig. 6). Formoterol alone did not affect these levels and did not add to the anti-inflammatory efficacy of budesonide. The employment of histamine lavages resulted in a stable yield of ECP and tryptase. Furthermore, they allowed for estimation of the exudative responsiveness of the mucosa. This was reduced by the topical corticosteroid interventions, but unaffected by formoterol.

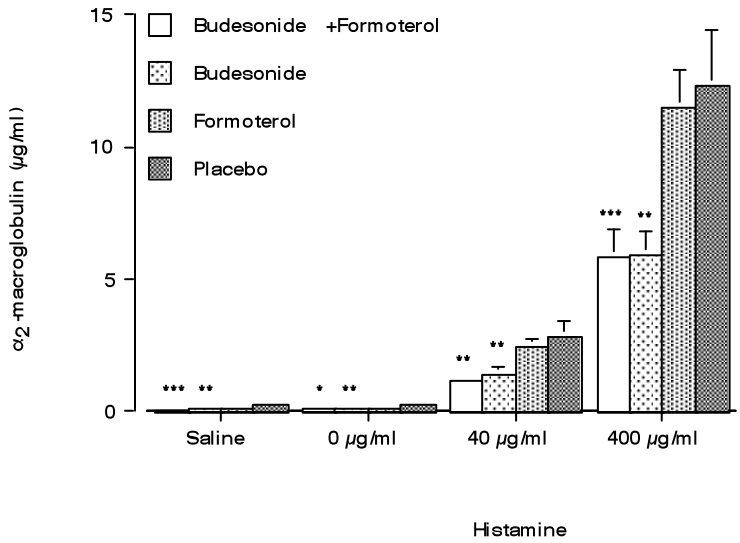

Fig. 6. Example of results showing reduced levels of α_2-macroglobulin by the topical corticosteroid (mean±SEM). In contrast, no such effects were observed for formoterol. Tryptase and ECP were similarly affected by the treatments (data not shown). (* Denotes $p<0.05$, ** denotes $p<0.01$, *** denotes $p<0.001$, c.f. placebo.)

Conclusions

Topical formoterol, in the present dose (above), does not affect allergic airway inflammation or symptoms of allergic rhinitis. Furthermore, formoterol does not add to the symptom reducing effects of budesonide. Precedent findings in acute challenge experiments in animals and humans did not translate into the present model of repeated allergen challenges. The present lack of clinical efficacy for a $ß_2$-agonist in allergic rhinitis is in agreement with the observations by Holt et al. (2000) at seasonal allergen exposure. Together, our data suggest that $ß_2$-agonists do not add a significant therapeutic value to the treatment of allergic rhinitis.

Study IV

Early phase resolution of mucosal eosinophilic inflammation in allergic rhinitis. Manuscript.

Aim

To determine epithelial and subepithelial eosinophil numbers and expression of CCL5 and CCL11 in human airway tissue at early phase of corticosteroid-induced resolution of established allergic inflammation.

Design

Twenty-one patients with birch or grass pollen allergic rhinitis were subjected to individualized allergen challenges for two seven days' periods separated by three weeks. Five days into the challenge periods, budesonide treatment was instituted and continued for six days in a double blinded, randomized, placebo-controlled, and crossover design (Table VII). The focus of the present report was on the analysis of the nasal biopsy material. Therefore, the presentation of results only covered the second challenge series since it was the only period followed by a biopsy. (Closely repeated biopsies were not considered as they likely affect nasal symptoms.)

Table VII. Study scheme. The scheme indicates one of two study periods. Note that biopsies were not obtained during the first treatment/challenge period.

	Study day									
	1	2	3	4	5	6	7	8	9	10
Treatment					X	X	X	X	X	X
Allergen	X	X	X	X	X	X	X			
Evaluation point										X
Nasal lavages					(X)	(X)		(X)		X
Nasal biopsy										X

(X): Data not included in the present report.

Nasal symptoms were registered during the challenge periods. Nasal lavages were performed at four occasions (study days 5, 6, 8, and 10) and the lavage fluids were analysed for CCL5 and CCL11. In nasal biopsies obtained on study day ten, tissue

indices of allergic inflammation, including eosinophil numbers and expression of CCL5 and CCL11, were determined.

Results

Tissue expression of CCL5 was reduced in the corticosteroid group, but the level of CCL11 was not affected (Fig. 7). In parallel, the treatment accelerated the resolution of the mucosal eosinophilia (Fig 8). In contrast to the tissue observations, nasal lavage fluids levels of CCL5 and CCL11 did not differ between the treatments (data not shown).

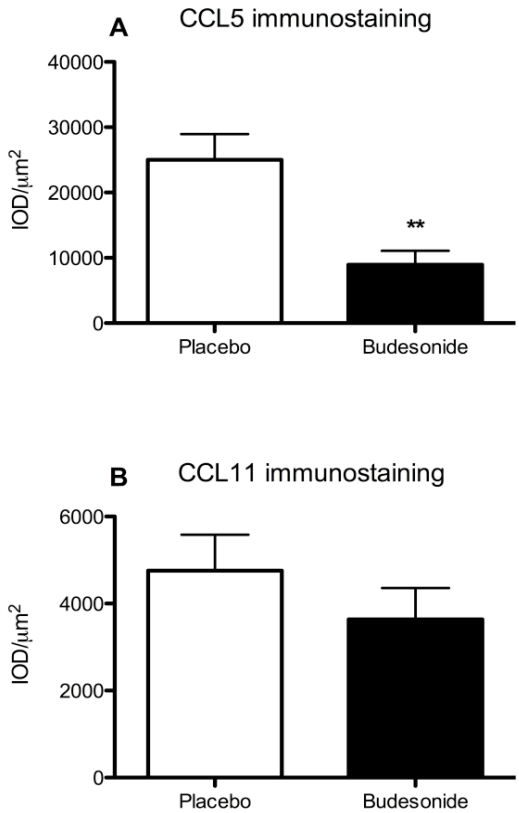

Fig. 7. CCL5 (A) was reduced in the corticosteroid group (mean±SEM). In contrast, CCL11 (B) was not affected. IOD: Integrated Optical Density. (** Denotes $p<0.01$, c.f. placebo.)

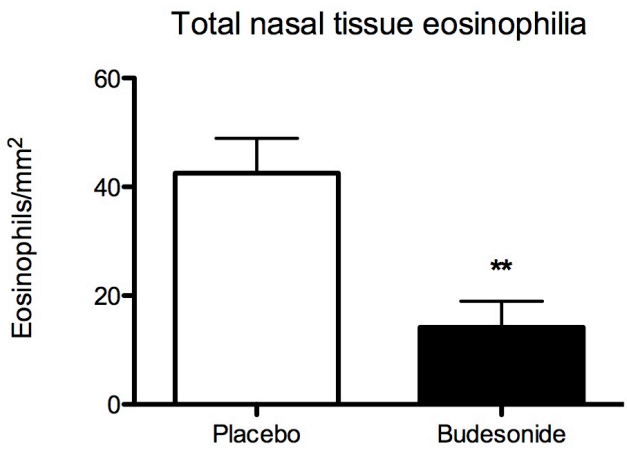

Fig. 8. Numbers of eosinophils in nasal tissue (mean±SEM). The topical corticosteroid reduced the eosinophil numbers, suggesting an accelerated resolution of allergic inflammation. (** Denotes $p<0.01$, c.f. placebo.)

Conclusions

Early phase of corticosteroid induced resolution of established human allergic airway inflammation may involve inhibition of CCL5-dependent cell recruitment. This agrees with similar experimental observations in animals demonstrating selective effects on CCL5 (Uller et al. 2006a). Taken together, these studies contrast the global effects on Th2 cytokines and CC-chemokines of corticosteroids observed in experimental studies where treatment has been given prior to allergen exposure. The present finding suggests that CCL5 may be a valid target candidate in allergic airway conditions. The observation also suggests the possibility that corticosteroids can be used experimentally to identify this type of treatment targets.

Discussion

Repeated allergen challenges in allergic rhinitis

Limitations associated with studies at natural pollen exposure prompted us to explore repeated allergen challenges as experimental test model in allergic rhinitis as suggested by Rouquet et al. (1996), Andersson et al. (2000), and Schmidt et al. (2001). Specific for the present model was the use of individualized allergen doses, carefully defined in a titration procedure, and a focus on symptoms around the clock. In Study I, the model's power was indicated by a demonstration of dose-dependent effects of a topical corticosteroid. It has not been possible to generate such information at natural allergen exposure (Bronsky et al. 1997, Stern et al. 1997, Meltzer 1998.)

The need for crossover designs was suggested in Study IV (c.f. I and III), where significant effects of a topical corticosteroid on symptoms of allergic rhinitis was not observed in a parallel group design, albeit under the condition that the treatment commenced at on-going established disease. In Study III and IV, the model generated information on aspects of allergic inflammation under very controlled conditions. These studies indicated that besides disease-like around-the-clock symptoms, the model featured aspects of allergic airway inflammation similar to those observed in patients at natural disease, i.e., mast cell activity, eosinophil activity, and plasma exudation (II, III).

During the course of the studies (I, III, IV), several advantageous features of the challenge model became apparent. The allergen challenges were well tolerated and the challenge series could be repeated up to four times without troublesome dropout rates. No untoward effects (expect for symptoms of allergic rhinitis) were noticed, and the model might now be considered as safe. The latter aspect was also suggested by later studies in this model (Korsgren et al. 2007, Widegren et al. 2009). For example, it was interesting to learn that different groups of airway pharmaceuticals have different effect profiles in the model (Korsgren et al. 2007).

In the present studies (I, III), the repeated challenge model was used to compare treatments. Focusing on differences between treatments, it is important to consider that potency of interventions may be clinically relevant particularly if symptoms are monitored rather than inflammatory indices (with unknown relevance to the

clinical presentation of the disease). However, true differences between treatments can be estimated only when dose-response relationships for the drugs are known and when differences in onset of action as well as in time to development of full efficacy can be accommodated.

Alternative to the present allergen challenge model are other experimental models as outlined in Table I. All of these models have their own merits and disadvantages. The choice of model may depend on whether or not a study has a focus on symptoms or inflammatory markers, on what type of pharmaceutical that is studied etc. For example, focusing on pharmacological onset of action, the pollen chamber may offer specific advantages (Couroux et al. 2009). When comparisons between active drugs and placebo are made it is important to realize that the present model, or any other model, does not replace studies at natural allergen exposure.

Eosinophil degranulation in allergic rhinitis

Experimental possibilities

In the present studies (II-IV), eosinophil aspects of allergic airway inflammation were explored. This was done to examine pathophysiological features of allergic disease, including eosinophil activation mechanisms and factors associated with corticosteroid-induced resolution of allergic inflammation (II, IV), and to employ ECP as a surrogate marker for allergic airway inflammation (III). The analytical methods used involved comprised an ELISA, light microscopy/immunohisto-chemistry, and TEM. Furthermore, corticosteroid intervention was included as an experimental tool (IV).

Degranulation of eosinophils

Through a very detailed ultrastructural analysis, Study II demonstrated the degranulation status of tissue eosinophils in allergic rhinitis prior to and during seasonal allergen exposure. The results indicated that while mildly degranulated eosinophils might be present already at asymptomatic baseline conditions, eosinophil numbers and their degranulation were markedly increased during the pollen season to an extent where nearly every eosinophil granule exhibited signs of extensive protein loss. This picture was complemented by an increased occurrence of tissue areas with intense ECP immunoreactivity and increased nasal mucosal output of ECP. Hence, during active allergic rhinitis, accumulation of tissue eosinophils and extensive degranulation produced high levels of extra-cellular depositions of eosinophil granule products in the target tissue, an event that may cause tissue disturbance.

Baseline eosinophil degranulation

Previous studies examining patients with allergic rhinitis demonstrated that even at off-seasonal conditions the number of tissue eosinophils might be elevated compared to healthy individuals (Togias et al. 1988). Our study confirmed an occurrence of mild off-season eosinophilia and showed that the cells often were of a degranulating phenotype, although the level of degranulation was weak. Several facts suggested that this feature represented true degranulation compared with a proper baseline such as circulating blood eosinophils (Malm-Erjefält et al. 2005), which lack the granule alterations observed in the present study. Also, occurrence of abundant small vesicles in the cytoplasm, as observed before the season (II), is known to be associated with on-going degranulation (Dvorak et al. 1993). Hence, a low-grade eosinophil degranulation may occur after the cells have reached the airway tissue even before symptoms develop in seasonal rhinitis.

Piecemeal degranulation index

The only method available that accurately can identify and quantify different modes of eosinophil degranulation is ultrastructural analysis by TEM. This technique reveals in detail the degranulation status of single eosinophils (Erjefält & Persson 2000). Accordingly, quantification of major modes of eosinophil degranulation in vivo, i.e., piecemeal degranulation (PMD) and cytolysis, can be performed (Erjefält et al. 1998). Focusing on PMD, a calculated index (PMDi) defined as the percentage of granules displaying morphological signs of protein release can be employed (Erjefält et al. 1998). In Study II, a three-fold increase in PMDi was observed at seasonal allergen exposure. This in combination with a seven-fold increase in eosinophil numbers underscores that on-going allergic rhinitis is characterized by a particularly marked eosinophil activity.

Lamina propria lavage

In Study II and III, we confirmed that increased nasal lavage fluid levels of α_2-macroglobulin and ECP, reflecting plasma exudation and eosinophil activity, respectively, characterize allergic rhinitis. In further agreement, a significant correlation between the luminal levels of these markers was observed (Meyer et al. 1999). The co-appearance of α_2-macroglobulin and ECP on the mucosal surface supports the hypothesis that plasma exudation facilitates luminal entry of cellular mediators released in the airway tissue. Accordingly, during the plasma exudation response there is a unidirectional (from the tissue to the airway lumen) bulk flow of plasma containing specific binding proteins that may contribute to an efficient rinsing of extracellular tissue spaces of the airway mucosa (Persson et al. 1998).

As indicated by the present data (II), the increase in degranulation of individual cells in combination with increased cell numbers produced tissue areas with very high local depositions of extracellular granule proteins. The capacity of extra-

vasating plasma to move ECP into the nasal cavity was suggested by the observation that seasonal levels of ECP recorded at histamine-challenge were high despite the fact that these lavages followed directly upon saline lavages (which likely removed any accumulated luminal ECP). Accordingly, luminal ECP, as recorded after a combination of saline and histamine lavages, may reflect tissue levels of extracellular ECP. In agreement, in Study II, it was only the levels of ECP recorded at histamine challenge (before and during the pollen season taken together) that correlated significantly to the PMDi. A potential implication would be to use histamine challenges as a "lamina propria lavage".

Future aspects

In light of the debate on the pathogenic role of eosinophils, prompted by results from studies with anti-IL-5 in asthma (Leckie et al. 2000, Kips et al. 2003), it may be stressed that a presence of eosinophils must probably be complemented with data on their degranulation in order to indicate an involvement of these cells in disease processes. In agreement, degranulation may not be taken for granted just because the eosinophilic tissue is inflamed. Thus, it has been demonstrated that eosinophilic conditions are characterized by a marked heterogeneity in degranulation levels (Erjefält et al. 2001).

