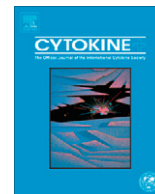




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Spatial and temporal expression of CCR3 and the common beta chain of the IL-3, IL-5 and GM-CSF receptor in the nasal epithelium and lymphoid tissues in a rat model of allergic rhinitis

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ABSTRACT

Background: Allergic rhinitis (AR) and asthma are closely related conditions that often co-exist, and are characterized by a Th2 inflammatory response where eosinophils occupy a predominant role. Strategies aimed at blocking signaling through the CC chemokine receptor 3 (CCR3) and/or the common beta chain of the IL-3, IL-5 and GM-CSF receptor (βc) efficiently reduced eosinophilic inflammation in both animal models and in asthmatic patients. This study was therefore aimed at characterizing the spatio-temporal expression pattern of βc and CCR3 using a rat model of AR.

Methods: Sensitized rats were challenged with ovalbumin and sacrificed at 2 h, 8 h, 16 h or 24 h post-challenge. Nasal tissues were microdissected and used for mRNA quantification by QPCR, while histological evaluation determined the presence of eosinophils and mucosubstances.

Results: Allergen-induced recruitment of eosinophils in the distal septum and turbinates was maximal at 8 h post-challenge, and was correlated with 2–4-fold increase in CCR3 and βc mRNA. Recruitment of eosinophils was also accompanied by upregulated IL-5, IL-4R α , TNF- α and IFN- γ mRNA at early time-points. In contrast, IL-13 and MUC5AC mRNA, as well as production of mucosubstances were maximal at 24 h.

Conclusions: βc and CCR3 could play important roles in the modulation of the allergic response, and their inhibition may represent a promising therapeutic approach for AR.

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1. Introduction

Allergic rhinitis (AR) is a chronic inflammatory condition of the upper airways, characterized by rhinorrhea, sneezing, nasal itching and obstruction following contact of the nasal mucosa with environmental allergens. AR shares numerous immunopathological and pathophysiological features with asthma [1,2]. As such, these conditions have been described as manifestations of one syndrome in two regions of the respiratory tract, leading to a chronic allergic respiratory syndrome or also referred to as “one airway, one disease” [3–5]. Furthermore, the connection between AR and asthma extends to the fact that more than 80% of patients with allergic asthma have AR and that AR is a risk factor for the development of asthma [6,7].

In sensitized individuals, the allergic cascade is induced when the allergen is deposited in the nasal mucosa and activates mast cells by

cross-linking of their surface IgE receptors. When activated, mast cells rapidly degranulate and release a variety of mediators, including histamine, prostaglandins, leukotrienes and proteases, resulting in immediate nasal symptoms (early response) [6]. In addition, resident dendritic cells act to present antigen to T helper lymphocytes, which orchestrate the inflammatory reaction by releasing key regulatory cytokines such as IL-4 and IL-5. IL-4 induces IgE isotype switching and promotes differentiation of lymphocytes into T helper type 2 (Th2) lymphocytes, leading to further secretion of Th2-types cytokines (IL-4, IL-5 and IL-13) and recruitment of other inflammatory cells, including eosinophils, basophils and T cells. By their ability to secrete additional histamine and leukotrienes, as well as pro-inflammatory cytokines and chemokines (such as eotaxin), these cells therefore contribute to promote the late phase response, that usually takes place 6–9 h after the initial contact with the allergen [6]. Other cytokines such as TNF- α and IFN- γ , predominantly associated with Th1-mediated inflammation and which are released early in allergic responses, may also contribute to the development of AR by modulating expression of adhesion molecules and eosinophil recruitment to the nasal mucosa [8,9].

