

Effects of a Low-Molecular-Weight CCR-3 Antagonist on Chronic Experimental Asthma

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Eosinophils represent one of the main effector cell populations of allergic airway inflammation and allergic bronchial asthma. Their infiltration correlates with many characteristics of the disease, including airway hyperresponsiveness (AHR) and increased mucus production. CCR-3 is the principle chemokine receptor involved in eosinophil attraction into inflamed tissue. Therefore, antagonizing CCR-3 could be a novel promising approach toward asthma therapy. We investigated the effect of a low-molecular-weight CCR-3 antagonist on established airway inflammation in a chronic model of experimental bronchial asthma. For this purpose, BALB/c mice intraperitoneally sensitized with ovalbumin (OVA) were chronically challenged with OVA aerosol to induce chronic airway inflammation and airway remodeling. The effect of antagonizing CCR-3 on asthma pathology was examined in BAL and lung histology. Airway reactivity was assessed by head-out body plethysmography. Treatment with the CCR-3 antagonist resulted in a marked reduction of eosinophils in the bronchoalveolar lumen and in airway wall tissue, whereas infiltration of lymphocytes or macrophages remained unchanged. The reduction in eosinophil infiltration was accompanied by normalization of AHR and prevention of goblet cell hyperplasia, indicating reduced mucus production. Furthermore, antagonizing CCR-3 prevented airway remodeling as defined by subepithelial fibrosis and increased accumulation of myofibrocytes in the airway wall of chronically challenged mice. These data demonstrate that antagonism of CCR3 reduces eosinophil numbers, which is accompanied by diminution of asthma pathology in a mouse model of established chronic experimental asthma. Therefore, antagonizing CCR-3 represents a new approach toward a promising asthma therapy.

Keywords: CCR-3; chronic asthma; eosinophils; remodeling

Human bronchial asthma is a chronic inflammatory disease of the airways that is characterized by several major findings: chronic airway inflammation, airway hyperresponsiveness (AHR), and airway remodeling (1). Since eosinophils have been found in great quantities in lung parenchyma and in bronchoalveolar lavage (BAL) fluids, these cells are suspected to act as eminent effector cells of the inflammatory process. Nevertheless, the level of contribution by these cells still remains controversial. Eosinophils contain a variety of cytotoxic molecules, such as eosinophil cationic protein (ECP), which cause tissue damage and promote inflammation (2). Furthermore, the eosinophil and its mediators seem to be involved in the development of AHR, because AHR is absent in mice whose eosinophils are completely ablated (3).

CLINICAL RELEVANCE

Since eosinophils represent the main effector cells in asthma pathology, ablation of these cells seems to be a promising approach towards asthma therapy. This could be achieved by antagonizing CCR-3, the main receptor involved in eosinophil chemotaxis.

On the other hand, mice with genetically targeted GATA-1 promoter showing impaired eosinophil lineage develop AHR after allergen challenge (4). Several studies indicate that eosinophils may activate fibroblasts in the inflamed tissue (2, 5), suggesting that eosinophils may be involved in airway remodeling. For example, eosinophils produce TGF- β , which has profibrotic effects on fibroblasts (6, 7). Therefore, eosinophils seem to be promising targets for therapeutic intervention in allergic asthma.

A number of different therapeutic strategies that interfere either with eosinophil maturation or eosinophil recruitment have been explored. One target molecule is IL-5, since it represents the major maturation factor for eosinophils (8) and primes eosinophils to respond to chemotactic factors (9). Whereas animal studies provided evidence that IL-5 neutralization may be an effective approach toward asthma therapy, the effects of IL-5 neutralization on human airway eosinophilia and AHR did not reach the expected level of clinical significance (10).

Although IL-5 is essential for maturation and differentiation of eosinophils, the principal chemokine receptor responsible for eosinophil attraction into inflamed tissue has been identified as CCR3 (11). Major ligands for CCR-3 are eotaxin-1 (CCL11), eotaxin-2 (CCL24), eotaxin-3 (CCL26), RANTES (CCL5), and monocyte chemoattractant protein (MCP)-2 (CCL8), MCP-3 (CCL7), and MCP-4 (CCL13) (12). The importance of CCR-3 and its ligands in asthma pathology has already been shown in human and mouse studies. mRNAs of eotaxin and CCR-3 are expressed in bronchial mucosa of patients with asthma, and their abundance correlates with the severity of AHR (13). The impact of CCR-3 on the development of AHR in animal models of experimental asthma remains controversial. Depending on which sensitization and challenge protocol was used, development of AHR was present (14) or absent (15) in CCR-3-deficient mice. Based on these data, CCR-3 may represent a new and promising target for therapeutic intervention of asthma. In the present study we examined the effect of the low-molecular-weight antagonist LH31407 on chronic experimental asthma in a therapeutic setting (16). The CCR-3 antagonist is a substituted piperidine derivative that displaces eotaxin with a K_i of 14 nM as revealed by scintillation proximity assay on eosinophils. It also inhibits eotaxin-induced calcium influx in human eosinophils with an IC₅₀ of 10.9 nM. In addition, LH31407 is highly selective against related chemokine receptors (> 1,000-fold against CCR1, CCR2B, CCR4, > 50-fold against CCR5; Göggel and coworkers, unpublished observations).