It is unfortunate that the studies aiming at IL-5 and the eosinophil component of airway inflammation were performed in asthma, since this disease is characterized by moderate eosinophil degranulation: PMDi in asthma may be 18% (Erjefält et al. 2001) whereas the corresponding figure in on-going allergic rhinitis is 82% (II). Accordingly, based on the present demonstration of a high degree of eosinophil activity in allergic rhinitis (II), we suggest that allergic rhinitis is a condition particularly suited for studies of the pathogenic role of eosinophils and for early testing anti-eosinophilic drugs. Such studies may include IL-5 and CCL11 active drugs and, based on Study IV, interventions with CCL5 active drugs.

ß$_2$-Agonist intervention in allergic rhinitis

Anti-permeability and mast cell stabilizing effects?

In Study III, nasal mucosal output of tryptase, ECP, α_2-macroglobulin was monitored. Tryptase and α_2-macroglobulin were chosen as markers of mast cell activity and plasma exudation, respectively, based on precedent reports on mast cell stabilizing and anti-permeability effects of ß$_2$-agonists (Svensson et al. 1995a, Proud et al. 1998). ECP was employed as a surrogate marker of allergic inflammation (III). Baseline mucosal output of these markers was not monitored. Accordingly, we cannot firmly conclude that the challenge series produced allergic

inflammation. However, it is strongly suggested by the results of the corticosteroid intervention, which reduced the mucosal output of plasma and the plasma exudation producing effect of histamine (III). These are typical features of allergic airway inflammation and not seen at baseline conditions (Svensson et al. 1990). Later studies in the present model, employing baseline sampling, indicate that it is characterized by increases in nasal mucosal output of tryptase, ECP, and α_2-macroglobulin, indicating allergic inflammation (Greiff et al. unpubl).

Plasma exudation produced by the allergen challenge series, as indicated by α_2-macroglobulin, was reduced by the corticosteroid intervention (III). Tryptase and ECP were also reduced, but these changes failed to reach statistical significance. Arguably, this reflected that a number of observations were below limit of quantification (possibly due to the use of high volume lavages). However, when histamine lavages were employed, as "lamina propria lavage", it was evident that also mast cell and eosinophil activities were reduced by the corticosteroid intervention. In contrast, the ß$_2$-agonist (formoterol) failed to affect the mucosal output of tryptase and α_2-macroglobulin. This is at variance with the previous observations in allergic rhinitis on mast cell stabilizing and anti-permeability effects of ß$_2$-agonists (Svensson et al. 1995a, Proud et al. 1998). The difference between the previous and present reports may be explained by a development of tachyphylaxis. Our observations suggest that formoterol, in the present dosage, does not exert mast cell stabilizing or anti-permeability effects in allergic rhinitis. The absence of anti-inflammatory actions is supported by the observation that budesonide but not formoterol reduced the luminal entry of ECP (III).

Lack of clinical effects in allergic rhinitis

In the study by Svensson et al. (1995a), four 1.0 mg doses of terbutaline were employed in an acute allergen challenge model. Parallel to an anti-permeability effect, symptoms of allergic rhinitis in response to high dose allergen were reduced: From a score of 8 (range 0-9), symptoms were reduced by 25%. Similarly, Borum & Mygind (1980) showed that topical fenoterol reduced symptoms of allergic rhinitis. In Study III, formoterol given repeatedly to the nasal mucosal surface had no effects on symptoms of allergic rhinitis. As discussed above tachyphylaxis may explain the discrepant findings and the different challenge models must also be considered. We cannot exclude that a higher dose of formoterol or more frequent administrations would have produced a symptom reducing effect, but we regard the present topical dose as high. Our observations suggest that ß$_2$-agonists, in agreement with observations at seasonal allergen exposure (Svensson 1982, Holt et al. 2000), are not viable treatment options for allergic rhinitis. Moreover, formoterol did not add to the clinical efficacy of budesonide. This finding is in agreement with parallel work in an acute challenge

model on effects of concomitant treatment with intranasal fluticasone and salmeterol (Parameswaran et al. 2006). Accordingly, in this context, indirect interplay between the glucocorticosteroid receptor and the $ß_2$-receptor may be of little relevance (Eickelberg et al. 1999, Baraniuk et al. 1997, Mak et al. 1995).

Corticosteroid induced resolution of allergic airway inflammation

Early resolution

Except for observations in an animal model (Uller et al. 2006a), there was little prior information regarding early resolution of allergic eosinophilic airway inflammation in vivo. In Study IV, early resolution was examined in allergic rhinitis and original information on the action of a corticosteroid in this context was revealed. The consideration that inflammatory indices recovered on the nasal mucosal surface reflect corresponding reductions in the airway tissue might be particularly complicated during a resolution phase of an inflammatory process. Hence, Study IV focused on airway tissue indices. Indeed, while several tissue indices were affected by corticosteroid intervention, there was no corresponding trend at reductions in the nasal lavage fluids.

In Study IV, several methodological considerations were made. The institution of treatment had to be optimal in order to pick up early phase resolution effects and at the same time allow for an onset of action of the corticosteroid treatment. Again, this was a field where helpful precedents were scarce (Uller et al. 2006a). As it turned out, tissue indices of eosinophilic inflammation remained present in the subjects receiving placebo, whereas biopsies obtained from individuals receiving the corticosteroid exhibited signs of a accelerated resolution (c.f. placebo). Yet, we cannot exclude that the effects of the corticosteroid in part reflected attenuation of allergen-induced effects during the first two days after starting the treatment when allergen challenges were still delivered.

Elimination of tissue eosinophils

Whereas anti-inflammatory pharmaceuticals might produce a general reduction in mucosal “injury” and associated occurrence of apoptosis in diseased airways, it has been forwarded that apoptosis specifically of airway tissue eosinophils is increased by corticosteroids (Haslett 1999, Vignola et al. 2000, Druilhe et al. 2003). Inducement of eosinophil apoptosis (followed by a postulated engulfment of apoptotic cells) has been advocated as a major pharmacological mechanism through which airway eosinophilic inflammation is resolved (Haslett 1999, Vignola et al. 2000, Druilhe et al. 2003). By contrast, animal data have scarcely

supported a role of apoptosis in the pharmacology of eosinophil elimination (Erjefält et al. 1998, Ikeda et al. 2003, Uller et al. 2004, 2006b). Similarly, in Study IV, apoptotic tissue eosinophils were not detected in corticosteroid-treated subjects. Such negative data are strengthened by concomitant demonstrations that other types of apoptotic cells can be observed in the allergic tissues (IV).

The anti-CCL5 effect

Airway tissue indices of allergic eosinophilic inflammation were reduced by therapeutic corticosteroid intervention five days after its institution (IV). Also, a degree of selectivity in the corticosteroid action was shown in that tissue CCL5 expression, but not CCL11, was reduced along with attenuated tissue eosinophilia. The reduced eosinophilia in the corticosteroid group would in part reflect reduced recruitment of these cells during the period of corticosteroid treatment. Although many locally present chemoattractants might contribute. CCL5 and CCL11 have been pointed out as two major chemokines involved in recruiting circulating eosinophils to allergic airway tissues (Lloyd et al. 2002). The present demonstration of a reduced expression of CCL5, occurring simultaneously to a reduced tissue eosinophilia, suggests that CCL5, with its proposed roles in eosinophil and lymphocyte recruitment, could be of particular importance as treatment target.

Currently entertained molecular actions of corticosteroids suggest that these drugs exert non-selective inhibition of the generation of inflammatory chemokines (Barnes & Adcock 1993). Hence, the mechanism behind the particular anti-CCL5 action of corticosteroids in animal (Uller et al. 2006a) and human (IV) allergic airways remains challenging. Other selective actions of corticosteroids have been shown including observations that they, despite their potent anti-inflammatory effects, may spare leukocyte and microvascular innate responses mobilised in relation to epithelial repair and microbial defence (Erjefält et al. 1995, Zhang et al. 2007). Clearly, the inhibition of CCL5 in animal and human airway tissues with resolving allergic inflammation, and the association between increase in airway CCL5 and deterioration of asthma at corticosteroid withdrawal (Castro et al. 2004), support the view that this chemokine may have a central role in maintaining allergic airway inflammation.

Conclusions

- Individualized, repeated allergen challenges can be employed in allergic rhinitis to produce symptoms and eosinophilic inflammation that mimic symptoms/inflammation at natural pollen exposure. This model is suited for accurate comparisons of pharmacological treatments for allergic rhinitis (I, III).

- Piecemeal degranulation is a key activation mechanism for eosinophils in allergic rhinitis. It may occur already outside the pollen season, but is markedly increased at seasonal allergen exposure. This, in combination with eosinophil recruitment, results in extracellular deposition of potent eosinophil mediators including ECP (II).

- The degree of piecemeal degranulation correlates with eosinophil activity as monitored through analysis of ECP in histamine lavages carried out after preceding saline lavages. Arguably, histamine lavages can be used to monitor tissue eosinophil activity (II).

- Using ECP in histamine lavages as surrogate marker of allergic inflammation, and with focus on symptoms of allergic rhinitis, effects of a $ß_2$-agonist (formoterol) was examined. Formoterol treatment did not affect symptoms or eosinophilic inflammation. Furthermore, it did not add to the efficacy of a topical corticosteroid (III).

- Topical corticosteroid treatment accelerates the resolution of an established eosinophilic inflammation in allergic rhinitis. Potentially, this effect was mediated through CCL5. Inferentially, CCL5 might be a valid treatment target for allergic airway inflammation (IV).

References

Ahlström-Emanuelsson C, Andersson M, Persson CG, Schrewelius C, Greiff L. Topical treatment with aqueous solutions of rofleponide palmitate and budesonide in a pollen-season model of allergic rhinitis. Clin Exp Allergy 2004; 34: 731-5.

Andersson M, Svensson C, Persson CG, Åkerlund A, Greiff L. Dose-dependent effects of budesonide aqueous nasal spray on symptoms in a daily nasal allergen challenge model. Ann Allergy Asthma Immunol 2000; 85: 279-83.

Baluk P, McDonald DM. The beta-2-adrenergic receptor agonist formoterol reduces microvascular leakage by inhibiting endothelial gap formation. Am J Physiol 1994; 266: L461-8.

Baraniuk JN, Ali M, Brody D, Maniscalco J, Gaumond E, Fitzgerald T, et al. Glucocorticoids induce beta-2-adrenergic receptor function in human nasal mucosa. Am J Respir Crit Care Med 1997; 155: 704-10.

Baraniuk JN. Pathogenesis of allergic rhinitis. J Allergy Clin Immunol 1997; 99: S763-72.

Barnes PJ, Adcock I. Anti-inflammatory actions of steroids: molecular mechanisms. Trends Pharmacol Sci 1993; 14: 436-41.

Barnes PJ. Scientific rationale for inhaled combination therapy with long-acting beta-2-agonists and corticosteroids. Eur Respir J 2002; 19: 182-91.

Baysal C, Atilgan AR. Elucidating the structural mechanisms for biological activity of the chemokine family. Proteins 2001; 43: 150-160.

Bernstein DI, Schoenwetter WF, Nathan RA, Storms W, Ahlbrandt R, Mason J. Efficacy and safety of fexofenadine hydrochloride for treatment of seasonal allergic rhinitis. Ann Allergy Asthma Immunol 1997; 79: 443-8.

Borum P, Mygind N. Inhibition of the immediate allergic reaction in the nose by the beta-2 adrenostimulant fenoterol. J Allergy Clin Immunol 1980; 66: 25-32.

Bousquet J, Van Cauwenberge P, Bachert C, Canonica GW, Demoly P, Durham SR, et al. Requirements for medications commonly used in the treatment of allergic rhinitis. European Academy of Allergy and Clinical Immunology (EAACI), Allergic Rhinitis and its Impact on Asthma (ARIA). Allergy 2003; 58: 192-7.

Bousquet PJ, Combescure C, Neukirch F, Klossek JM, Mechin H, Daures JP, et al. Visual analog scales can assess the severity of rhinitis graded according to ARIA guidelines. Allergy 2007; 62: 367-72.

Bronsky EA, Aaronson DW, Berkowitz RB, Chervinsky P, Graft D, Kaiser HB, et al. Dose ranging study of mometasone furoate (Nasonex) in seasonal allergic rhinitis. Ann Allergy Asthma Immunol 1997; 79: 51-6.

Busse WW, Sedgwick JB, Jarjour NN, Calhoun WJ. Eosinophils and basophils in allergic airway inflammation. J Allergy Clin Immunol 1994; 94: 1250-4.

Castells M, Schwartz LB. Tryptase levels in nasal-lavage fluid as an indicator of the immediate allergic response. J Allergy Clin Immunol 1988; 82: 348-55.

Castro M, Bloch SR, Jenkerson MV, DeMartino S, Hamilos DL, Cochran RB, et al. Asthma exacerbations after glucocorticoid withdrawal reflects T cell recruitment to the airway. Am J Respir Crit Care Med 2004; 169: 842-49.

Chong LK, Cooper E, Vardey CJ, Peachell PT. Salmeterol inhibition of mediator release from human lung mast cells by beta-adrenoceptor-dependent and independent mechanisms. Br J Pharmacol 1998; 123: 1009-15.

Ciprandi G, Cirillo I, Klersy C, Marseglia GL, Caimmi D, Vizzaccaro A. Nasal obstruction is the key symptom in hay fever patients. Otolaryngol Head Neck Surg 2005; 133: 429-35.

Collins PD, Marleau S, Griffiths-Johnson DA, Jose PJ, Williams TJ. Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation in vivo. J Exp Med 1995; 182: 1169-74.

Couroux P, Kunjibettu S, Hall N, Wingertzahn MA. Onset of action of ciclesonide once daily in the treatment of seasonal allergic rhinitis. Ann Allergy Asthma Immunol 2009; 102: 62-8.

Day JH, Briscoe MP, Welsh A, Smith JN, Clark A, Ellis AK, et al. Onset of action, efficacy, and safety of a single dose of fexofenadine hydrochloride for ragweed allergy using an environmental exposure unit. Ann Allergy Asthma Immunol 1997; 79: 533-40.

Druilhe A, Cai Z, Haile S, Chouaib S, Pretolani M. Fas-mediated apoptosis in cultured human eosinophils. Blood 1996; 87: 2822-30.

Druilhe A, Letuve S, Pretolani M. Glucocorticoid-induced apoptosis in human eosinophils: mechanisms of action. Apoptosis 2003; 8: 481-95.

Dvorak AM, Onderdonk AB, McLeod RS, Monahan-Earley RA, Antonioli DA, Cullen J, et al. Ultrastructural identification of exocytosis of granules from human gut eosinophils in vivo. Int Arch Allergy Immunol 1993; 102: 33-45.

Egesten A, Alumets J, von Mecklenburg C, Palmegren M, Olsson I. Localization of eosinophil cationic protein, major basic protein, and eosinophil peroxidase in human eosinophils by immunoelectron microscopic technique. J Histochem Cytochem 1986; 34: 1399-403.

Eickelberg O, Roth M, Lorx R, Bruce V, Rudiger J, Johnson M, et al. Ligand-independent activation of the glucocorticoid receptor by beta-2-adrenergic receptor agonists in primary human lung fibroblasts and vascular smooth muscle cells. J Biol Chem 1999; 274: 1005-10.