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Eosinophils are considered to be the primary effector cells in the pathogenesis of AR. In humans, AR is associated with a significant accumulation of eosinophils in the nasal submucosa and epithelium [2,10–12]. In the late phase of the allergic reaction, infiltrated eosinophils degranulate and release cytotoxic proteins that cause epithelial damage, excess mucin secretion into the airway lumen, hyper-responsiveness of nasal mucosa and swelling [13–15]. While migration of eosinophils is mostly dependent on signaling of several chemokines acting through the CC chemokine receptor 3 (CCR3), their survival, proliferation and activation are largely due to the cytokines IL-3, IL-5 and GM-CSF, which stimulate their respective receptors composed of a common β chain (β c) and a specific α chain [15–21]. Until now, inhibitors of IL-5 signaling, such as inhibitor of cytokine synthesis or humanized blocking IL-5 mAbs have been exhaustively evaluated for their ability to reduce allergen-induced airway response and inflammation [22–25]. In addition, there is a growing body of evidence that shows increased expression of CCR3 in atopic allergic conditions such as asthma and atopic dermatitis, suggesting the importance of CCR3 in allergic conditions [26–31]. These observations led to recent efforts focusing on finding effective CCR3 antagonists for the treatment of allergic diseases of the airway [32–37].

TPI ASM8 is an antisense drug candidate targeting both CCR3 and β c, that has been found to be effective at blocking eosinophilic inflammation in both animal models of asthma [32,34,38] and in asthmatic patients [39]. Given that AR and asthma share the common feature of persistent eosinophilic inflammation, we sought to investigate whether β c and CCR3 could also represent potential therapeutic targets for AR. In the present study, we used an intranasal allergen challenge model in sensitized Brown Norway (BN) rats [40] to examine the spatial and temporal modulation of gene expression of β c and CCR3, as well as other inflammatory cytokines and receptors, and their associations with the infiltration of eosinophils and alterations to the mucus-secreting epithelium after provocation with an allergic stimulus. Site-specific nasal epithelial and lymphoid tissues were microdissected at different time-points after allergen exposure, and β c and CCR3 mRNA levels, as well as other important inflammatory mediators, were measured by quantitative real-time PCR (QPCR). We also used histochemical detection for eosinophils and epithelial mucus, image analysis, and morphometric techniques to characterize the development of inflammation and morphologic alterations in the nasal epithelium.

2. Materials and methods

2.1. Animals

Male Brown Norway (BN) rats (Charles River), 10–12 weeks of age, were randomly assigned to 1 of 16 experimental groups ($n = 6$ per group). Rats were free of pathogens and respiratory disease, and used in accordance with guidelines set forth by the All-University Committee on Animal Use and Care at Michigan State University. Animals were housed two per cage in polycarbonate boxes, covered with filter lids, and had free access to tap water and food (Tek Lad 1640, Harlan Sprague–Dawley). The room temperature and relative humidity were maintained between 21–24 °C and 40–55%, respectively. Room lights were set on a 12 h light/dark cycle beginning at 6:00 A.M.

2.2. Allergen sensitization and challenge

Rats were sensitized by subcutaneous injection of 1 ml of saline containing 1 mg of chicken egg OVA (Sigma–Aldrich) and 3.5 mg of aluminum hydroxide gel (Sigma–Aldrich). Fourteen days after OVA sensitization, rats were anesthetized with 4% halothane in oxygen,

and 50 μ l of either pyrogen-free saline or an ovalbumin solution (1%, prepared in pyrogen-free saline) was instilled into each nasal airway passage of rats.

2.3. Necropsy and tissue preparation

Rats were necropsied 2 h, 8 h, 16 h and 24 h following OVA challenge. Half of the animals were dedicated for histopathological analysis, while the other half were used for assessment of gene expression. Rats were injected intraperitoneally with sodium pentobarbital (50 mg/kg), a midline laparotomy was performed, and euthanized by exsanguination by cutting the abdominal aorta. The head of each animal was removed from the carcass, and the lower jaw and skin were removed. For animals dedicated to gene expression analysis, heads were split open to reveal the right nasal cavity by cutting just off the mid-sagittal plane using the natural fissure separating nasal from maxillary and premaxillary bones of the proximal skull. The nasoturbinate, maxilloturbinate, and ethmoids were carefully cut away from the exposed lateral wall of the nasal cavity (Fig. 1A) and placed in RNALater solution (Qiagen) and stored at 4 °C until RNA was isolated. Epithelium covering the nasopharynx, which also includes nasal associated lymphoid tissue (NALT) was also removed and placed in RNALater. The epithelium on the exposed right aspect of the proximal and distal nasal septum was then microdissected, and the olfactory epithelium dorsal aspect of the distal septum removed. The septum was then removed and the epithelium and nasal structures from the left nasal cavity were collected and added to the respective tubes with RNALater.