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MATERIALS AND METHODS

Animals

BALB/c mice were purchased from Harlan Winkelmann (Hannover, Germany). Female animals aged 6–8 wk were used in all experiments and were maintained under pathogen-free conditions. Water and ovalbumin (OVA)-free diet were supplied *ad libitum*. All mouse experiments were reviewed and approved by the local authorities (Regierungspräsidium Giessen).

OVA Sensitization and Challenge

Acute experimental asthma (Figure 1A) was induced in BALB/c mice as previously described (17). To generate chronic airway inflammation accompanied by airway remodeling (Figure 1B), OVA aerosol challenge was performed for at least 8 wk with two consecutive challenges a week.

In the acute protocol the CCR-3 antagonist (LH31407), a substituted piperidine derivative, was administered in doses of 10 or 30 mg/kg body weight (BW) together with Natrosol (hydroxyethylcellulose; Merk Schuchardt OHG, Hohenbrunn, Germany) as a carrier substance by intragastric instillation 1 h before and 5 h after each allergen aerosol challenge (Figure 1A), whereas control animals were sham-treated by intragastric application of Natrosol.

For treatment of chronic experimental asthma LH31407 was administered from Weeks 5–8. Animals received 30 mg/kg BW LH31407 together with Natrosol intragastrically. CCR3 antagonist was applied 1 h before and 5 h after each OVA aerosol challenge (Figure 1B). Control animals were treated by intragastric application of Natrosol. All analyses were performed 24 h after the last OVA or PBS aerosol challenge.

Assessment of Leukocyte Distribution in BAL Fluid

BAL was performed as previously described (18). The total number of leukocytes was determined by using a Casy TT cell counter (Schaefer Systems, Reutlingen, Germany). BAL cells were differentially stained with Diff-Quick (DADE Diagnostics, Unterschleissheim, Germany).

Measurements of Cytokines in BAL Fluid

IL-4, IL-5, IFN- γ , and TNF- α were measured in cell-free BAL fluids by cytometric bead array (CBA) (BD Biosciences, San Diego, CA) according to the manufacturer's protocol. IL-13 concentration in BAL samples was determined by ELISA-technique as previously described (19). The detection limits were 2.5 pg/ml for IL-13 and 5 pg/ml for all other cytokines analyzed with the CBA technique.

Lung Histology

Lungs were fixed *in situ* with 4% (wt/vol) formaldehyde via the trachea. Lung tissues were paraffin-embedded, and 3- μ m sections were stained with hematoxylin and eosin (H&E) or periodic acid Schiff (PAS) as

previously described (20). Cell profiles of inflammatory cells were counted in H&E-stained sections, using high-power field microscopy ($\times 100$ magnification analyzing five airway cross-sections/mouse). The number of infiltrating leukocytes is expressed as cells per high-power field (HPF). Goblet cell hyperplasia was assessed in PAS-stained lung sections and expressed as cells/100 μ m basement membrane according to the method of Foster and coworkers (21). Detection of collagen fibrils was performed by Sirius red/Fast green staining according to the method of Uhal and colleagues (22). Indirect immunohistochemistry was used to stain lung cryosections for α -smooth muscle actin (mouse monoclonal IgG against α -SMA, clone 1A4; Immunotech, Marseilles, France), as described earlier (23). To distinguish smooth muscle cells from myofibroblasts, a rat monoclonal IgG against fibroblast-specific peptide (clone ER-TR7; Biogenesis, Poole, UK) was used.

Measurement of Serum Immunoglobulins

Total IgE and allergen-specific IgE, IgG₁, and IgG_{2a} antibody concentrations were measured by ELISA as previously described (18). The antibody titers of the samples were compared with pooled standard serum and are expressed as U/ml. Total IgE levels were calculated with a known IgE standard.

Measurement of Airway Reactivity to Methacholine

Airway reactivity (AR) to methacholine was assessed using head-out body-plethysmography as previously described (24). Briefly, the system consists of a glass made head-out body plethysmograph to which is attached to aerosol exposure chamber (Forschungsstaetten, Medical School Hannover, Germany). The mouse was positioned in the head-out body plethysmograph while the head of the animal protruded through a neck collar (9 mm inner diameter, dental latex dam; Roeko, Langenau, Germany) into the aerosol exposure chamber, which was ventilated by continuous airflow of 200 ml/min.