Erin EM, Zacharasiewicz AS, Nicholson GC, Tan AJ, Higgins LA, Williams et al. Topical corticosteroid inhibits interleukin-4, -5 and -13 in nasal secretions following allergen challenge. Clin Exp Allergy 2005; 35: 1608-14.

Erjefält I, Persson CG. Long duration and high potency of antiexudative effects of formoterol in guinea-pig tracheobronchial airways. Am Rev Respir Dis 1991; 144: 788-91.

Erjefält JS, Erjefält I, Sundler F, Persson CG. Effects of topical budesonide on epithelial restitution in vivo in guinea pig trachea. Thorax 1995; 50: 785-92.

Erjefält JS, Andersson M, Greiff L, Korsgren M, Gizycki M, Jeffery PK, et al. Cytolysis and piecemeal degranulation as distinct modes of activation of airway mucosal eosinophils. J Allergy Clin Immunol 1998; 102: 286-94.

Erjefält JS, Greiff L, Andersson M, Matsson E, Petersen H, Linden M, et al. Allergen-induced eosinophil cytolysis is a primary mechanism for granule protein release in human upper airways. Am J Respir Crit Care Med 1999; 160: 304-12.

Erjefält JS, Persson CG. New aspects of degranulation and fates of airway mucosal eosinophils. Am J Respir Crit Care Med 2000; 161: 2074-85.

Erjefält JS, Greiff L, Andersson M, Ädelroth E, Jeffery PK, Persson CG. Degranulation patterns of eosinophil granulocytes as determinants of eosinophil driven disease. Thorax 2001; 56: 341-4.

Eum SY, Maghni K, Hamid Q, Eidelman DH, Campbell H, Isogai S, et al. Inhibition of allergic airways inflammation and airway hyperresponsiveness in mice by dexamethasone: role of eosinophils, IL-5, eotaxin, and IL-13. J Allergy Clin Immunol 2003; 111: 1049-61.

Foresi A, Teodoro C, Leone C, Pelucchi A, D'Ippolito R, Chetta A, et al. Eosinophil apoptosis in induced sputum from patients with seasonal allergic rhinitis and with asymptomatic and symptomatic asthma. Ann Allergy Asthma Immunol 2000; 84: 411-6.

Fransson M, Benson M, Wennergren G, Cardell LO. A role for neutrophils in intermittent allergic rhinitis. Acta Otolaryngol 2004; 124: 616-20.

Fokkens WJ, Vroom TM, Gerritsma V, Rijntjes E. A biopsy method to obtain high quality specimens of nasal mucosa. Rhinology 1988; 26: 293-5.

Freeland HS, Pipkorn U, Schleimer RP, Bascom R, Lichtenstein LM, Naclerio RM, et al. Leukotriene B4 as a mediator of early and late reactions to antigen in humans: the effect of systemic glucocorticoid treatment in vivo. J Allergy Clin Immunol 1989; 83: 634-42.

Gauvreau GM, Doctor J, Watson RM, Jordana M, O'Byrne PM. Effects of inhaled budesonide on allergen-induced airway responses and airway inflammation. Am J Respir Crit Care Med 1996; 154: 1267-71.

Gauvreau GM, Wood LJ, Sehmi R, Watson RM, Dorman SC, Schleimer RP, et al. The effects of inhaled budesonide on circulating eosinophil progenitors and their expression of cytokines after allergen challenge in subjects with atopic asthma. Am J Respir Crit Care Med 2000; 162: 2139-44.

Gleich GJ, Adolphson CR, Leiferman KM. The biology of the eosinophilic leukocyte. Annu Rev Med 1993; 44: 85-101.

Greiff L, Pipkorn U, Alkner U, Persson CG. The "nasal pool" device applies controlled concentrations of solutes on human nasal airway mucosa and samples its surface exudations/secretions. Clin Exp Allergy 1990; 20: 253-9.

Greiff L, Svensson C, Andersson M, Persson CG. Effects of topical capsaicin in seasonal allergic rhinitis. Thorax 1995a; 50: 225-9.

Greiff L, Persson CG, Svensson C, Enander I, Andersson M. Loratadine reduces allergen-induced mucosal output of alpha-2-macroglobulin and tryptase in allergic rhinitis. J Allergy Clin Immunol 1995b; 96: 97-103.

Greiff L, Andersson M, Svensson C, Åkerlund A, Alkner U, Persson CG. Effects of two weeks of topical budesonide treatment on microvascular exudative responsiveness in healthy human nasal airways. Eur Respir J 1997; 10: 841-5.

Greiff L, Erjefält JS, Andersson M, Svensson C, Persson CG. Generation of clusters of free eosinophil granules (Cfegs) in seasonal allergic rhinitis. Allergy 1998; 53: 200-3.

Greiff L, Petersen H, Mattsson E, Andersson M, Erjefält JS, Linden M, et al. Mucosal output of eotaxin in allergic rhinitis and its attenuation by topical glucocorticosteroid treatment. Clin Exp Allergy 2001; 31: 1321-7.

Greiff L, Andersson M, Erjefält JS, Svensson C, Persson CG. Loss of size-selectivity at histamine-induced exudation of plasma proteins in atopic nasal airways. Clin Physiol Funct Imaging 2002; 22: 28-31.

Haslett C. Granulocyte apoptosis and its role in the resolution and control of lung inflammation. Am J Respir Crit Care Med 1999; 160: S5-11.

Hellgren J, Jarlstedt J, Dimberg L, Toren K, Karlsson G. A study of some current methods for assessment of nasal histamine reactivity. Clin Otolaryngol Allied Sci 1997; 22: 536-41.

Holgate ST, Bodey KS, Janezic A, Frew AJ, Kaplan AP, Teran LM. Release of RANTES, MIP-1 alpha, and MCP-1 into asthmatic airways following endobronchial allergen challenge. Am J Respir Crit Care Med 1997; 156: 1377-83.

Holmström M, Scadding GK, Lund VJ, Darby YC. Assessment of nasal obstruction. A comparison between rhinomanometry and nasal inspiratory peak flow. Rhinology 1990; 28: 191-6.

Holt S, Suder A, Dronfield L, Holt C, Beasley R. Intranasal beta-agonist in allergic rhinitis. Allergy 2000; 55: 1198.

Howarth PH. The cellular basis for allergic rhinitis. Allergy 1995; 50: 6-10.

Ikeda RK, Nayar J, Cho JY, Miller M, Rodriguez M, Raz E, et al. Resolution of airway inflammation following ovalbumin inhalation: comparison of ISS DNA and corticosteroids. Am J Respir Cell Mol Biol 2003; 28: 655-63.

Jahnsen F, Halstensen TS, Brandtzaeg P. Immunostaining with monoclonal antibodies to eosinophil cationic protein (EG1 and EG2) does not distinguish between resting and activated eosinophils in formalin-fixed tissue specimens. Adv Exp Med Biol 1995; 371A: 283-6.

Kautz J, Demarsh QB. An electron microscope study of sectioned cells of peripheral blood and bone marrow. Blood 1954; 9: 24-38.

Kimura K, Adachi M, Kubo K, Ikemoto Y. The basal plasma histamine level and eosinophil count in allergic and non-allergic patients. Fukuoka Igaku Zasshi 1999; 90: 457-63.

Kips JC, O'Connor BJ, Langley SJ, Woodcock A, Kerstjens HA, Postma DS, et al. Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study. Am J Respir Crit Care Med 2003; 167: 1655-9.

Klementsson H, Svensson C, Andersson M, Venge P, Pipkorn U, Persson CG. Eosinophils, secretory responsiveness and glucocorticoid-induced effects on the nasal mucosa during a weak pollen season. Clin Exp Allergy 1991; 21: 705-10.

Korsgren M, Andersson M, Borgå O, Larsson L, Alden-Raboisson M, Malmqvist U, et al. Clinical efficacy and pharmacokinetic profiles of intranasal and oral cetirizine in a repeated allergen challenge model of allergic rhinitis. Ann Allergy Asthma Immunol 2007; 98: 316-21.

Kowalski ML, Dietrich-Milobedzki A, Majkowska-Wojciechowska B, Jarzebska M. Nasal reactivity to capsaicin in patients with seasonal allergic rhinitis during and after the pollen season. Allergy 1999; 54: 804-10.

Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. Lancet 2000; 356: 2144-8.

Linden M, Svensson C, Andersson E, Andersson M, Greiff L, Persson CG. Immediate effect of topical budesonide on allergen challenge-induced nasal mucosal fluid levels of granulocyte-macrophage colony-stimulating factor and interleukin-5. Am J Respir Crit Care Med 2000; 162: 1705-8.

Linder A, Venge P, Deuschl H. Eosinophil cationic protein and myeloperoxidase in nasal secretion as markers of inflammation in allergic rhinitis. Allergy 1987; 42: 583-90.

Lloyd C. Chemokines in allergic lunf inflammation. Immunology 2002; 105: 144-54.

Lukacs NW. Role of chemokines in the pathogenesis of asthma. Nat Rev Immunol 2001; 1: 108-16.

Majno G, Joris I. Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol 1995; 146: 3-15.

Mak JC, Nishikawa M, Shirasaki H, Miyayasu K, Barnes PJ. Protective effects of a glucocorticoid on downregulation of pulmonary beta-2-adrenergic receptors in vivo. J Clin Invest 1995; 96: 99-106.

Malm-Erjefält M, Greiff L, Ankerst J, Andersson M, Wallengren J, Cardell L-O, et al. Circulating eosinophils in asthma, allergic rhinitis, and atopic dermatitis lack morphological signs of degranulation. Clin Exp Allergy 2005; 35: 1334-40.

Marcucci F, Sensi LG, Migali E, Coniglio G. Eosinophil cationic protein and specific IgE in serum and nasal mucosa of patients with grass-pollen-allergic rhinitis and asthma. Allergy 2001; 56: 231-6.

Meltzer EO, Weiler JM, Widlitz MD. Comparative outdoor study of the efficacy, onset and duration of action, and safety of cetirizine, loratadine, and placebo for seasonal allergic rhinitis. J Allergy Clin Immunol 1996; 97: 617-26.

Meltzer EO. Clinical and antiinflammatory effects of intranasal budesonide aqueous pump spray in the treatment of perennial allergic rhinitis. Ann Allergy Asthma Immunol 1998; 81: 128-34.

Message SD, Laza-Stanca V, Mallia P, Parker HL, Zhu J, Kebadze T, et al. Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production. Proc Natl Acad Sci USA 2008; 105: 13562-7.

Meyer P, Persson CG, Andersson M, Wollmer P, Linden M, Svensson C, et al. Alpha-2-macroglobulin and eosinophil cationic protein in the allergic airway mucosa in seasonal allergic rhinitis. Eur Respir J 1999; 13: 633-7.

Meyer P, Andersson M, Persson CG, Greiff L. Steroid-sensitive indices of airway inflammation in children with seasonal allergic rhinitis. Pediatr Allergy Immunol 2003; 14: 60-5.

Mullol J, Valero A, Alobid I, Bartra J, Navarro AM, Chivato T, et al. Allergic Rhinitis and its Impact on Asthma update (ARIA 2008). The perspective from Spain. J Investig Allergol Clin Immunol 2008; 18: 327-34.

Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF, Jr., Meyers DA, Norman PS, et al. Mediator release after nasal airway challenge with allergen. Am Rev Respir Dis 1983; 128: 597-602.

Nials AT, Ball DI, Butchers PR, Coleman RA, Humbles AA, Johnson M, et al. Formoterol on airway smooth muscle and human lung mast cells: a comparison with salbutamol and salmeterol. Eur J Pharmacol 1994; 251: 127-35.

Nihlén U, Greiff L, Montnémery P, Löfdahl C-G, Johannisson, A, Persson CG, Andersson M. Incidence and remission of self-reported allergic rhinitis symptoms in adults. Allergy 2006; 61: 1299-304.

Pawankar R, Yamagishi S, Takizawa R, Yagi T. Mast cell-IgE and mast cell structural cell interactions in allergic airway disease. Curr Drug Tarhets Inflamm Allergy 2003; 2: 303-12.

Parameswaran K, Fanat A, O'Byrne PM. Effects of intranasal fluticasone and salmeterol on allergen-induced nasal responses. Allergy 2006; 61: 731-6.

Peters MS, Rodriguez M, Gleich GJ. Localization of human eosinophil granule major basic protein, eosinophil cationic protein, and eosinophil-derived neurotoxin by immunoelectron microscopy. Lab Invest 1986; 54: 656.62.

Peterson CG, Venge P. Interaction and complex-formation between the eosinophil cationic protein and alpha-2-macroglobulin. Biochem J 1987; 245: 781-7.

Pipkorn U, Proud D, Lichtenstein LM, Kagey-Sobotka A, Norman PS, Naclerio RM. Inhibition of mediator release in allergic rhinitis by pretreatment with topical glucocorticosteroids. N Engl J Med 1987; 316: 1506-10.

Persson CG, Erjefalt I, Andersson P. Leakage of macromolecules from guinea-pig tracheobronchial microcirculation. Effects of allergen, leukotrienes, tachykinins, and anti-asthma drugs. Acta Physiol Scand 1986; 127: 95-105.

Persson CG, Erjefält JS. Eosinophil lysis and free granules: an in vivo paradigm for cell activation and drug development. Trends Pharmacol Sci 1997; 18: 117-23.

Persson CG, Erjefält JS, Greiff L, Andersson M, Erjefält I, Godfrey RW, et al. Plasma-derived proteins in airway defence, disease and repair of epithelial injury. Eur Respir J 1998; 11: 958-70.

Proud D, Reynolds CJ, Lichtenstein LM, Kagey-Sobotka A, Togias A. Intranasal salmeterol inhibits allergen-induced vascular permeability but not mast cell activation or cellular infiltration. Clin Exp Allergy 1998; 28: 868-75.

Pullerits T, Lindén A, Praks L, Cardell LO, Lötvall J. Upregulation of nasal mucosal eotaxin in patients with allergic rhinitis during grass pollen season: effects of a local glucocorticosteroid. Clin Exp Allergy 2000; 30: 1469-75.

Reed CE. The importance of eosinophils in the immunology of asthma and allergic disease. Ann Allergy 1994; 72: 376-80.

Resnick MB, Weller PF. Mechanisms of eosinophil recruitment. Am J Respir Cell Mol Biol 1993; 8: 349-55.

Raphael GD, Igarashi Y, White MV, Kaliner M. The pathophysiology of allergic rhinitis. V. Sources of proteiin in allergen-induced nasal secretions. J Allergy Clin Immunol 1991; 88: 33-42.

Roquet A, Ihre E, van Hage-Hamsten M, Halldén G, Zetterström O. Allergen-induced inflammation in the nose: a comparison of acute and repeated low-dose allergen exposure. Allergy 1996; 51: 42-8.

Rothenberg ME. Eosinophilia. N Engl J Med 1998; 338: 1592-600.

Sarin S, Undem B, Sanico A, Togias A. The role of the nervous system in rhinitis. J Allergy Clin Immunol 2006; 118: 999-1016.

Schleimer RP, Bochner BS. The effects of glucocorticoids on human eosinophils. J Allergy Clin Immunol 1994; 94: 1202-13.