For animals dedicated to histopathological analysis, the nasal cavity was flushed retrograde with 1 ml of neutral-buffered formalin and then immersed in a large volume of the same fixative. Twenty-four hours later, the heads were decalcified in 13% formic acid for five days, and then rinsed in water prior to sectioning. Two tissue blocks were removed from the anterior nasal cavity by making cuts perpendicular to the hard palate: immediately posterior to the upper incisors, and at the level of the incisive papilla (dotted

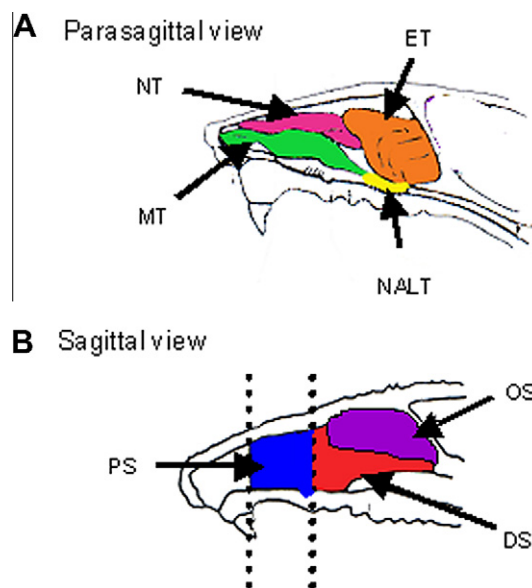


Fig. 1. Location of nasal tissues selected for analysis. Parasagittal (A) and sagittal (B) representations of the nasal epithelial and lymphoid tissues. The vertical lines indicate the anterior surface of tissue block from proximal and distal nasal airway used for morphometric study. NT, nasoturbinate; MT, maxilloturbinate; ET, ethmoids; PS, proximal septum; DS, distal septum; OS, olfactory septum; and NALT, nasal associated lymphoid tissue.

lines in Fig. 1B). The tissue blocks were embedded in paraffin, and 5–6 μm -thick sections were cut from the anterior surface. Nasal sections were stained with hematoxylin and eosin (H&E) for routine histology, with Alcian Blue (AB) (pH 2.5)/Periodic Acid-Schiff (PAS) to detect intra-epithelial mucosubstances (IM), or with May-Grunwald stain to detect mucosal eosinophils. Site-specific morphometry of IM or eosinophils was conducted for the epithelium and underlying mucosa lining the proximal and distal septum, nasoturbinates and maxilloturbinates.

2.4. Morphometry of mucosal eosinophil infiltration and intra-epithelial mucosubstances

The effect of ovalbumin challenge on eosinophil influx was determined in May-Grunwald stained sections by counting the total number of cells within the mucosa of the septum (area between the airway and septal cartilage) or mucosa of the nasoturbinate and maxilloturbinate (area between the airway and the turbinate bone).

To estimate the amount of the intra-epithelial mucosubstances in the respiratory epithelium of the septum, the volume density of AB/PAS-stained mucosubstances was quantified using computerized image analysis and standard morphometric techniques. Briefly, the area of AB/PAS-stained mucosubstance was estimated by setting colorized thresholds to highlight only IM, and these areas were then automatically calculated using the public domain NIH Image program (written by Wayne Rasband, US National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/nih-image/>). The length of the basal lamina underlying the surface epithelium was calculated from the contour length of the digitized image of the basal lamina. The volume of stored mucosubstances per unit length of surface area of epithelial basal lamina was determined as described previously [41]. It was expressed as nl of intra-epithelial mucosubstances per mm^2 of basal lamina (i.e. volume density).