For airflow measurement, a calibrated pneumotachograph (PTM 378/1.2; Hugo Sachs Elektronik, March-Hugstetten, Germany) and a differential pressure transducer (8T-2; Gaeltec, Dunvegan, Scotland) coupled to an amplifier (HSE-IA; Hugo Sachs Elektronik) were attached to the top port of each plethysmograph. For each animal the amplified analog signal from the pressure transducer was digitized via an A/D converter (DT301 PCI; Data Translation, Marlboro, MA) at a sampling rate of 2,000/s. Notocord hem 3.5 (Notocord, Paris, France) was used for data calculation.

During continuous assessment of EF₅₀, mice were exposed to MCh aerosols with different concentrations (12.5, 25, 50, 75, 100, or 125 mg/ml), and airway reactivity was expressed as the concentration of methacholine that caused a 50% reduction in baseline midexpiratory airflow (MCH₅₀ mg/ml).

Statistics

One-way ANOVA test was used to determine the level of difference between the experimental groups.

RESULTS

Antagonizing CCR-3 Reduces Acute Allergic Airway Inflammation and AHR

A murine model of acute experimental asthma was used to test whether antagonizing CCR-3 has any effect on the development of acute airway inflammation and AHR (Figure 1A). Leukocyte distribution and cytokine concentrations in BAL fluids (BALF) as well as airway reactivity to β -methylacetylcholine (MCh) were analyzed. In contrast to sham-sensitized animals, OVA-sensitized animals revealed markedly increased numbers of eosinophils and lymphocytes as well as increased levels of IL-4, IL-5, IL-13, and TNF- α in BALF after OVA aerosol challenges. OVA-sensitized animals were significantly more reactive to MCh as compared with control mice (Table 1). To investigate the effect of the CCR-3 antagonist LH31407, doses of 10 mg/kg or 30 mg/kg LH31407 were applied intragastrically 1 h before and 5 h after each OVA allergen challenge. Whereas administration of 10 mg/kg of the CCR-3 antagonist showed only a slight reduction of airway

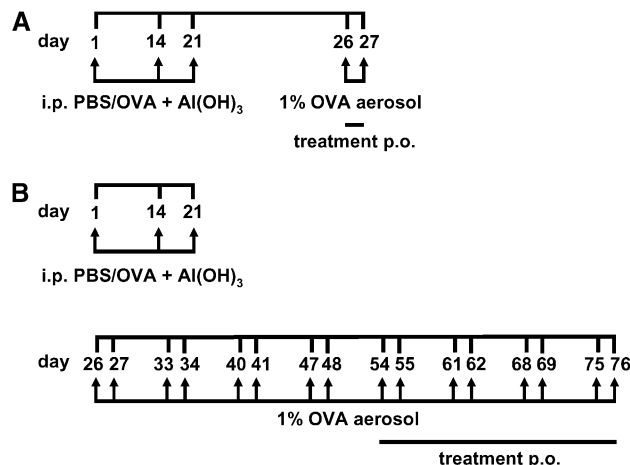


Figure 1. Sensitization, challenge, and treatment protocols for the mouse model of acute (A) and chronic experimental asthma (B).

TABLE 1. THE ANTI-INFLAMMATORY EFFECT OF CCR-3 ANTAGONIST ON ACUTE EXPERIMENTAL ASTHMA

	PBS	OVA	CCR-3 (10 mg/kg)	CCR-3 (30 mg/kg)
BAL cells (10 ⁴ /BAL)*				
Leukocytes	4.98 ± 1.12	172.17 ± 9.88 [§]	139.58 ± 8.67 [§]	118.90 ± 9.10 ^{§††}
Eosinophils	0.01 ± 0.01	148.06 ± 8.20 [§]	94.83 ± 6.63 ^{§††}	85.10 ± 7.85 ^{§††}
Lymphocytes	0.08 ± 0.04	14.33 ± 1.85 [§]	22.99 ± 2.47 [§]	15.13 ± 2.49 [§]
Macrophages	4.89 ± 1.08	9.77 ± 1.63 [§]	20.89 ± 1.81 ^{§††}	18.67 ± 1.9 ^{§††}
BAL cytokines (pg/ml) [†]				
IL-4	Below DL	41.2 ± 5.9 [§]	37.8 ± 7.1 [§]	35.0 ± 5.5 [§]
IL-5	Below DL	140.1 ± 14.9 [§]	133.7 ± 18.6 [§]	119.5 ± 17.0 [§]
IL-13	Below DL	72.2 ± 4.9 [§]	68.9 ± 6.1 [§]	65.8 ± 7.0 [§]
IFN- γ	25.8 ± 2.9	27.7 ± 5.5	22.8 ± 4.0	30.2 ± 6.2
TNF- α	Below DL	63.5 ± 15.7	55.7 ± 13.4	51.6 ± 9.1 [§]
MCh ₅₀ (mg/ml) [‡]	88.9 ± 7.8	54.4 ± 2.8 [§]	59.5 ± 6.6	78.4 ± 2.5 ^{**}

Definition of abbreviations: BAL, bronchoalveolar lavage; DL, detection limit; MCh₅₀, concentration of methacholine that caused a 50% reduction in baseline midexpiratory airflow; OVA, ovalbumin.