Schmidt BM, Kusma M, Feuring M, Timmer WE, Neuhauser M, Bethke T, et al. The phosphodiesterase 4 inhibitor roflumilast is effective in the treatment of allergic rhinitis. J Allergy Clin Immunol 2001; 108: 530-6.

Shen H, O'Byrne PM, Ellis R, Wattie J, Tang C, Inman MD. The effects of intranasal budesonide on allergen-induced production of interleukin-5 and eotaxin, airways, blood, and bone marrow eosinophilia, and eosinophil progenitor expansion in sensitized mice. Am J Respir Crit Care Med 2002; 166: 146-53.

Stern MA, Dahl R, Nielsen LP, Pedersen B, Schrewelius C. A comparison of aqueous suspensions of budesonide nasal spray (128 micrograms and 256 micrograms once daily)

and fluticasone propionate nasal spray (200 micrograms once daily) in the treatment of adult patients with seasonal allergic rhinitis. Am J Rhinol 1997; 11: 323-30.

Stübner P, Zieglmayer R, Horak F. A direct comparison of the efficacy of antihistamines in SAR and PAR: randomised, placebo-controlled studies with levocetirizine and loratadine using an environmental exposure unit - the Vienna Challenge Chamber (VCC). Curr Med Res Opin 2004; 20: 891-902.

Svensson C, Andersson M, Persson CG, Venge P, Alkner U, Pipkorn U. Albumin, bradykinins, and eosinophil cationic protein on the nasal mucosal surface in patients with hay fever during natural allergen exposure. J Allergy Clin Immunol 1990; 85: 828-33.

Svensson C, Klementsson H, Andersson M, Pipkorn U, Alkner U, Persson CG. Glucocorticosteroid-induced attenuation of mucosal exudation of fibrinogen and bradykinins in seasonal allergic rhinitis. Allergy 1994; 49: 177-83.

Svensson C, Greiff L, Andersson M, Alkner U, Grönneberg R, Persson CG. Antiallergic actions of high topical doses of terbutaline in human nasal airways. Allergy 1995a; 50: 884-90.

Svensson C, Grönneberg R, Andersson M, Alkner U, Andersson O, Billing B, et al. Allergen challenge-induced entry of alpha-2-macroglobulin and tryptase into human nasal and bronchial airways. J Allergy Clin Immunol 1995b; 96: 239-46.

Svensson C, Andersson M, Greiff L, Alkner U, Persson CG. Exudative hyper-responsiveness of the airway microcirculation in seasonal allergic rhinitis. Clin Exp Allergy 1995c; 25: 942-50.

Svensson G. Topical treatment of seasonal allergic rhinitis with a beta-adrenoceptor stimulant (KWD 2131). Rhinology 1982; 20: 159-66.

Tai PC, Sun L, Spry CJ. Effects of IL-5, granulocyte/macrophage colony stimulating factor (GM-CSF) and IL-3 on the survival of human blood eosinophils in vitro. Clin Exp Allergy 1991; 85: 312-6.

Togias A, Naclerio RM, Proud D, Pipkorn U, Bascom R, Iliopoulos O, et al. Studies on the allergic and nonallergic nasal inflammation. J Allergy Clin Immunol 1988; 81: 782-90.

Tokuyama K, Lötvall JO, Löfdahl CG, Barnes PJ, Chung KF. Inhaled formoterol inhibits histamine-induced airflow obstruction and airway microvascular leakage. Eur J Pharmacol 1991; 193: 35-9.

Uller L, Persson CG, Källström L, Erjefält JS. Lung tissue eosinophils may be cleared through luminal entry rather than apoptosis: effects of steroid treatment. Am J Respir Crit Care Med 2001; 164: 1948-56.

Uller L, Andersson M, Greiff L, Persson CG, Erjefält JS. Occurrence of apoptosis, secondary necrosis, and cytolysis in eosinophilic nasal polyps. Am J Respir Crit Care Med 2004; 170: 742-7.

Uller L, Lloyd CM, Rydell-Törmänen K, Persson CG, Erjefält JS. Effects of steroid treatment on lung CC chemokines, apoptosis and transepithelial cell clearance during development and resolution of allergic airway inflammation. Clin Exp Allergy 2006a; 36: 111-21.

Uller L, Persson CG, Erjefält JS. Resolution of airway disease: removal of inflammatory cells through apoptosis, egression or both? Trends Pharmacol Sci 2006b; 27: 461-6.

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

Walsh GM, Sexton DW, Blaylock MG. Corticosteroids, eosinophils and bronchial epithelial cells: new insights into the resolution of inflammation in asthma. J Endocrinol 2003; 178: 37-43.

Wang D, Smitz J, Derde MP, Clement P. Concentrations of myeloperoxidase in nasal secretions of atopic patients after nasal allergen challenge and during natural allergen exposure. Int Arch Allergy Immunol 1996; 110: 85-90.

Weido AJ, Reece LM, Alam R, Cook CK, Sim TC. Intranasal fluticasone propionate inhibits recovery of chemokines and other cytokines in nasal secretions in allergen-induced rhinitis. Ann Allergy Asthma Immunol 1996; 77: 407-15.

Weiler CR, Kita H, Hukee M, Gleich GJ. Eosinophil viability during immunoglobulin-induced degranulation. J Leukoc Biol 1996; 60: 493-501.

Widegren H, Andersson M, Greiff L. Effects of Clara cell 10 (CC10) protein on symptoms and signs of allergic rhinitis. Ann Allergy Asthma Immunol 2009; 102: 51-6.

Zhang N, Truong-Tran QA, Tancowny B, Harris KE, Schleimer RP. Glucocorticoids enhance or spare innate immunity: effects in airway epithelium are mediated by CCAAT/enhancer binding proteins. J Immunol 2007; 179: 578-89.

Populärvetenskaplig sammanfattning

Förekomsten av allergisk snuva (rinit) har under senaste tioårsperioden ökat. Detta har gjort det allt viktigare att studera mekanismer och behandlingar relevanta för sjukdomen.

Utvärdering av en pollenprovokationsmodell

När man studerar allergisk rinit orsakad av pollen så kan man antingen utnyttja den naturliga pollensäsongen eller experimentellt utsätta patienter för det ämne (allergen) som framkallar deras symptom (allergenprovokationer). Den naturliga säsongen är mest relevant, men den är oförutsägbar eftersom den varierar i start, intensitet och längd. Detta medför stora skillnader i symptom vilket i sin tur gör det svårt att genomföra kontrollerade behandlingsstudier. Man har vid naturlig exposition t.ex. inte kunnat visa att en dosering av ett lokalt kortisonpreparat (”kortisonnässpray”) är bättre än en annan klart lägre dosering av samma preparat.

När allergenprovokationer utförs i ett laboratorium så kan patienter utsättas för standardiserade doser av allergen. Oftast görs detta som enstaka provokationer, men sådana modeller liknar inte helt en naturlig pollensäsong. I *Studie I* använde vi lokal kortisonbehandling för att utvärdera en modell där upprepade och individuellt utprovade doser av allergen gavs dagligen under en vecka. Detta upprepades under fyra perioder och fyra olika behandlingar gavs. Vi mätte nässymptom och kunde visa att modellen kännetecknades av säsongslika symptom dygnet runt. Vidare kunde vi påvisa dosberoende effekter av ett lokalt kortison-preparat.

Eosinofil aktivitet vid allergisk rinit

Eosinofiler är vita blodkroppar som har fått sitt namn av att de innehåller kapslar (granuler) som karakteristiskt färgas in av färgämnet eosin. Allergisk rinit kännetecknas av ansamling av eosinofiler till nässlemhinnan. Antalet celler kan räknas i vävnadsprov med hjälp av ljusmikroskopi, men en sådan analys klargör inte om cellerna är aktiverade. När graden av eosinofil aktivitet skall bestämmas använder man sig vanligtvis av nässköljningar och analys av något av de proteiner som finns i eosinofilens granuler och som frisätts när cellen aktiveras, t.ex. ”eosinophil cationic protein” (ECP). Vissa observationer talar nu för att man mycket exakt kan bedöma eosinofilens aktivitetsgrad genom elektronmikroskopi av vävnadsprover.

Det saknas information om sådana mått på aktiveringsgrad korrelerar till allergen-exposition.

Studie II genomfördes under en naturlig björkpollensäsong. Före och under säsongen togs vävnadsprov från nässlemhinnan och nässköljningar utfördes. Vi påvisade med elektronmikroskopi att en viktig eosinofil aktiveringsmekanism var att eosinofila granuler sönderförföll och frisatte proteiner på ett speciellt sätt (s.k. ”piecemeal degranulation”). Mängden protein som frisattes från eosinofila granuler till omgivande vävnad ökade markant under pollensäsongen. Vidare kunde vi relatera aktiveringen till andra mått på eosinofil förekomst/aktivitet.

Påverkas allergi av ß$_2$-agonister?

Histamin är ett kroppseget ämne som frisätts vid allergiska reaktioner och som framkallar nysningar, rinnsnuva och nästäppa. Experimentella studier har visat att ß$_2$-agonister (”luftrörsvidgare”) kan påverka mastceller (en cell som är central för allergiska reaktioner) så att de inte lika lätt frisätter histamin. Dessutom har det påvisats att ß$_2$-agonister kan minska den utsöndring av blodplasma i vävnaden som karakteriserar allergisk inflammation. Det finns data som visar att ß$_2$-agonister kan ha effekt på symtom vid allergisk rinit, men dessa observationer har framför allt gjorts i modeller där enstaka allergenprovokationer har använts. Vidare finns det observationer som talar för att ß$_2$-agonister skulle kunna göra så att kortison-behandling fungerar bättre.

I *Studie III* använde vi vår allergenprovokationsmodell för att studera effekten av lokal behandling (i näsan) med en ß$_2$-agonist vid allergisk rinit, ensam och i kombination med ett lokalt kortisonpreparat. Vi kunde inte påvisa någon effekt av ß$_2$-agonisten vare sig på allergiska nässymptom eller allergisk inflammation (där bl.a. ECP i nässköljningsvätska användes som markör för allergisk inflammation). Vi kunde inte heller visa att ß$_2$-agonisten hade någon tilläggseffekt till kortison-nässpray.

Hur påverkas allergisk inflammation av kortisonbehandling?

I både djurexperimentella modeller och i studier av patienter så har effekten av kortisonbehandling vid allergisk inflammation i huvudsak undersökts i situationer då behandlingen har påbörjats före exponeringen för allergen. I dessa studier har kortisonbehandling ofta en bred antiinflammatorisk effekt och det är svårt att identifiera mekanismer som är olika kortisonkänsliga och därmed kanske olika viktiga. Mer relevant vore kanske att studera effekten av kortisonbehandling när den ges till en redan etablerad allergisk inflammation.

I *Studie IV* använde vi oss av vår allergenprovokationsmodell. Efter att allergisk inflammation etablerats påbörjades behandling med ett lokalt kortisonpreparat. Vi

upphörde sedan med provokationerna medan kortisonbehandlingen fortsatte. På det sättet kunde vi studera hur behandlingen påverkade upplösningen av en etablerad allergisk inflammation. Vävnadsprov togs från näs别emhinnan och förekomst av ämnen som attraherar eosinofiler (”kemokiner”) mättes parallellt med mängden eosinofiler. Särskilt noterade vi att kortisonbehandling påverkade upplösningen av den allergiska inflammationen och sammanföll med en minskad förekomst i vävnaden av kemokinen CCL5.

Sammanfattning

Genom att använda ett lokalt kortisonpreparat har vi först utvärderat en allergen-provokationsmodell och visat att relativt små skillnader i behandlingseffekt vid allergisk rinit kan mätas. Vi har sedan använt denna modell och visat att en $ß_2$-agonist inte påverkar symptom eller eosinofil inflammation vid allergisk rinit. I samma modell har vi bekräftat att den allergiska inflammationen minskar vid behandling med av ett lokalt kortisonpreparat. Genom att studera upplösningen av allergisk inflammation identifierade vi den eosinofilattraherande kemokinen CCL5 som särkilt kortisonkänslig. Denna påverkan av CCL5 skulle kunna utnyttjas som ny behandlingsstrategi. Vidare har vi visat, under naturlig pollen säsong, att allergisk rinit karakteriseras av en kraftig aktivering eosinofila celler med sönderfall av eosinofila granuler och frisättning av potenta proteiner.

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Paper I

Establishing a model of seasonal allergic rhinitis and demonstrating dose-response to a topical glucocorticosteroid

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Background: Symptoms of seasonal allergic rhinitis may vary greatly. Hence, for research purposes, there is a need for disease-like models of allergic rhinitis. In a preliminary study, involving 7 days' challenge with allergen, promising symptom consistency was obtained and dose-response to a glucocorticosteroid could, in part, be demonstrated.

Objective: To establish this model of seasonal allergic rhinitis and test the hypothesis that mometasone furoate is more potent than budesonide as an antirhinitis drug.

Methods: Thirty-eight patients with seasonal allergic rhinitis received treatment with spray-formulations of placebo, budesonide 64 μg, budesonide 256 μg, and mometasone furoate 200 μg in a double-blind, crossover design. After 3 days' treatment, individualized nasal allergen-challenges were administered daily for 7 days while the treatment continued. Nasal symptoms and peak inspiratory flow (PIF) were recorded.

Results: During the last 3 days of allergen challenge without active treatment, consistent around-the-clock symptoms were recorded and recordings during these days were used in the analysis. With few exceptions the active treatments reduced nasal symptoms and improved nasal PIF (P values <0.001 to 0.05). Budesonide caused dose-dependent improvements in evening symptoms, morning nasal PIF, and nasal PIF recorded 10 minutes after allergen-challenge (P values <0.05). Budesonide 256 μg produced greater improvement than mometasone furoate 200 μg for nasal PIF 10 minutes after allergen-challenge ($P < 0.05$).

Conclusion: The present allergen challenge method, producing consistent symptoms and nasal PIF data, emerges as a model of seasonal allergic rhinitis well suited for exploring potency and efficacy of drug intervention. The present data do not support the view that mometasone furoate is a more potent antirhinitis drug than budesonide.

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INTRODUCTION

Studies of allergic rhinitis usually involve either the natural course of the disease during the pollen season or allergen challenges in the laboratory. However, the former is often hampered by unpredictable and highly variable exposure to allergen, whereas the latter method may not mimic the full spectrum of the disease. In an attempt at finding a useful intermediate test system of allergic rhinitis, we have exposed subjects with seasonal allergic rhinitis to daily, symptom-producing allergen challenges for 1 week. At the end of this week nasal inflammation can be documented, eg, cytolytic eosinophils abound in the mucosal tissue,[1] and around-the-clock symptoms of allergic rhinitis may be experienced by the test subjects.[2] Further, using this model, we demonstrated glucocorticosteroid-induced symptom reductions and, for some of the subjective variables, dose-dependent drug effects were recorded.[2] These preliminary observations are remarkable, as even large-scale clinical trials have frequently failed to demonstrate dose-response to nasal glucocorticosteroids.[3–5] Hence, the repeat challenge method showed promise in terms of disease-like symptoms, their duration, consistency, and sensitivity to glucocorticosteroid treatment.