2.5. mRNA quantification in nasal tissue

RNA was extracted using the RNeasy kit from Qiagen and cDNA prepared using the Superscript II reverse transcriptase (Invitrogen). Quantitative real-time PCR was performed using the LC FastStart SYBR Green and the LightCycler (Roche). The expression of the housekeeping gene peptidylprolyl isomerase B (Ppib) was used as a reference for normalization. The primers used in this study (TIB Mol BIOL) were as follows: CCR3 (forward: 5'-CTTAGCAAAATC ATCAGAGACAA-3'; reverse: 5'-TGATGCTGACTTTTCACAGG-3'), βc (forward: 5'-CATCTATGGGTGCAAGTTGTAC-3'; reverse: 5'-TCGTT GTCCTCCAGATGTGTAT-3'), IL-5 (forward: 5'-GTTGACGAGCAATGA GACGAT-3'; reverse: 5'-GCCACACTTCTCTTTTGTCC-3'), IL-13 (forward: 5'-AGGAGCTGAGCAACATCACAC-3'; reverse: 5'-CCATAGCGG AAAAGTTGCTT-3'), IFN- γ (forward: 5'-AGGAACTGGCAAAGGACG-3'; reverse: 5'-CGAACTGGCGATGCTCAT-3'), TNF- α (forward: 5'-GTAGCCACGTCGTAGCAA-3'; reverse: 5'-AAATGGCAATCGGCT GAC-3'), IL-4R α (forward: 5'-GTTCTCTGAAAACCTCACATGC-3'; reverse: 5'-TGGGGGCTTCACATTGTCA-3'), Muc5AC (forward: 5'-GTT CTGAGATGTCCCTCCACTG-3'; reverse: 5'-CAAATAAGCAGCCTTCAT-AGAAGT-3'), Ppib (forward: 5'-GCTACAGGAGAGAAAGGATTTG-3'; reverse: 5'-GCTTGAAGTTCTCATCTGGG-3').

2.6. Statistical analysis

Morphometric data are expressed as the mean $\pm$ the standard error of the mean (SEM) and were statistically analyzed using a completely randomized analysis of variance. Multiple comparisons were made by Student–Newman–Keuls *post hoc* test. In cases when measurements for a group was undetectable, multiple

comparisons were made with Kruskal–Wallis one way analysis of variance on ranks. mRNA quantification data are expressed as the mean fold increase of OVA-challenged over saline-challenged rats (mean fold increase $\pm$ SEM). *t*-Tests were used to compare OVA-challenged and saline-challenged animals for each time-point and each tissue. Criterion for significance was taken to be $p < 0.05$.

3. Results

3.1. Time course of eosinophils infiltration and mucus accumulation in nasal epithelium following allergen challenge

Systemic sensitization of BN rats to OVA followed by intranasal challenge 14 days later elicits an inflammatory response in nasal tissues that is similar to the phenotype seen in AR patients [42]. In order to determine the localization and timing of the inflammatory response following allergen challenge, site-specific analysis of distinct nasal epithelium and tissues were determined at different time-points (see location of nasal tissues in Fig. 1). Fig. 2 shows the time course of eosinophils influx following challenge in the mucosa associated with the proximal and the distal septum. Although eosinophils could be detected in the subepithelial lamina propria of the proximal septum (Fig. 2A), the eosinophilic response was more pronounced in the distal septum (Fig. 2B). Eosinophils numbers in the distal septum peaked at 8 h post-allergen challenge, and gradually decreased at 16 h and 24 h (Fig. 3B). Significant numbers of eosinophils were evident in the maxilloturbinates as early as 2 h after allergen provocation, and remained elevated through 24 h (Fig. 3C). Increases in nasoturbinates were not significant at 2 h, but were significant at all time-points thereafter (Fig. 3D).

Fig. 4 shows the volume density of intra-epithelial mucosubstance (IM) in the proximal and the distal septums following OVA challenge. Although higher amounts of IM were detected in the epithelium of the proximal septum (Figs. 2A and 4A), these remained unchanged upon challenge. In contrast, IM increased with time in the distal septum, with maximal detection at 24 h post-challenge (Fig. 4B). Thus, in response to acute provocation with OVA, both eosinophil influx and mucus production and storage were increased and co-localized in the distal septum. Increases in IM in maxillo- and nasoturbinates were not evident at 24 h (data not shown).