Sensitized animals (OVA and CCR-3, $n = 8$) were challenged twice with OVA aerosol and treatment with CCR-3 antagonist (CCR-3, $n = 8$) was performed according to the acute protocol as described in MATERIAL AND METHODS. Control (PBS, $n = 8$) animals were sensitized and challenged with PBS and sham-treated.

* Absolute numbers of eosinophils, lymphocytes, and macrophages in BAL samples.

† Concentrations of cytokines in BAL samples as quantified by CBA-technique.

‡ Airway constriction was measured as described in MATERIALS AND METHODS. Data are presented as mean ± SEM. The unpaired ANOVA test was used to determine the level of difference between the study groups.

§ $P < 0.001$, label significance relating to control group.

|| $P < 0.01$, label significance relating to control group.

|| $P < 0.05$, label significance relating to control group.

** $P < 0.05$, label significance relating to OVA group.

†† $P < 0.001$, label significance relating to OVA group.

‡‡ $P < 0.01$, label significance relating to OVA group.

inflammation and no significant changes in the reactivity to MCh, 30 mg/kg of the CCR-3 antagonist reduced the number of eosinophils in BALF significantly (Table 1). The number of lymphocytes as well as concentrations of IL-4, IL-5, IL-13, and TNF- α

remained unchanged by this treatment. These animals were significantly less reactive to MCh as compared with untreated animals (see Table 1). Therefore, the dose of 30 mg/kg LH31407 was selected for further experiments.

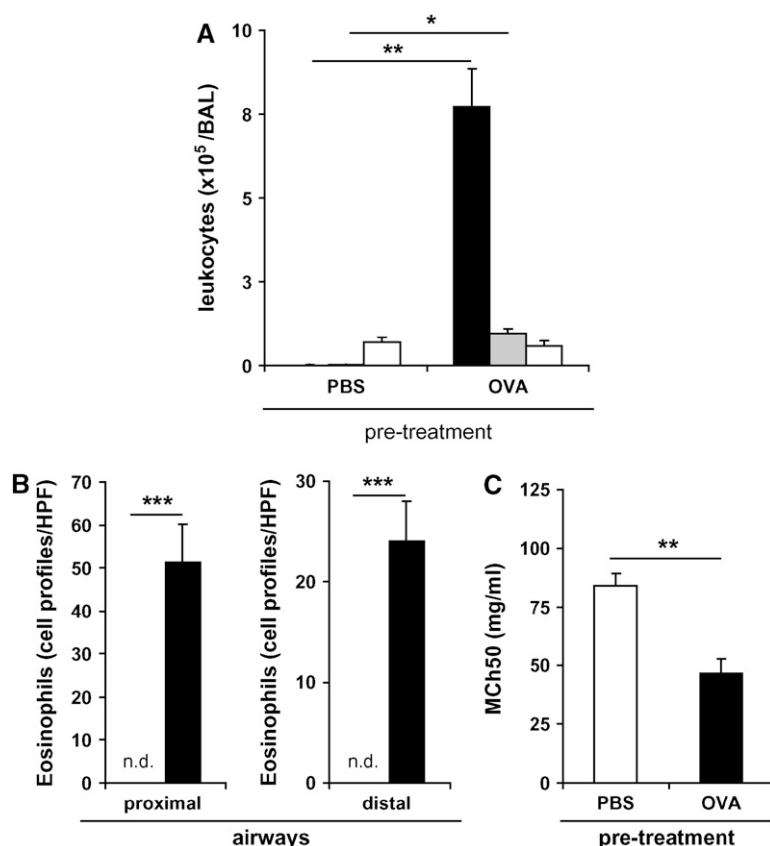


Figure 2. Acute allergic airway inflammation. (A) Absolute numbers of eosinophils (solid bars), lymphocytes (shaded bars), and macrophages (open bars) in BAL samples from sham-sensitized BALB/c mice challenged with aerosolized PBS and OVA-sensitized animals challenged with aerosolized OVA for 4 wk as described in MATERIALS AND METHODS. Control (PBS, $n = 8$) animals were sensitized and challenged with PBS. (B) Numbers of eosinophil cell profiles in airway mucosa of proximal and distal airways. Determination of eosinophil cell profiles was performed in hematoxylin/eosin stained whole-lung sections from BALB/c mice from the PBS (n.d.) or OVA group (solid bars) using high-power field (HPF) microscopy ($\times 100$ magnification). Eosinophil profile numbers are expressed as cells per HPF. The unpaired one-way ANOVA was used to compare differences between treatment and OVA groups. (C) Measurement of airway reactivity to β -methacholine in BALB/c mice PBS or OVA group. Airway constriction was measured as described in MATERIALS AND METHODS. Data are presented as mean ± SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ indicate significance relating to control group (n.d., not detectable).