The present study uses repeat nasal allergen challenges to provide a useful model of allergic rhinitis, and examines effects of two anti-inflammatory drugs. To this end we have thus determined subjective symptoms, as in our previous work,[2] but now we have included an objective determination of nasal peak inspiratory flow (PIF) changes. We have further increased the sample size in this study, to find out whether previously observed trends regarding effects of glucocorticosteroid drugs[2] are significant, and we have, mainly for utilitarian reasons, shortened the allergen challenge series (from 8 to 7 days) as well as the washout periods (from 4 to 2 weeks). We have studied two doses of budesonide to determine dose-response to this drug. Additionally, we have made a preliminary comparison between budesonide and mometasone. Because only a single dose of mometasone is included, this study is not a fully balanced comparison between the two glucocorticosteroids.[6] However, the chosen dose levels in this study may test the proposal that mometasone could be more potent than budesonide in allergic rhinitis.[7]

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METHODS

Study Design

The present study was of a double-blind, placebo-controlled, randomized, crossover design. It comprised four different 10-day treatment periods: once-daily treatment with placebo, budesonide 64 μg, budesonide 256 μg, and mometasone 200 μg. Three days into each treatment period, a 7-day nasal allergen challenge series was started. Washout periods of 2 weeks were instituted between the treatment periods. The study was performed during the pollen-free autumn and winter months. The study was approved by the ethics committee as well as the Swedish Medical Product Agency, and informed consent was obtained. The study was conducted according to the Declaration of Helsinki.

Subjects

Thirty-eight patients were randomized to the study. Nineteen patients were women and 19 were men. Mean age was 25 years (range 19 to 43 years).

Inclusion criteria were: 1) a history of birch and/or timothy pollen-induced seasonal allergic rhinitis for at least the previous 2 years, verified by a positive skin prick test; 2) a need of treatment for nasal symptoms at seasonal allergen exposure; and 3) a nasal allergen challenge (see below) resulting in at least five sneezes and/or a symptom score of at least 2 or more on a scale from 0 to 3 for either of the symptoms nasal blockage and runny nose. In addition, female subjects had to be postmenopausal, surgically sterile, or on regular oral contraceptive treatment.

Exclusion criteria were: 1) perennial allergic rhinitis except for cat and/or dog sensitivity under the condition that these patients were not exposed to cats and dogs; 2) structural abnormalities of the nose; 3) any upper respiratory tract infection during a period of 2 weeks before the start of the study; 4) asthma; 5) current cardiovascular, renal, liver, or endocrinologic disease conditions; 6) planned hospitalization or planned blood donation during the study period; 7) topical glucocorticosteroid treatment within 1 month before the start of the study; 8) systemic glucocorticosteroid treatment for any reason during a period of 6 weeks before the start of the study; 9) regular use of dermal or rectal glucocorticosteroids; 10) immunotherapy for seasonal allergies; 11) antihistamine treatment during a period of 1 week before the start of the study; 12) pregnancy or nursing; 13) alcohol or drug abuse; or 14) participation in other clinical studies during the study period.

Reasons for withdrawal from the study were: 1) lack of cooperation on behalf of the study subject; 2) requirement of nonpermitted medication; 3) development of exclusion criteria or concurrent disease; 4) development of intolerable adverse events; 5) failure to participate in less than three treatment periods; and/or 6) pregnancy. In addition, the study subjects were free to discontinue their participation in the study at any time.

Physical Examination

At the start of the study, an ear, nose, and throat examination was carried out. A skin prick test with a panel of common seasonal and perennial allergens (Soluprick, ALK, Hørsholm, Denmark) was also performed. None of the patients had any perennial allergies, and none presented structural abnormalities or signs of upper respiratory tract infection.

Titration of the Nasal Allergen Challenge Dose

Titration of the nasal allergen challenge dose has been described previously.[2] Briefly, increasing doses of allergens (Alutard, ALK, Denmark) were administered at 10-minute intervals using a nasal spray device. The spray device delivered 50 μL per actuation, and two puffs were sprayed into each nostril resulting in effective doses of 100, 300, 1,000, and 3,000 standardized quantity units (SQ-U) per nasal cavity. This scheme was followed until the subject responded acutely with at least five sneezes and/or a symptom score of at least 2 or more on a scale from 0 to 3 (see below) for either of the symptoms nasal blockage and runny nose. The allergen dose that produced this effect was chosen for the daily allergen challenge series. All nasal allergen challenges were carried out at morning visits to the clinic.

Investigational Drug Treatments

Aqueous nasal spray formulations of the glucocorticosteroid budesonide (Rhinocort Aqua, AstraZeneca, Lund, Sweden) was used in two different concentrations (0.64 and 1.28 mg/mL). The drugs were provided in glass bottles fitted with a mechanical spray device. Each actuation delivered 50 μL to the nasal cavity, giving effective doses of 32 and 64 μg per actuation. A spray identical in appearance and taste to the active treatment, but without any active drug, was used as a placebo.

The glucocorticosteroid mometasone furoate (Schering Plough, Brüssels, Belgium) was used in one concentration (0.5 mg/mL). To achieve identical appearance between the preparations of active mometasone furoate and placebo both formulations were filled into identical 10-mL brown glass bottles. Mometasone furoate suspension in original plastic bottles (Nasonex nasal spray, Schering-Plough) was thus refilled into the glass bottles. A placebo formulation identical to the mometasone furoate suspension except for the content of mometasone furoate was manufactured and filled into identical glass bottles. All bottles were equipped with new spray pumps (Valois VP3/93, Le Vaudreuil, France) of the identical quality of the original pumps for mometasone furoate suspension. The only difference was the crimp diameter that had increased from 13 to 20 mm to fit the larger neck of the glass bottle. Finally, all bottles were equipped with new applicators (Valois CB 18) identical to the original product (Nasonex) except for the fitting to the larger crimp diameter. Each actuation delivered 100 μL to the nasal cavity, giving a dose of 50 μg. A spray identical in appearance and taste to the active treatment, but without any active drug, was used as placebo.

The subjects received two identical bottles before each treatment period and were instructed to take one dose into each nostril from each bottle in the morning. In the placebo run, both bottles contained the placebo solution, and half of the patients received placebo for mometasone furoate and the other half placebo for budesonide. In the 64-μg budesonide run, one bottle contained the 0.64 mg/mL budesonide solution and the other the placebo solution. In the 256 μg budesonide run, both bottles contained the 1.28 mg/mL budesonide solution. In the mometasone run both bottles contained the 0.5 mg/mL mometasone furoate solution.

Therefore, the total doses of budesonide were 64 and 256 μg, once daily, respectively, and the total dose of mometasone furoate was 200 μg, once daily. Intake of the first dose of each study drug was carried out at the clinic under the supervision of the investigators. Medication then was taken at home every morning. Compliance was confirmed by interview at each visit. Treatment continued until the end of each allergen challenge series.

Clinical Measurements

The nasal symptoms—blocked nose, runny nose, and sneezy/itchy nose—were scored by the patients before the intake of the drug in the morning (rating symptoms during the preceding 12 hours) and 10 minutes after each allergen challenge. In addition, symptoms were scored in the evening (again, the rating reflected symptoms during the preceding 12 hours). The symptoms were each scored 0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms, 3 = severe symptoms. The scores were added to constitute a total daily score, which thus ranged from 0 to 9. Mean total nasal symptom scores, for morning recordings, for recordings 10 minutes after allergen challenge, and for evening recordings, respectively, of the last 3 days of each allergen challenge period were used in the analysis. Hence, treatment effects were evaluated when the "artificial pollen season" had developed for 5 to 7 days and when the drugs had been given for 8 to 10 days. In this situation, consistent and seasonal-like around-the-clock symptoms would likely have been developed.

Nasal PIF

Nasal PIF was measured by the patients before the intake of the drug in the morning, 10 minutes after each allergen challenge, and in the evening, and recordings of the last 3 days of each allergen challenge period were used in the analysis. The measurements were carried out using a nasal PIF-meter (Clement Clarke, Harlow, UK).

Statistics

The total nasal symptom score was defined as the sum of the individual nasal symptom scores obtained during last 3 days of each treatment and allergen challenge period. The symptoms were blocked nose, runny nose, and the most severe symptom of sneezing and itchy nose. The total nasal symptom score was calculated for the last 3 days of each allergen challenge period and the mean values were subjected to analysis of variance with treatment, subject, and period as factors in the model. To determine dose-response effects of budesonide, least squares estimates were used on comparisons between active treatments and placebo. Individual nasal symptom scores were analyzed in the same way. For nasal PIF measurements, mean values were calculated over last 3 days of each allergen challenge period. These mean values were subjected to analysis of variance with the same factors as described above. If appropriate, the baseline value was included as a covariate in the model. Parametric tests were used, and, in all tests of significance, two-tailed alternatives were considered. P values <0.05 were considered statistically significant. Data are presented as mean values $\pm$ SEM.

RESULTS

The nasal allergen challenge titration resulted in 2 subjects receiving allergen challenge at 100 SQ-U, 3 subjects at 300 SQ-U, 27 subjects at 1,000 SQ-U, and 6 subjects at 3,000 SQ-U. Thirteen subjects received challenges with birch pollen allergen, and 25 subjects with timothy pollen allergen. Thirty-eight patients were randomized to the study. Patients who had completed at least three study periods were considered eligible for analysis. Two subjects were withdrawn prematurely after having completed two treatment periods only. Thus, 36 patients were included in the statistical analysis. Six subjects completed only three treatment periods.

The 7 days of allergen challenges were well tolerated by the subjects and no untoward side effects occurred. In subjects receiving placebo, the nasal symptoms progressed gradually during the allergen challenge series, and during the last 3 days, around-the-clock symptoms were established (Fig 1). During the washout periods the nasal symptoms returned to baseline levels, ie, symptomless conditions (Fig 1).

Morning and Evening Recordings of Nasal Symptoms and Nasal PIF

Mean values, for the last 3 days of the allergen challenge series, of morning and evening symptoms as well as morning and evening nasal PIF are shown in Figure 2a-d. Budesonide 64 and 256 μg, as well as mometasone 200 μg, reduced morning and evening nasal symptoms compared with placebo (P values <0.001 for all comparisons except for evening nasal symptoms between budesonide 64 μg and placebo where $P < 0.01$). The reduction in evening nasal symptoms with budesonide 256 μg was significantly greater than the reduction with budesonide 64 μg ($P < 0.05$).

Morning nasal PIF was significantly improved by budesonide 256 μg compared with placebo ($P < 0.001$), whereas the effects of budesonide 64 μg and mometasone 200 μg did not reach statistical significance. The improvement in morning nasal PIF with budesonide 256 μg was significantly greater than the effects with budesonide 64 μg ($P < 0.05$). Evening nasal PIFs were significantly improved by budesonide 256 μg ($P < 0.01$) and mometasone 200 μg ($P < 0.05$) compared with placebo, whereas the effects of budesonide 64 μg failed to reach statistical significance.

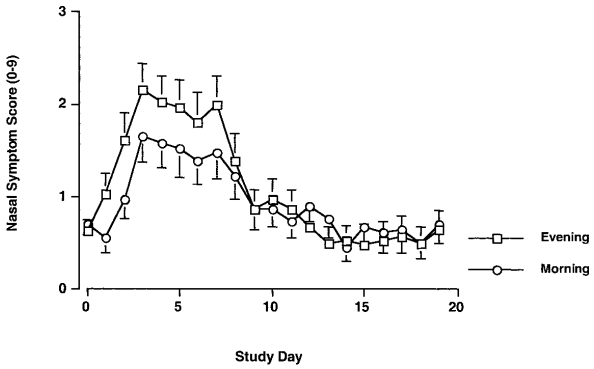

Figure 1. Mean ± SEM nasal symptom scores obtained from morning (circles) and evening (squares) diary recordings during the treatment and allergen challenge period as well as during the washout period in subjects receiving placebo treatment. The challenge period started on study day 1 and ended with a challenge given on day 7. (Note that the recording in the morning of study day 1 was made before any allergen challenge had been given.) The nasal symptoms progressed during the allergen challenge series, and during the last 3 days, around-the-clock symptoms were established. During the washout periods, the nasal symptoms returned to baseline levels.

Nasal Symptoms and Nasal PIF Recordings 10 Minutes after Challenge

Mean values, for the last 3 days of the allergen challenge series, of symptoms and nasal PIF 10 minutes after allergen challenge are shown in Figure 3a and b: budesonide 64 and 256 μg, as well as mometasone 200 μg, reduced nasal symptoms and improved nasal PIF compared with placebo (P values <0.001). The effect on nasal PIF 10 minutes after allergen challenge with budesonide 256 μg was significantly greater than the effects produced by budesonide 64 μg ($P < 0.05$) or mometasone 200 μg ($P < 0.05$).

DISCUSSION

The present study, involving subjects with seasonal allergic rhinitis repeatedly challenged with allergen outside the pollen season, confirms and expands our previous observations of dose-dependent effects of an aqueous nasal spray formulation of budesonide on allergic rhinitis symptoms. Further, significant and dose-dependent effects of budesonide on the nasal PIF are demonstrated. The present study also demonstrates significant effects on allergic rhinitis symptoms and nasal PIF of a once-daily-dose nasal spray formulation of mometasone furoate. This allergen challenge model may be useful in studies of the clinical pharmacology and efficacy of topical nasal glucocorticosteroids.

With the present allergen challenge model, we have aimed at producing consistent and well tolerated symptoms of allergic rhinitis maintained around the clock. After a nasal allergen challenge titration scheme, designed to reduce variability attributable to individual differences in allergen sensitivity, symptom-producing challenges above symptom threshold were administered once daily for 7 days. In confirmation of our previous study,[2] the present diary cards demonstrated mild to moderate nasal symptoms during day and evening hours. Importantly, around-the-clock symptoms characterized the last 3 days of the allergen challenge series, which equaled the present evaluation period. However, and importantly, after the condensed 7 days of artificial season a prompt return to symptomless conditions occurred. These data support the repeatability of the present artificial season when intervals of 2 weeks are allowed as used in this study. This interval was also considered to result in a reasonable and practical washout period for the glucocorticosteroid drugs that had been given during the 10 days only. Yet, further studies may be warranted to examine in detail the effect of different washout periods in this model.

We have previously demonstrated that repeated daily allergen challenges, in addition to evoking disease-like symptoms, produce a pathophysiology characterized by significant eosinophilia where virtually all mucosal eosinophils exhibit distinct signs of degranulation, including piecemeal degranulation and cytolysis with generation of free eosinophil granules.[1] Further, the challenged nasal mucosa in this model exhibits exudation of plasma and development of nasal mucosal hyperresponsiveness to histamine.[8] These signs of exudative and eosinophilic inflammation are similar to the features of real airway disease.[9,10] Several aspects of the pharmacology of inflammation associated with allergic rhinitis may be examined in this model. However, it is likely that interventions with nasal lavages, brushings, and biopsies, etc, would affect nasal symptom scores. Therefore, effects of glucocorticosteroids on allergic rhinitis symptoms and "inflammation," respectively, may best be examined by separate studies. In the present study the focus is on the effects of drug treatment on allergic rhinitis symptoms. However, nasal PIF measurements, reflecting an important clinical aspect of treatment efficacy, were included as an objective parameter in this study. Our observations on nasal PIF agreed well with the present symptom recordings, supporting the utility of nasal PIF in studies of allergic rhinitis.