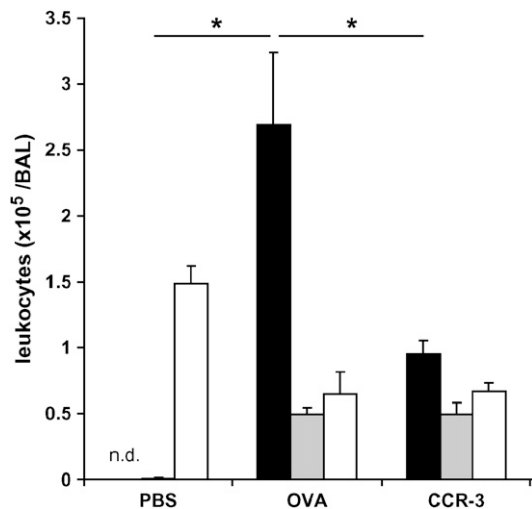


Figure 3. Antagonism of CCR-3 results in diminished infiltration of eosinophils into the airway lumen. Absolute numbers of eosinophils (solid bars), lymphocytes (shaded bars), and macrophages (open bars) in BAL samples from OVA-sensitized BALB/c mice are presented. Animals (OVA and CCR-3, $n = 8$) were sensitized and challenged with OVA and sham-treated (only OVA group) as described in MATERIALS AND METHODS. Oral treatment with 30 mg/kg CCR-3 antagonist (CCR-3, $n = 8$) was performed as described in MATERIALS AND METHODS. Control (PBS, $n = 8$) animals were sensitized and challenged with PBS and sham-treated. Data are presented as mean $\pm$ SEM. * $P < 0.05$ indicates significance (n.d., not detectable).

Antagonizing CCR-3 Reduces Progressive Airway Inflammation, AHR, and Airway Remodeling in a Model of Chronic Experimental Asthma

To examine the effect of CCR-3 antagonization on an established inflammation, we used a murine model of chronic experimental asthma that reflects progressive airway inflammation together with AHR and shows development of airway remodeling as indicated by subepithelial fibrosis and increased numbers of airway myofibrocytes (Figure 1B) (20). In this model, OVA-

sensitized BALB/c mice were challenged on two consecutive days per week over a period of 8 wk. Treatment with 30 mg/kg CCR-3 antagonist was performed during the last 4 wk of the protocol. The analysis of leukocyte distribution in BALF and lung histology before treatment revealed marked influx of eosinophils into the airway lumen (Figure 2A) as well as the airway tissue (Figure 2B). Furthermore, these mice were significantly more reactive to MCh as compared with the control group, indicating development of increased AHR (Figure 2C). After the next 4 wk of OVA challenge, infiltration of the airway lumen was less pronounced as compared with animals at Week 4 (compare cell numbers in Figure 3 to numbers in Figure 2B). Although the absolute numbers of eosinophils and lymphocytes were significantly reduced, they are still higher in comparison to the control group (see Figure 3).

Nevertheless, treatment with the CCR-3 antagonist significantly lowered the number of eosinophils in BALF, while absolute lymphocyte numbers remained unchanged (Figure 3). The further analysis of the cytokine levels in BAL samples revealed no effect of the CCR-3 antagonist on IL-13 production (OVA group: 14.1 ± 0.6 pg/ml versus CCR-3-treated group: 14.1 ± 0.4 pg/ml; PBS-group: below detection limit). IL-4, IL-5, and TNF- α levels were below detection limit in all groups. In addition, a pronounced reduction of airway wall inflammation (Figure 4) was observed. In sham-treated OVA-sensitized mice, AHR was observed throughout all 8 wk of OVA challenge. In contrast, treatment with the CCR-3 antagonist completely normalized AHR (Figure 5).

Evaluation of the lung histology revealed marked reduction in mucus-producing cells within the airway epithelium (Figures 6A, 6D, 6G, and 7). OVA challenges over 8 wk resulted in development of airway remodeling including (1) goblet cell hyperplasia, (2) subepithelial fibrosis, and (3) increased emersion of smooth-muscle actin (SMA)-containing myofibroblasts as indicated by staining with (1) PAS (Figures 6A, 6D, and 6G), (2) Sirius red (Figures 6B, 6E, and 6H), or (3) immunostaining with an antibody against SMA, respectively (Figures 6C, 6F, and 6I). CCR-3 antagonist-treated animals revealed significantly less mucus-producing goblet cells per airway cross-section (Figure 7). Only in sham-treated OVA-sensitized mice, large amounts of collagen fibers and SMA were detected in the airway wall

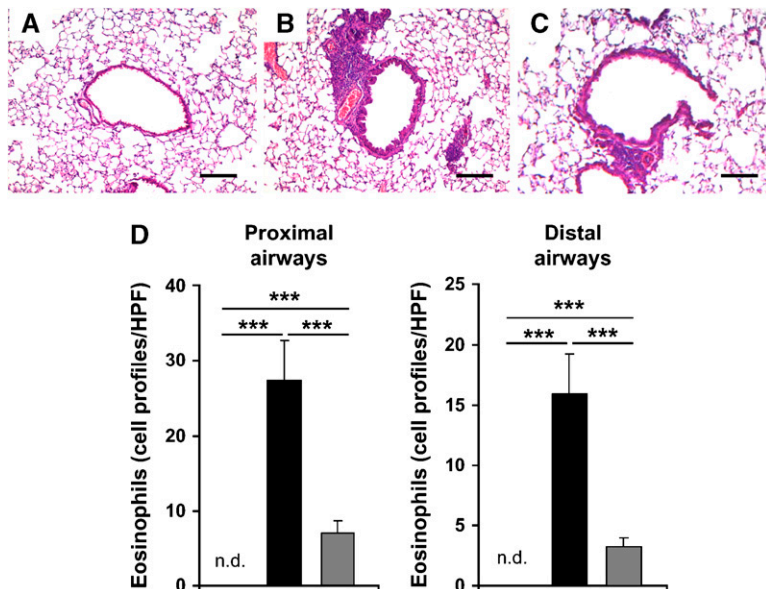


Figure 4. Antagonism of CCR-3 results in diminished infiltration of eosinophils into the airway tissue. (A–C) Lung histology from BALB/c mice. Sensitized animals (OVA [B] and CCR-3 [C]) were challenged with OVA aerosol on two consecutive days a week for at least 8 wk. Treatment with 30 mg/kg CCR-3 antagonist (CCR-3, $n = 8$) or Natrosol (OVA, $n = 8$) started after 4 wk of OVA aerosol challenge and lasted until the end of the experiment. Control (PBS, $n = 8$ [A]) animals were sensitized and challenged with PBS and sham-treated. Representative photomicrographs of paraffin-embedded whole lung sections stained with H&E are presented. Scale bar = 100 μ m. (D) Numbers of eosinophil cell profiles in airway mucosa of proximal and distal airways. Determination of eosinophil cell profiles was performed in H&E-stained whole-lung sections from sham-sensitized BALB/c mice challenged with aerosolized PBS and OVA-sensitized animals challenged two times with aerosolized OVA and treated with Natrosol (solid bars) or CCR-3 antagonist (shaded bars) per os using HPF microscopy ($\times 100$ magnification). Eosinophil profile numbers are expressed as cells per HPF. *** $P < 0.001$ indicates significance (n.d., not detectable).

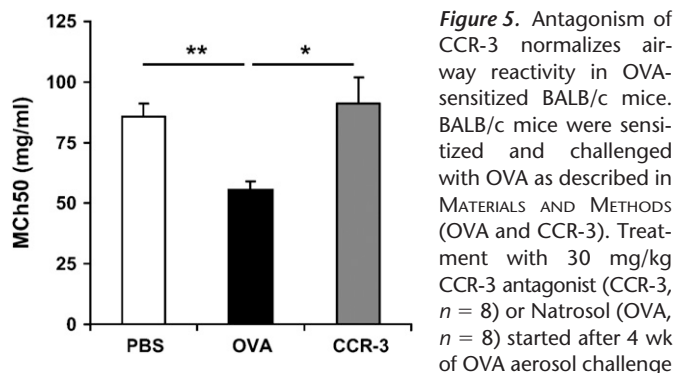


Figure 5. Antagonism of CCR-3 normalizes airway reactivity in OVA-sensitized BALB/c mice. BALB/c mice were sensitized and challenged with OVA as described in MATERIALS AND METHODS (OVA and CCR-3). Treatment with 30 mg/kg CCR-3 antagonist (CCR-3, $n = 8$) or Natrosol (OVA, $n = 8$) started after 4 wk of OVA aerosol challenge

and lasted until the end of the experiment. Control (PBS, $n = 8$) animals were sensitized and challenged with PBS and sham-treated. Airway constriction was measured as described in MATERIALS AND METHODS. Data are presented as mean $\pm$ SEM. * $P < 0.05$ and ** $P < 0.01$ indicate significance.

(Figures 6E and 6F). In contrast, in CCR-3 antagonist-treated animals all the signs of airway remodeling were absent (Figures 6H and 6I).