The data obtained by nasal PIF measurements 10 minutes post challenge represent an experimental situation and may not entirely mimic natural disease. However, in addition to being an objective parameter, these measurements were included also to provide us with an evaluation point where consistently strong allergic reactions were induced and where the room for graded improvement by drug treatment would be

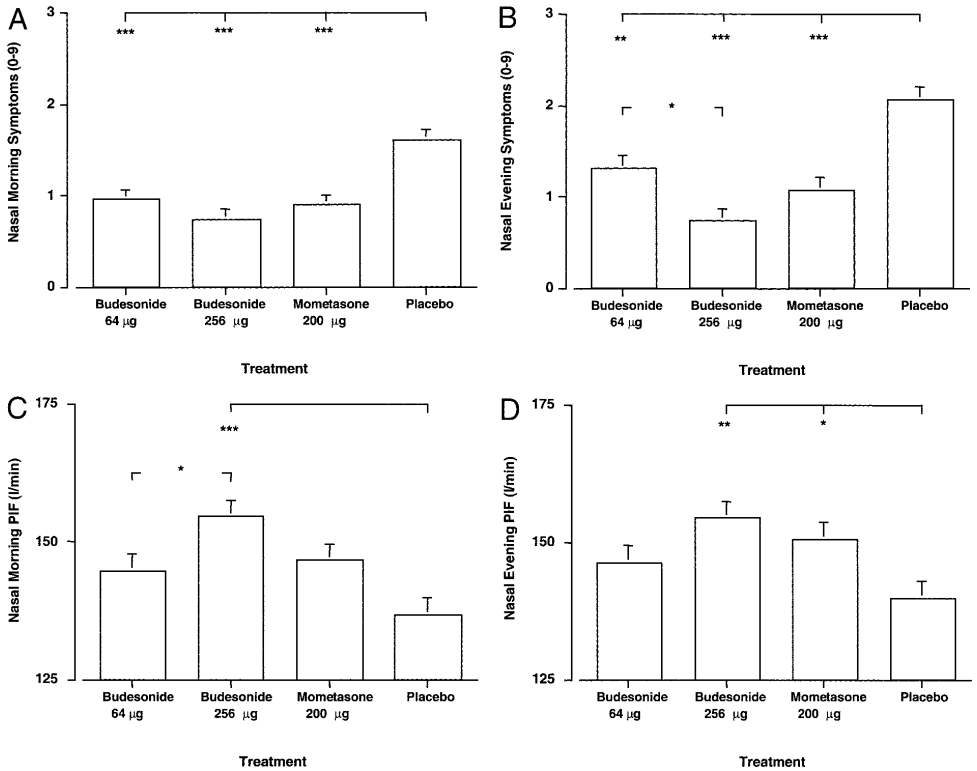

Figure 2. Mean ± SEM values of total nasal symptom scores (*A* and *B*) and nasal PIF (*C* and *D*) recorded in the morning (*A* and *C*) and in the evening (*B* and *D*), respectively, obtained from the last 3 days of the allergen challenge period. Budesonide and mometasone reduced morning and evening nasal symptoms compared with placebo. The reduction in evening nasal symptoms with budesonide 256 μg was significantly greater than the reduction with budesonide 64 μg. Morning nasal PIF was significantly improved by budesonide 256 μg compared with placebo. This improvement in nasal PIF with budesonide 256 μg was significantly greater than the effects with budesonide 64 μg. Evening nasal PIF was significantly improved by budesonide 256 μg and mometasone 200 μg compared with placebo. (Comparisons are made between active treatment and placebo unless otherwise indicated; *, **, and *** denote $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.)

desirably great. Further, we have previously demonstrated that the allergen challenge-induced effect is greater during the allergic season than outside the season,[11] and therefore, the 10 minutes postchallenge measurements in this study may reveal a degree of specific allergic nasal hyperresponsiveness and its inhibition by drugs.

Glucocorticosteroids are widely used as effective treatments of allergic rhinitis and asthma. It is important to evaluate the dose-response relationships for these drugs to avoid unnecessarily high doses as well as undertreatment. In allergic rhinitis, it has been difficult to demonstrate dose-response during natural allergen exposure.[3–5] By contrast, in the present model, dose-dependent effects of budesonide on nasal symptoms are repeatedly demonstrated.[2] In this study, dose-dependent drug effects were thus noted for evening symptoms, morning nasal PIF, and nasal PIF 10 minutes after allergen challenge: 64 μg of the nasal spray formulation of budesonide was effective and further significant effects were gained by increasing the dose fourfold to 256 μg. The nasal spray formulation of mometasone also reduced nasal symptoms and nasal PIF. Using datapoints where a clear dose-response effect of budesonide was demonstrated (ie, evening symptoms, morning nasal PIF, and nasal PIF 10 minutes after allergen challenge) and assuming a near linear dose-response relationship in the 64- to 256-μg interval, a rough estimation of a potency ratio between budesonide and mometasone could not support the notion that mometasone would be more potent than budesonide. Rather, the effect

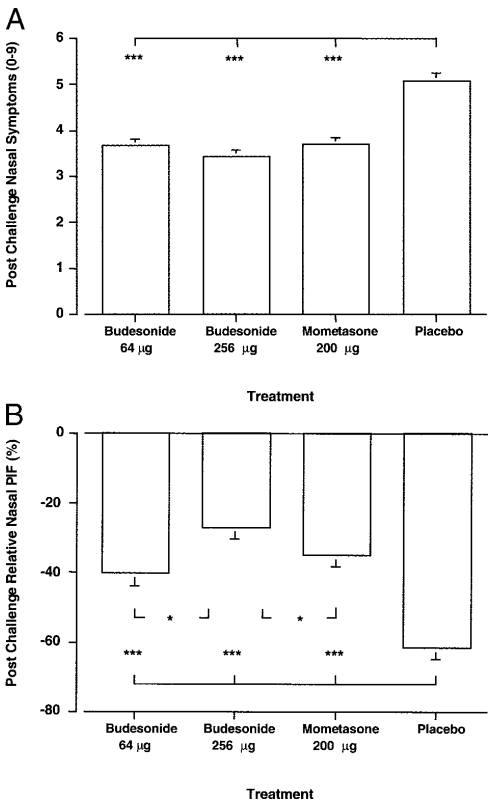

Figure 3. Mean ± SEM values of total nasal symptom scores (*A*) and nasal PIF (*B*), recorded 10 minutes after allergen challenge, obtained from the last 3 days of the allergen challenge period. Budesonide and mometasone reduced nasal symptoms and improved nasal PIF compared with placebo. The effect on nasal PIF 10 minutes after allergen challenge with budesonide 256 μg was significantly greater than the effects with budesonide 64 μg and mometasone 200 μg. (Comparisons are made between active treatment and placebo unless otherwise indicated; * and *** denote $P < 0.05$ and $P < 0.001$, respectively.)

of mometasone 200 μg fell closer to effects obtained by 64 μg than 256 μg of budesonide.

Potency merely gives information about the dose of a drug that is required to produce a certain level of effect. Whether symptoms, inflammatory indices, or other variables are recorded is not crucial. Thus, the present indication of dose-dependent effects of nasal budesonide in reducing symptoms informs us about the potency of this glucocorticosteroid drug formulation under the conditions of the present study. We submit that potency in this regard would be much closer to clinical efficacy than any effect on mechanisms with unknown real importance in disease. However, the general term efficacy is the level of effect that is reachable with a given drug, and is thus in a strict and theoretical sense independent of a drug's potency. Clearly, our study was not designed to determine any difference in clinical efficacy between glucocorticosteroid drugs, nor would we hypothesize that a difference in clinical efficacy would be seen between these two drugs that belong to the same class of pharmacologic agents. The potency ratio between budesonide and mometasone needs to be addressed in further studies where dose-response is established also with mometasone, and where potential differences in onset of action as well as in time to development of full efficacy can be accommodated. Also, studies where identical doses are given may be warranted. A known potency ratio may be of some practical importance when switching treatment drugs. Also, it is only when accurate potency ratios have been established that meaningful comparisons concerning side effects of nasal glucocorticosteroids can be made. This study was not designed to explore side effects, which may require both larger glucocorticosteroid doses and longer-term treatment periods than used in the present study.

Using the present model, an artificial season of allergic rhinitis is created in individuals with strictly seasonal disease. This artificial season provides several advantages. Allergen exposure and symptom levels are standardized, providing consistent objective as well as subjective symptom recordings with reduced variability compared with most natural courses of seasonal allergic rhinitis. Further, the present protocol involves a crossover design that eliminates interindividual variability. Indeed, the demonstration in this study of the clinical efficacy of glucocorticosteroids including evident dose-dependency of the effects of budesonide aqueous nasal spray attests to the utility of the present model of repeat allergen challenge. The sensitivity to anti-inflammatory glucocorticosteroids in this study is evident by the demonstration of clinical efficacy of budesonide already at the 64-μg dose level. Hence, it is likely that a fuller spectrum of disease features develop by the present protocol than seen in single allergen challenge studies where nasal glucocorticosteroids may exhibit poor effects.[12]

CONCLUSION

We conclude that the daily allergen challenge model as used and developed in this study is well tolerated, mimics important features of natural seasonal allergic rhinitis, is suited for crossover designs, and has utility in exploration of clinical efficacy and clinical pharmacologic properties of anti-inflammatory drugs. The advantages of the model were in part borne out by the demonstration of significant reduction of symptoms and increase in PIF by both budesonide and mometasone furoate. Further, dose-dependency on several points of the antirhinitis action of budesonide was established.

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REFERENCES

1. Erjefält JS, Greiff L, Andersson M, et al. Allergen-induced eosinophil cytolysis is a primary mechanism for granule protein release in human upper airways. Am J Respir Crit Care Med 1999;160:304–312.
2. Andersson M, Svensson C, Persson CG, et al. Dose-dependent effects of budesonide aqueous nasal spray on symptoms of allergic rhinitis in a daily nasal allergen challenge model. Ann Allergy Asthma Immunol 2000;85:279–283.
3. Bronsky EA, Aaronson DW, Berkowitz RB, et al. Dose ranging study of mometasone furoate (Nasonex) in seasonal allergic rhinitis. Ann Allergy Asthma Immunol 1997;79:51–56.
4. Stern MA, Dahl R, Nielsen LP, et al. A comparison of aqueous suspensions of budesonide nasal spray (128 micrograms and 256 micrograms once daily) and fluticasone propionate nasal spray (200 micrograms once daily) in the treatment of adult patients with seasonal allergic rhinitis. Am J Rhinol 1997;11: 323–330.
5. Meltzer EO. Clinical and antiinflammatory effects of intranasal budesonide aqueous pump spray in treatment of perennial allergic rhinitis. Ann Allergy Asthma Immunol 1998;81: 128–134.
6. Pedersen S, O'Byrne P. A comparison of the efficacy and safety of inhaled corticosteroids in asthma. Allergy 1997;52(Suppl): 1–34.
7. Davies RJ, Nelson HS. Once-daily mometasone furoate nasal spray: efficacy and safety of a new intranasal glucocorticoid for allergic rhinitis. Clin Ther 1997;19:27–38.
8. Svensson C, Andersson M, Greiff L, Persson CG. Physiological and cellular changes in a model of daily nasal allergen challenges: an experimental pollen season. Eur Respir J 1995; 8:124S.
9. Naclerio RM, Meier HL, Kagey-Sobotka A, et al. Mediator release after nasal airway challenge with allergen. Am Rev Respir Dis 1983;128:597–602.
10. Greiff L, Erjefält J, Andersson M, et al. Generation of clusters of free eosinophil granules (Cfegs) in seasonal allergic rhinitis. Allergy 1998;53:200–203.
11. Svensson C, Andersson M, Greiff L, et al. Exudative hyperresponsiveness of the airway microcirculation in seasonal allergic rhinitis. Clin Exp Allergy 1995;25:942–950.
12. Andersson M, Rimmer J, Salome C, et al. Dual symptomatic and exudative nasal responses are not characteristics of perennial allergic rhinitis. Acta Otolaryngol 2001;121:407–413.

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Paper III

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Effects of topical formoterol alone and in combination with budesonide in a pollen season model of allergic rhinitis

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KEYWORDS
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Summary
Background: β_2-Agonists may exert mast cell stabilizing and anti-plasma exudation effects. While available data suggest no or only marginal effects of β_2-agonists on symptoms of allergic rhinitis, little is known about whether these drugs may add to the efficacy of anti-rhinitis drugs.
Objective: To examine effects of a β_2-agonist, alone and in combination with an intranasal glucocorticosteroid, on symptoms and signs of allergic rhinitis.
Methods: Patients were examined in a pollen season model. Budesonide 64 μg, alone and in combination with formoterol 9 μg, as well as formoterol 9 μg alone was given in a placebo-controlled and crossover design. After 7 days of treatment, the patients received allergen challenges for 7 days. Symptoms and nasal peak inspiratory flow (PIF) were recorded. Nasal lavages with and without histamine were carried out at the end of each challenge series. These lavages were analysed for tryptase, eosinophil cationic protein (ECP), and α_2-macroglobulin as indices of mast cell activity, eosinophil activity, and plasma exudation, respectively.
Results: Budesonide reduced symptoms of allergic rhinitis and improved nasal PIF in the morning, in the evening as well as post allergen challenge. Formoterol alone did not affect symptoms or nasal PIF and did not affect the efficacy of budesonide. Tryptase, ECP, and α_2-macroglobulin were significantly reduced by budesonide. Formoterol alone did not affect these indices and did not affect the anti-inflammatory effect of budesonide.
Conclusion: The present dose of formoterol does not affect symptoms and inflammatory signs of allergic rhinitis and does not add to the efficacy of topical budesonide.

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Introduction

β_2-Agonists have been demonstrated to exert effects that may be characterized as anti-inflammatory. Initial airway observations in vivo comprised work on animals in which terbutaline and salmeterol, and later formoterol, were shown to possess anti-plasma exudation properties.[1–7] These observations were extended into studies on human nasal airways where terbutaline and salmeterol were demonstrated to reduce allergen challenge-induced plasma exudation in allergic rhinitis.[8,9] In the study by Svensson et al.,[8] reduced nasal lavage fluid levels of tryptase were also reported with terbutaline treatment, suggesting a β_2-agonist-induced mast cell stabilizing effect. The latter possibility was supported by in vitro observations focusing on β_2-agonist actions and mast cell histamine release.[10] Taken together, the above observations suggest that β_2-agonists may exert an anti-inflammatory action in allergic rhinitis.

Focusing on a potential clinical efficacy of β_2-agonists in allergic rhinitis, studies employing allergen-challenges have demonstrated reductions in acutely induced nasal symptoms by high doses of nasal β_2-agonists (terbutaline, fenoterol).[8,11] However, negative studies are also available: For example, in a study involving 15 patients with allergic rhinitis examined at seasonal allergen exposure, formoterol administered nasally twice daily for 1 week failed to affect symptoms of allergic rhinitis compared with placebo.[12] Whereas β_2-agonists thus may have no or only marginal effects on symptoms of allergic rhinitis, little is known about whether or not this class of drugs would add to the efficacy of anti-rhinitis drugs. In the present study, we have hypothesized that the use of a β_2-agonist in combination with an intranasal glucocorticosteroid (GCS) might lead to a potentiation of the GCS-induced anti-inflammatory effect and possibly to some degree of improved clinical efficacy.