DISCUSSION

The presence of eosinophils in the airways is predictive for asthma severity and occurs even in mild cases of the disease. In addition, the recruitment of eosinophils to the lung tissue has been closely correlated with asthma pathology (25). Furthermore, it is not possible to induce experimental asthma in mice, which were lacking eosinophils (3). Therefore, ablation of eosinophils from lung tissues seems to be a promising approach toward asthma therapy.

Recent studies elucidated the central role of CCR-3 for the recruitment of eosinophils into inflamed tissue. FACS analysis revealed that CCR-3 is intensely expressed on the surface of eosinophils (26). Thus, CCR-3 may represent a new target for

the inhibition of airway eosinophilia in allergic asthma. Justice and coworkers demonstrated in a murine asthma model that monoclonal anti-CCR-3 antibodies (α -CCR-3 mAb) ablated eosinophils from circulation as well as from lung tissue (27). The same study revealed that the use of an anti-CCR-3 strategy had no influence on the underlying mechanisms leading to the induced Th2-mediated inflammatory response. However, that model did not show signs of airway remodeling.

We used a murine model of chronic allergen challenge to investigate whether blocking CCR-3 in a therapeutic setting would suppress the development of airway remodeling. Here we demonstrate for the first time the positive effect of a low-molecular-weight antagonist on experimental asthma in a therapeutic setting resulting in a 50–60% reduction of tissue eosinophilia in parallel with a marked reduction of airway remodeling.

The incomplete reduction of infiltrating eosinophils is likely due to our experimental design. We started the treatment in mice with already established allergic airway inflammation, which reflects the clinical situation more closely. Nevertheless, even in CCR-3-deficient mice experimental asthma is associated with marked influx of eosinophils into the airways, indicating that eosinophil trafficking into the lung does not exclusively depend on CCR-3 (14).

Unique features of this model of experimental asthma are the pathological signs of airway remodeling, especially in distal airways that were absent in animals treated with the CCR-3 antagonist. This indicates that CCR-3 expression plays an important role in the development of this asthma pathology. Since it was demonstrated that eosinophils produce TGF- β along with cysteinyl leukotrienes (6, 28), eosinophils may have a direct effect on airway fibrosis. Several studies reviewed by Gharaee-Kermani and Phan (2) support this concept by demonstrating interactions of eosinophils and fibroblasts. Levi-Schaffer and colleagues were able to show that lysates from eosinophils stimulated fibroblasts *in vitro* to increase their proliferation and collagen production (7). Furthermore, Humbles and coworkers could demonstrate that airway eosinophilia is essential for airway remodeling in a mouse model of experimental chronic asthma (4).