It is difficult to demonstrate an increase in efficacy by a combination of a non-GCS drug and a GCS over the GCS alone at seasonal allergen exposure. This reflects uncertainties concerning onset and intensity of the allergen exposure. Additionally, it reflects that it is difficult to perform studies of crossover designs during the pollen season. Finally, parallel group studies are hampered by interindividual differences in allergen sensitivity. In order to overcome these problems, we have employed once daily challenges with individualized allergen doses carried out for seven consecutive days as a pollen-season model.[13] The evaluation period in our model, i.e., the last 3 days of the challenge series, is characterized by around-the-clock symptoms. Using this model, we have shown dose–response relationships for a topical GCS,[13] indicating that even small changes in efficacy can be detected. It seems likely, therefore, that the pollen-season model would be well suited for the present comparison (below).

In the present study, involving patients with allergic rhinitis, we have examined effects of budesonide alone and in combination with a topically high dose of formoterol, as well as of formoterol alone, on symptoms of allergic rhinitis and nasal peak inspiratory flow (PIF) in our pollen season model. Furthermore, we have carried out nasal saline lavages at the end of each treatment/challenge period and monitored levels of α_2-macroglobulin and tryptase as indices of plasma exudation and mast cell activity, respectively. Moreover, we have included an analysis of eosinophil cationic protein (ECP) as a marker of the increased eosinophil granulocyte activity that characterizes allergic rhinitis. In order to improve the lavage fluid yield of cellular markers (i.e., tryptase and ECP), we have carried out histamine lavages. This experimental measure produces plasma exudation, a process that may move free tryptase and ECP from the lamina propria into the nasal lumen.[14] In addition, the employment of histamine challenges allows for an estimation of the nasal exudative responsiveness to histamine.

Material and methods

Study design (Table 1)

The study was of a randomized, placebo-controlled (double-dummy), double-blinded, and crossover design. It comprised four 15-day treatment periods, all carried out in the pollen-free Scandinavian autumn/winter months. The treatments were budesonide plus formoterol, budesonide plus placebo, formoterol plus placebo, and placebo plus placebo, all given topically once daily. Wash-out periods of at least 2 weeks were instituted between the treatment periods. After 1 week of treatment (*Study day 8*), a series of individualized, once-daily allergen challenges commenced while the treatment continued. The allergen challenges were given for 7 days and symptom scores and nasal PIF rates of the last 3 days of the challenge series were used in the analysis. In addition, on *Study day 15*, nasal lavages with and without histamine were carried out and analysed for tryptase, ECP, and α_2-macroglobulin as indices of mast cell activity, eosinophil activity, and plasma exudation, respectively. The study was approved by the Regional Ethics Committee and the Swedish Medical Product Agency. It was conducted according to the Declaration of Helsinki and informed consent was obtained .

Subjects

Forty patients were recruited to the study (25 men and 15 women, 18–37 years old). All patients presented with a history of allergic rhinitis during the birch and/or timothy pollen season requiring treatment with nasal GCSs for at least two pollen seasons preceding the study. According to the ARIA document,[15] all patients were classified as intermittent allergic rhinitis of moderate to severe intensity. A skin prick test was carried out (Soluprick, ALK, Horsholm, Denmark) and all subjects had positive reactions to birch and/or timothy allergen, defined as a weal and flare response of greater diameter than that of a histamine skin prick test. Patients with skin reactions to house dust mite were not included in the study. Individuals with reactions to cat and/or dog dander were included only if they were not regularly exposed to these animals. All subjects underwent an ear, nose, and throat examination and those with signs of upper respiratory tract infection or significant structural nasal abnormalities were excluded from the study. Patients with a history of asthma or other chronic diseases were also excluded from the study. Pharmacological anti-rhinitis

Table 1 The table describes treatment, allergen challenges, and nasal lavage for one of the four treatment/challenge periods.

	Study day													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Treatment	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Allergen challenge							X	X	X	X	X	X	X		
Nasal lavage															X

treatment, except for the study drugs, was not allowed during the study period.

Allergen challenge model

In order to establish individually tolerable, repeatable, yet symptom-producing allergen challenge doses, a nasal titration procedure was performed. The allergen that produced the largest skin test reaction was chosen as challenge agent (Alutard, ALK, Horsholm, Denmark). Increasing doses of the allergen were administrated at 10 min intervals using a spray-device delivering 100 μL per actuation. One actuation was administered into each nostril resulting in effective doses of 100, 300, 1.000, and 3.000 standardized quantity (SQ) units per nasal cavity. This scheme was followed until the subject responded with at least 5 sneezes or recorded a symptom score of 2 or more on a scale from 0 to 3 for either of the symptoms nasal secretion or nasal blockage. The allergen dose that produced this effect was chosen for the allergen challenge series and was given once daily for 7 days (*Study days 8–14*).

Investigational treatments

An aqueous suspension of budesonide (Rhinocort® Aqua, AstraZeneca, Lund, Sweden) was used in a concentration of 0.64 mg/mL. The suspension was provided in glass bottles fitted with a mechanical spray-pump delivering 32 μg budesonide per actuation. The placebo solution for budesonide was provided in identical glass bottles. Formoterol (AstraZeneca, Lund, Sweden) was provided in a multidose, inspiratory flow-driven, dry powder inhaler fitted with a nasal adapter delivering 4.5 μg formoterol per inhalation. An inhaler identical in appearance and taste to the active treatment was used for administration of the placebo powder. The subjects received one nasal spray-device and one dry powder inhaler at the start of each treatment period and were instructed to administer one dose from each device into each nostril every morning. In the budesonide plus formoterol run both devices contained active substance and in the placebo run both contained placebo. In the mixed runs the devices contained budesonide plus placebo and placebo plus formoterol, respectively. The total daily doses administered in the active drug runs were 64 μg for budesonide and 9 μg for formoterol. The first dose of each study drug was taken at the clinic under supervision of the investigator and subsequent doses were taken at home in the morning. Treatment started on *Study day 1* and continued through *Study day 15*.

Clinical measurements

Throughout the study, including washout periods, the subjects scored nasal symptoms every morning and evening. The scores were entered into diary cards and each registration reflected the preceding 12 h. The symptoms nasal secretion and nasal blockage as well as the most severe of the symptoms sneezing and itching were each scored on a 4-point scale where 0 = no symptoms, 1 = mild symptoms, 2 = moderate symptoms, and 3 = severe symptoms. Symptom scores for morning and evening recordings, respectively, were added to a daily nasal symptom score. Morning and evening scores, respectively, from the 5th, 6th, and 7th challenge day were added and divided by three resulting in a mean total nasal symptom score (TNSS) (range 0–9). Nasal symptoms were also scored at 10 and 20 min post allergen challenge. The symptoms nasal secretion and nasal blockage were scored as described above, whereas the number of sneezes were counted and translated into a sneezing score by the investigator: 0 sneezes = 0, 1–4 sneezes = 1, 5–9 sneezes = 2, and 10 or more sneezes = 3. Mean TNSS for these post challenge symptom scores were calculated from observations made on the 5th, 6th, and 7th challenge day as described above.

Nasal PIF was measured every morning and evening as well as 20 min post allergen challenge. Nasal PIF was measured using a nasal inspiratory flow meter (Clement-Clark, Harlow, UK) and the highest flow of three measurements was registered. The assessments were carried out after scoring of nasal symptoms but before administration of study drug. Mean nasal PIF (for morning, evening, and post challenge observation, respectively) from observations made on the 5th, 6th, and 7th challenge day were calculated in the same way as described for symptoms above.

Nasal lavages

Nasal saline lavages with and without histamine (40 and 400 μg/mL) (Sigma, St. Louis, MO) were carried out on the last day of each treatment period (*Study day 15*). A nasal pool-device containing 15 mL of fluid was used to perform these lavages.[14] The first lavage was a 2.5 min saline lavage. This lavage was immediately followed by two 1 min saline lavages, carried out to remove solutes from the mucosal surface and thereby establishing a low and stable baseline. (These two lavages were discarded.) Thereafter, three 5 min lavages with saline, 40 μg/mL histamine, and 400 μg/mL histamine, respectively, were carried out. The latter challenges/lavages were carried out in rapid succession. The lavage fluids were centrifuged (105*g*, 10 min, 4 °C) and samples were obtained from the supernatant and frozen (−20 °C) awaiting analyses of tryptase, ECP, and α_2-macroglobulin.

Analyses

α_2-Macroglobulin was measured using a radioimmunoassay sensitive to 7.8 ng/mL. As antiserum a rabbit anti-human

α_2-macroglobulin (Dakopatts, Copenhagen, Denmark) was used and as standard a human serum (Boehringerwerke-Diagnostica, Marburg, Germany). Human α_2-macroglobulin (Cappel/Organon-Teknika, Turnhout, Belgium) was iodinated using the lactoperoxidase method. Tracer and standard (or sample) were mixed with antiserum before adding goat anti-rabbit antiserum (AstraZeneca, Lund, Sweden). The bound fraction was measured using a gamma counter (Pharmacia-Diagnostics, Uppsala, Sweden) The intra- and inter-assay coefficients of variation were between 3.8–6.0% and 3.1–7.2%, respectively.

Nasal lavage fluid levels of ECP were measured using a flouroimmunoassay (Pharmacia-Diagnostics, Uppsala, Sweden) with a sensitivity of 2.0 ng/mL. Tryptase was measured using a modified radioimmunoassay with a detection limit of 0.5 ng/mL (Pharmacia-Diagnostica, Uppsala, Sweden). α_2-Macroglobulin and ECP were analyzed in the lavage fluids as they were, whereas samples of the lavage fluids were concentrated 20 times before the analysis of tryptase.

Statistics

Mean values and standard deviations (S.D.) were calculated for TNSS and nasal PIF, for morning, evening, and post-challenge observations respectively, reflecting observations made on the 5th, 6th, and 7th challenge days of each treatment period. The intention-to-treat population was the primary analysis population. For the primary variable, i.e., mean TNSS, a two-sided test was used. The test was carried out using a primary statistical model of one-way analysis of covariance with change from baseline as dependent variable, subject, treatment, and periods as study factors, and baseline value for Mean TNSS as covariate. In order to address the multiple comparison issue, comparisons between treatment groups progressed in a stepwise fashion. The first comparison was between the budesonide 64 μg plus formoterol 9 μg group and placebo. If a significant difference was demonstrated at the $\alpha = 0.05$ level, then the budesonide 64 μg plus formoterol 9 μg group was compared with the budesonide 64 μg group. *P*-values were calculated for the comparisons. Nasal PIF was analyzed using the same model and *p*-values were calculated.

Mean values and standard error of the mean (S.E.M.) were calculated for lavage fluid levels of tryptase, ECP, and α_2-macroglobulin, respectively. Differences between the treatment groups were analyzed using the Friedman test and, if statistical significance emerged, using the Wilcoxon signed rank-test. A *p*-value of less than 0.05 was considered statistically significant.

Results

Forty patients were randomized and 38 completed three or more study periods. These 38 subjects were considered eligible for analysis. The two subjects who completed only one or two treatment periods were excluded because of common cold symptoms at the start of more than one study-period. Data on numbers of subjects who completed each treatment period are given in Table 2.

In the present study, 26 patients received timothy-pollen allergen and 14 birch-pollen allergen. The titration procedure resulted in that 5, 14, 5, and 2 subjects received challenges with 100, 300, 1000, and 3000 SQ-U, respectively, in the timothy-allergen group. The corresponding figures in the birch allergen group were 1, 5, 6, and 2. The allergen challenge series were well tolerated and no unexpected side effects occurred. In the placebo run, the challenge series produced mild to moderate around-the-clock symptoms during the evaluation period (Table 2). During the washout periods, nasal symptoms returned to symptomless baseline levels (data not shown).

Mean TNSS in the evening and in the morning were significantly reduced by budesonide alone and by budesonide in combination with formoterol (Tables 2 and 3). Also, these treatments significantly reduced TNSS 10 as well as 20 min post challenge. In contrast, formoterol alone did not affect evening or morning TNSS or TNSS post challenge (Tables 2 and 3). Moreover, formoterol did not add to the symptom-reducing efficacy of budesonide (Tables 2 and 3).

Nasal PIF recorded in the evening and morning was improved by budesonide alone and by budesonide in combination with formoterol (Tables 2 and 3). These changes all reached statistical significance except for nasal PIF recorded in the morning, which reached borderline significance. Also, these treatments significantly improved nasal PIF 20 min post challenge. Formoterol alone did not affect evening or morning nasal PIF or nasal PIF post challenge (Tables 2 and 3). Formoterol did not add to the PIF-improving efficacy of budesonide (Tables 2 and 3).

Nasal lavage fluid levels of tryptase were significantly reduced by budesonide alone and by budesonide in combination with formoterol in the second saline lavage

Table 2 Mean TNSS ± S.D. for total nasal symptoms and PIF (L/min) based on recordings obtained during the last 3 days of each allergen challenge series.

	BANS+Formoterol ($n = 37$)	BANS ($n = 34$)	Formoterol ($n = 37$)	Placebo ($n = 38$)
Evening TNSS	0.69 ± 0.85	0.72 ± 0.87	1.80 ± 1.62	1.97 ± 1.88
Morning TNSS	0.70 ± 0.84	0.85 ± 0.82	1.59 ± 1.42	1.74 ± 1.58
Evening PIF	179 ± 55	176 ± 58	159 ± 60	154 ± 58
Morning PIF	166 ± 52	165 ± 55	152 ± 55	152 ± 56
TNSS 10 min post challenge	3.84 ± 2.11	3.91 ± 1.80	5.90 ± 1.93	6.43 ± 1.95
TNSS 20 min post challenge	1.76 ± 1.39	1.89 ± 1.27	3.20 ± 1.62	3.46 ± 1.57
PIF 20 min post challenge	131 ± 56	128 ± 63	107 ± 52	105 ± 51

BANS = Budesonide aqueous nasal spray. S.D. = Standard deviation. TNSS = Total nasal symptom score. PIF = Peak inspiratory flow.

Table 3 *P*-values for paired comparisons between the different treatments.

	BANS+Formoterol vs. placebo	BANS+Formoterol vs. BANS	Formoterol vs. placebo	BANS vs. placebo
Evening TNSS	0.0000	0.7133	0.4570	0.0001
Morning TNSS	0.0000	0.4203	0.4787	0.0003
Evening PIF	0.0000	0.4827	0.5882	0.0003
Morning PIF	0.0063	0.4904	0.5156	0.0502
TNSS 10 min post challenge	0.0000	0.9485	0.0785	0.0000
TNSS 20 min post challenge	0.0000	0.6771	0.2862	0.0000
PIF 20 min post challenge	0.0001	0.6441	0.7800	0.0000

BANS = Budesonide aqueous nasal spray. TNSS = Total nasal symptom score. PIF = Peak inspiratory flow.

($p<0.05-0.01$) (Fig. 1A). In addition, tryptase was reduced by these treatments in the histamine lavages ($p<0.05-0.001$) (Fig. 1A). In contrast, formoterol alone did not affect the levels of tryptase (*c.f.* placebo) and formoterol did not affect the tryptase-reducing effect of budesonide (Fig. 1A). Levels of ECP were significantly reduced by budesonide alone and by budesonide in combination with formoterol in the histamine lavages ($p<0.05-0.001$) (Fig. 1B). Formoterol alone did not affect the levels of ECP (*c.f.* placebo), and formoterol did not affect the ECP-reducing effect of budesonide (Fig. 1B). Levels of α_2-macroglobulin were significantly reduced by budesonide alone and by budesonide in combination with formoterol in the first saline lavage ($p<0.01-0.001$) and this effect was also maintained in the second saline lavage ($p<0.05-0.01$) (Fig. 1C). In addition, these treatments reduced the exudative responsiveness to histamine, i.e., the ability of histamine to produce plasma exudation ($p<0.01-0.001$) (Fig. 1C). Formoterol alone did not affect the levels of α_2-macroglobulin (*c.f.* placebo) and formoterol did not affect the α_2-macroglobulin-reducing effect of budesonide (Fig. 1C).

Discussion

The present study, involving patients with allergic rhinitis examined in a pollen season model, has confirmed that topical treatment with budesonide attenuates symptoms of this condition and reduces its inflammatory features. Furthermore, it has shown that a topically high dose of formoterol does not affect symptoms and allergic inflammation in allergic rhinitis and that formoterol does not add to the efficacy of budesonide. The present data are potentially of interest in terms of discarding β_2-agonists as a treatment of allergic rhinitis and as a class of drugs possessing significant anti-inflammatory properties in this condition.

In the present study, we have utilized a pollen season model of allergic rhinitis and we have focused part of our evaluation, i.e., symptoms and nasal PIF, on the last 3 days of the 7 days' allergen challenge series (paired comparisons of four series). In agreement with our previous observations in this model,[13] around-the-clock symptoms were produced during the evaluation period and rapid returns to baseline values were apparent during the washout periods. Also in agreement with previous observations,[13] significant reductions in nasal PIF were produced by the allergen challenge series. Notably, symptom scores and nasal PIF levels reached at placebo treatment were very similar to those recorded earlier in this model.[13,16] In the present study, using nasal saline lavages with and without histamine, features of allergic airway inflammation were for the first time monitored in our model. Thus, as would be expected, we could demonstrate that the allergen challenge series produced airway inflammation that was characterized by increased mast cell activity, increased eosinophil activity, plasma exudation, and a development of exudative hyperresponsiveness to topical histamine (*c.f.* budesonide treatment). The strategy to employ lavages and histamine challenges was not associated with any carry-over effects in terms of heightened symptom scores at the start of the following allergen challenge series.

Symptoms of allergic rhinitis were attenuated by the present topical budesonide treatment and corresponding improvements in nasal PIF were observed (*c.f.* placebo). In contrast, topical formoterol (as a single treatment) did not affect these clinical indices. Hence, previous reports on reductions of nasal symptom at β_2-agonist treatment in acute allergen challenge experiments did not translate into the present season-like model. Taken together our observations suggest that this class of drugs, in agreement with recent observations at seasonal allergen exposure,[12] is not a treatment option in allergic rhinitis. We cannot exclude that a higher dose of formoterol or more frequent administrations would have produced a symptom reducing effect. However, we regard the present topical dose of formoterol as high. For instance, the dose used in our study is of the same range as what would be given to patients with asthma, even if in asthmatics it would be deposited over a much greater airway surface area compared with the present small nasal mucosal surface area.

In allergic rhinitis, pharmacological studies at natural allergen exposure are hampered by difficulties in carrying out trials of crossover designs and therefore, when resorting to parallel-group comparisons, by differences in disease severity between individuals. Also, these studies are hampered by unpredictable and variable allergen exposure. Reflecting these features, even very large-scale clinical trials have failed to show dose–response relationships in terms of effects of GCSs on total nasal symptoms.[17–19] In the present study, we demonstrated that topical formoterol did not add to the clinical efficacy of topical budesonide in allergic rhinitis. The relatively mild symptomathology

produced by the present allergen challenge regimen may suggest that there is not room for a greater improvement (*c.f.* placebo) than that produced by budesonide 64 μg (o.d.). However, in contrast, we have repeatedly shown dose-dependent effects of budesonide treatment in the presently employed pollen season model, e.g., that budesonide 256 μg has a significantly greater effect on total nasal symptoms than 128 μg.[13] The high degree of sensitivity by which symptoms can discriminate between two treatments in our model suggests that the present observation that formoterol did not improve on the efficacy of budesonide is accurate.

Extravasation, lamina propria distribution, and luminal entry of bulk plasma is a key innate airway defense mechanism as well as a prominent feature of airway inflammation.[20] We have previously demonstrated that this process, particularly when induced by histamine-lavages, moves non-plasma tissue solutes (released from inflammatory cells) from the lamina propria into the airway lumen whenever these solutes are freely available in the tissue such as at ongoing airway inflammation.[20] The benefit of this experimental tool is that it can detect a low-grade inflammatory activity that would not be detectable by plain saline lavages. In the present study, we employed histamine challenges and confirmed their exudative effect. It was largely only at histamine challenge observations that we could demonstrate that mast cell (tryptase) and eosinophil (ECP) features of allergic inflammation were reduced by budesonide treatment (*c.f.* placebo). Given the present study design, we cannot in a strict sense conclude that the allergen challenge series produced allergic inflammation. However, when comparing placebo and budesonide observations our findings suggest that this was the case.

In the present study, levels of tryptase and α_2-macroglobulin were monitored in nasal lavage fluids as indices of mast cell activity and plasma exudation, respectively. The selection of tryptase and α_2-macroglobulin was based on previous reports demonstrating that such markers of airway inflammation can be reduced by β_2-agonists in acute challenge models.[8,9,21] In our previous study,[8] we could not exclude the possibility that the reduced plasma exudation observed at high-dose terbutaline treatment was secondary to a mast cell effect leading to reduced release of mast cell mediators. However, we regarded the anti-permeability action primarily as an effect at the level of the permeability regulating endothelial cells, since β_2-agonists also can inhibit plasma exudation induced by histamine, a mediator that acts directly on post-capillary endothelial cells.[22–24] In the present study, topical budesonide reduced the mucosal output of tryptase and α_2-macroglobulin as would be expected. In contrast, neither the levels of tryptase nor α_2-macroglobulin were affected by formoterol. Our observation suggests that repeated administration of a β_2-agonist does not have mast-cell stabilizing or anti-plasma exudation effects. The difference between the present observation and previous reports may be

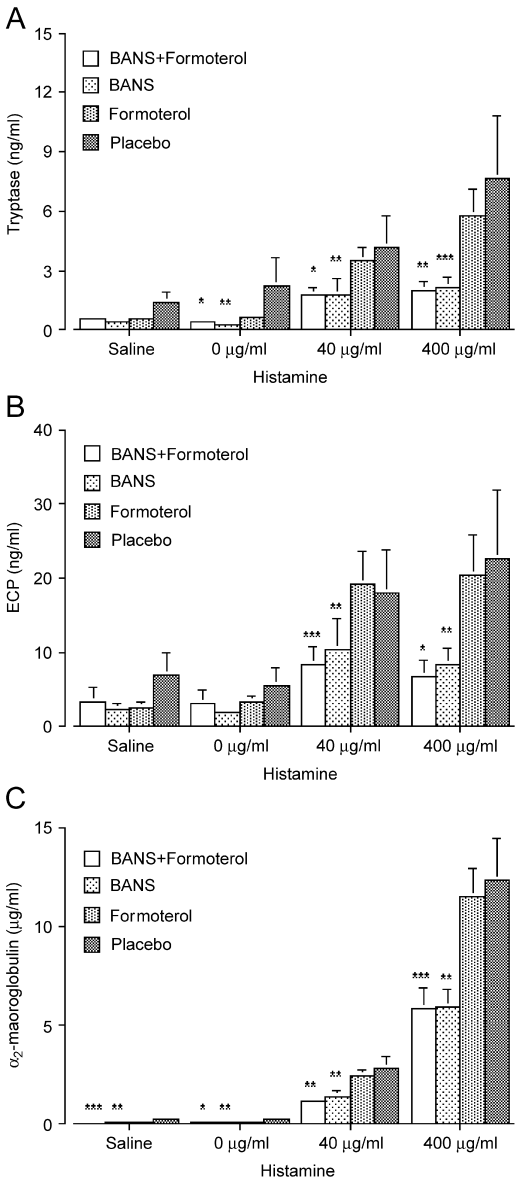

Figure 1 Levels of tryptase (A), ECP (B), and α_2-macroglobulin (C) in nasal saline lavages with and without histamine carried out at the end of each treatment and challenge period (*Study day 15*). In the initial saline lavage, α_2-macroglobulin was reduced by the corticosteroid treatment, i.e., BANS = Budesonide aqueous nasal spray, either given as a single treatment or in combination with formoterol. In the second saline lavage, this effect was maintained and in addition levels of tryptase were reduced. In the histamine lavages, levels of tryptase as well as ECP were reduced by the corticosteroid treatment, whereas formoterol exerted no such effects. The exudative responsiveness to histamine was reduced by the GCS treatment. (*$p<0.05$, **$p<0.01$, and ***$p<0.001$.)

explained by a development of tachyphylaxis. Taken together, our observations suggest that formoterol, in the present dosage, does not exert an anti-inflammatory efficacy in allergic rhinitis. This is also supported by the present observation that budesonide but not formoterol reduced the luminal entry of ECP.

We conclude that topical formoterol, in the present dosage, does not affect symptoms and signs of inflammation in allergic rhinitis, and that formoterol does not add to the efficacy of topical budesonide. We also conclude that the present allergen challenge model is characterized by allergic airway inflammation and that features of this inflammation can be monitored using nasal saline lavages with and without histamine.

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References

1. Persson CGA, Erjefält I, Andersson P. Leakage of macromolecules from guinea-pig tracheobronchial microcirculation: effects of allergen, leukotrienes, tachykinins and anti-asthma drugs. *Acta Physiol Scand* 1986;**127**:95–105.
2. Erjefält I, Persson CGA. Long duration and high potency of anti-exudative effects of formoterol in guinea-pig tracheobronchial airways. *Am Rev Respir Dis* 1991;**144**:788–91.
3. Tokuyama K, Lötvall JO, Löfdahl C-G, Barnes PJ, Chung KF. Inhaled formoterol inhibits histamine-induced airflow obstruction and airway microvascular leakage. *Eur J Pharmacol* 1991; **193**:35–9.
4. Advenier C, Qian Y, Koune JD, Molimard M, Candenas ML, Naline E. Formoterol and salbutamol inhibit bradykinin- and histamine-induced airway microvascular leakage in guinea-pig. *Br J Pharmacol* 1992;**105**:792–8.
5. Inoue H, Aizawa H, Matsumoto K, et al. Effect of β_2-agonists on histamine-induced airway microvascular leakage in ozone-exposed guinea pigs. *Am J Respir Crit Care Med* 1997;**156**: 723–7.
6. O'Donnell SR, Anderson GP. The effects of formoterol on plasma exudation produced by a localized acute inflammatory response to bradykinin in the tracheal mucosa of rats in vivo. *Br J Pharmacol* 1995;**116**:1571–6.
7. Baluk P, McDonald DM. The β_2-adrenergic receptor agonist formoterol reduces microvascular leakage by inhibiting endothelial gap formation. *Am J Physiol* 1994;**266**:L461–8.
8. Svensson C, Greiff L, Andersson M, Alkner U, Grönneberg R, Persson CGA. Antiallergic actions of high topical doses of terbutaline in human nasal airways. *Allergy* 1995;**50**: 884–90.
9. Proud D, Reynolds CJ, Lichtenstein M, Kagey-Sobotka A. Intranasal salmeterol inhibits allergen-induced vascular permeability but not mast cell activation or cellular infiltration. *Clin Exp Allergy* 1998;**28**:868–75.
10. Assem ESK, Schildt HO. Inhibition by sympathomimetic amines of histamine release induced by antigen in passively sensitized human lung. *Nature* 1969;**224**:1028–9.
11. Borum P, Mygind N. Inhibition of the immediate allergic reaction in the nose by the beta-2-adrenostimulant fenoterol. *J Allergy Clin Immunol* 1980;**66**:25–32.
12. Holt S, Suder A, Dronfield L, Holt C, Beasley R. Intranasal β-agonist in allergic rhinitis. *Allergy* 2000;**55**:1198–205.
13. Ahlström-Emanuelsson C, Persson CGA, Svensson C, et al. Establishing a model of seasonal allergic rhinitis and demonstrating dose–response to a glucocorticosteroid. *Ann Allergy Asthma Immunol* 2002;**89**:159–65.
14. Greiff L, Andersson M, Persson CGA. Nasal secretions/exudations: collection and approaches to analysis. In: Rogers D, Donnelly L, editors. *Methods in molecular medicine. Vol. 56: Human airway inflammation*. Totowa (NJ): Humana Press Inc; 2001. p. 61–73.
15. Bousquet J. Allergic rhinitis and its impact on asthma (ARIA). *Clin Exp Allergy* 2003;**3**:43–5.
16. Ahlström-Emanuelsson C, Andersson M, Persson CGA, Schrewelius C, Åkerlund A, Greiff L. Topical treatment with aqueous solutions of rofleponide palmitate and budesonide in a pollen-season model of allergic rhinitis. *Clin Exp Allergy* 2004;**34**: 731–5.
17. Stern MA, Dahl R, Nielsen LP, Pedersen B, Schrewelius C. A comparison of aqueous suspensions of budesonide nasal spray (128 μg and 256 μg once daily) and fluticasone propionate nasal spray (200 μg once daily) in the treatment of adult patients with seasonal allergic rhinitis. *Am J Rhinol* 1997;**11**:323–30.
18. Bronsky EA, Aaronson DW, Berkowitz RB, et al. Dose ranging study of mometasone furoate (Nasonex) in seasonal allergic rhinitis. *Ann Allergy Asthma Immunol* 1997;**79**:51–6.
19. Meltzer EO. Clinical and antiinflammatory effects of intranasal budesonide aqueous pump spray in treatment of perennial allergic rhinitis. *Ann Allergy Asthma Immunol* 1998;**81**:128–34.
20. Greiff L, Andersson, Erjefält JS, Persson CGA, Wollmer P. Airway microvascular extravasation and luminal entry of plasma. *Clin Physiol Funct Imag* 2003;**23**:301–6.
21. Russo C, Zeng D, Prosperini G, Spicuzza L, Guarino F, Polosa R. Effect of salbutamol on nasal symptoms and mast cell degranulation induced by adenosine 5′ monophosphate nasal challenge. *Clin Exp Allergy* 2005;**35**:1192–6.
22. Greiff L, Wollmer P, Andersson M, Svensson C, Persson CGA. Effects of formoterol on histamine challenge-induced plasma exudation in induced sputum from normal subjects. *Thorax* 1998;**53**:1010–3.
23. Majno G, Palade GE, Schoefl GI. Studies on inflammation II. The site of action of histamine and serotonin along the vascular tree: a topographic study. *J Biophys Biochem Cytol* 1961;**11**: 607–26.
24. Grega GJ, Persson CGA, Svensjö E. Endothelial cell reactions to inflammatory mediators assessed by fluid and solute flux analysis. In: Ryan US, editor. *Endothelial cells*. Boca Raton: CRC Press; 1988. p. 103–19.