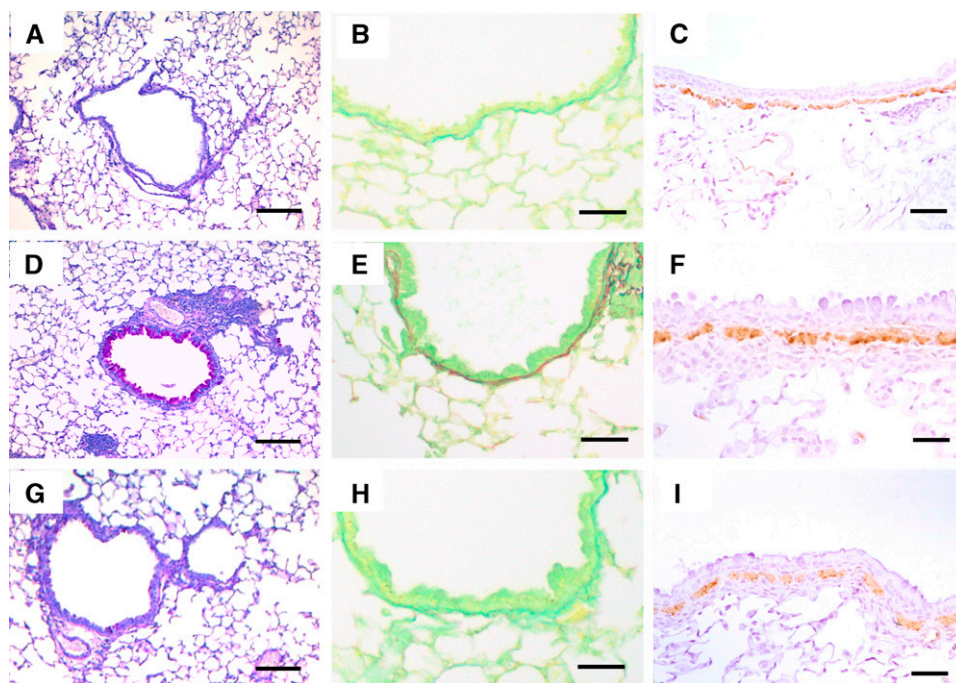


Figure 6. Antagonism of CCR-3 reduces airway remodelling in OVA-sensitized BALB/c mice. BALB/c mice were sensitized and challenged with OVA as described in MATERIALS AND METHODS (OVA and CCR-3). Treatment with 30 mg/kg CCR-3 antagonist (CCR-3, $n = 8$ [G, H, I]) or Natrosol (OVA, $n = 8$ [D, E, F]) started after 4 wk of OVA aerosol challenge and lasted until the end of the experiment. Control (PBS, $n = 8$ [A, B, C]) animals were sensitized and challenged with PBS and sham-treated. Representative photomicrographs of paraffin-embedded whole lung sections stained with PAS (A, D, G; scale bar = 100 μ m), with Sirius red and Fast green to identify collagen fibers (B, E, H; scale bar = 25 μ m), or stained against SMA to identify myofibroblasts (C, F, I; scale bar = 50 μ m) are presented.

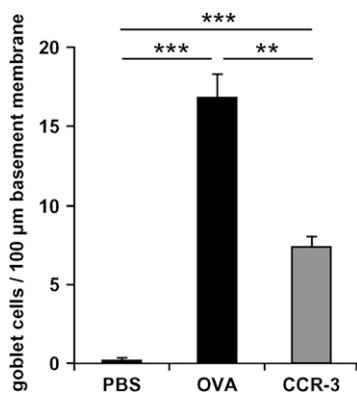


Figure 7. Antagonism of CCR-3 reduces goblet cell hyperplasia in OVA-sensitized BALB/c mice. Animals (OVA, and CCR-3) were sensitized and challenged with OVA. Treatment with 30 mg/kg CCR-3 antagonist (CCR-3, $n = 8$) or Natrosol (OVA, $n = 8$) started after 4 wk of OVA aerosol challenge and lasted until the end of the experiment. Control (PBS, $n = 8$) animals were sensitized, challenged, and treated with PBS. Absolute numbers of goblet

cell profiles in airway mucosa were counted in airway (AW) cross-sections of OVA-sensitized BALB/c mice treated with Natrosol (solid bar) or 30 mg/kg CCR-3 antagonist (shaded bar) using HPF microscopy (magnification $\times 100$). Data are presented as mean $\pm$ SEM. ** $P < 0.01$ and *** $P < 0.001$ indicate significance (n.d., not detectable).

It is remarkable that the effects on AHR and airway remodeling were observed in parallel to an only ~ 50 – 60% reduction of eosinophils. It is, therefore, also possible that the observed suppressive effects may not be entirely due to a reduction in eosinophil numbers. This concept receives further support from studies showing that asthma pathology and AHR is dissociated from airway eosinophilia (29–31). In particular, the role of IL-5 in asthma pathology still remains controversial. Several rodent models have been used to show that neutralizing IL-5 or a deficiency in IL-5 is associated with a strong reduction in airway eosinophilia after allergen sensitization and airway challenge. However, AHR was not absent in all these models and was dependent upon the strain of mouse and the experimental setup used (3, 4, 30, 32). In addition to IL-5, the Th2 cytokine IL-13 has been closely linked to asthma pathology and especially to development of AHR (33, 34). Nevertheless, we did not observe any effect of the CCR-3 administration on the local production of IL-5 or IL-13, indicating that further factors than Th2 cytokines or eosinophils might be involved in the development of AHR as a part of chronic experimental asthma.

In mice, CCR-3 is not exclusively expressed on eosinophils but also on mast cells (14) and smooth muscle cells (35). Since mast cells have been reported to play a critical role in the development of AHR in several mouse models of experimental asthma (36, 37), normalization of AHR in our model may be explained by a direct effect of the CCR-3 antagonist on mast cells. One mode of action could be, in particular, stabilization of the mast cells as it has been shown in a mouse of allergic rhino-conjunctivitis (38). Moreover, a recent study reported on a high level expression of CCR-3 on smooth muscle cells from patients with asthma. This could further be associated with a positive chemotactic response of these cells to eotaxin and with increased intracellular calcium production (39). Together with these findings, the lack of increased SMA in the airway wall after CCR-3 antagonization could be a link to the normalization of AHR.

In conclusion, we demonstrated here for the first time the therapeutic effect of a CCR-3 antagonist on asthma pathology that includes reduction of airway eosinophilia in particular in the tissue, reduced mucus production, improved lung function, and prevention of airway remodeling. Therefore, the use of CCR-3 antagonists may be a promising approach toward a novel asthma therapy.

Conflict of Interest Statement: M.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. R. G. is an employee of Boehringer Ingelheim Pharma, Biberach, Germany. S.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. S.S. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. K.J.E. is an employee of Boehringer Ingelheim Pharma, Biberach, Germany. F.K. is an employee of Boehringer Ingelheim Pharma, Biberach, Germany. H.R. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. H.G. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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