3.2. Allergen-induced spatial and temporal modulation of βc and CCR3 mRNA as well as key inflammatory mediators in nasal epithelial and lymphoid tissues

Chemokines, cytokines and their receptors play central roles in establishing and maintaining the inflammatory response in allergic conditions. In order to better understand the roles of two key cytokines and chemokines receptors in AR, namely βc and CCR3, we investigated their spatial and temporal expression profile in the nasal epithelial and lymphoid tissues following nasal challenge with allergen. Results indicate that βc and CCR3 had unique spatial and temporal expression profiles upon allergen challenge (Fig. 5). In OVA-challenged animals, βc expression was increased 2 h post-challenge, but this increase was limited to the ethmoid turbinate and the olfactory septum. However, 8 h following allergen challenge, βc upregulation could be measured throughout the nasal epithelium and lymphoid tissues, with significant increases in the proximal (3.3-fold, $p < 0.05$) and distal septums (2.5-fold, $p < 0.01$), in the naso- (3.6-fold, $p < 0.01$) and maxilloturbinates (4-fold, $p < 0.05$) as well as in the ethmoid turbinate (2-fold, $p < 0.05$) and NALT (1.7-fold, $p < 0.001$) (Fig. 5). The peak of induction for βc mRNA (8 h) correlated with the peak of eosinophil recruitment in the distal septum, maxilloturbinate and nasoturbinate (Fig. 3).

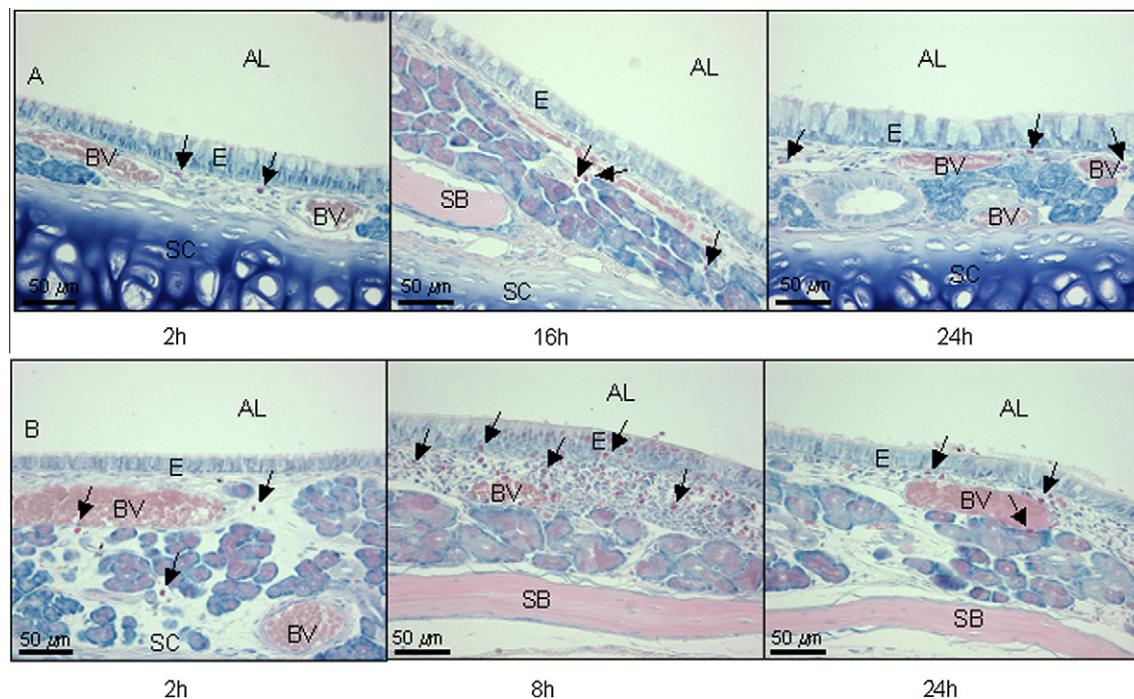


Fig. 2. Effects of allergen challenge on recruitment of eosinophils into the nasal septum. Light photomicrographs of representative tissue sections from the proximal septum (A) and the distal septum (B) showing eosinophil infiltration (arrows) at different time-points following challenge. Airway lumen (AL), epithelium (E), blood vessels (BV), septum bone (SB) and septum cartilage (SC).

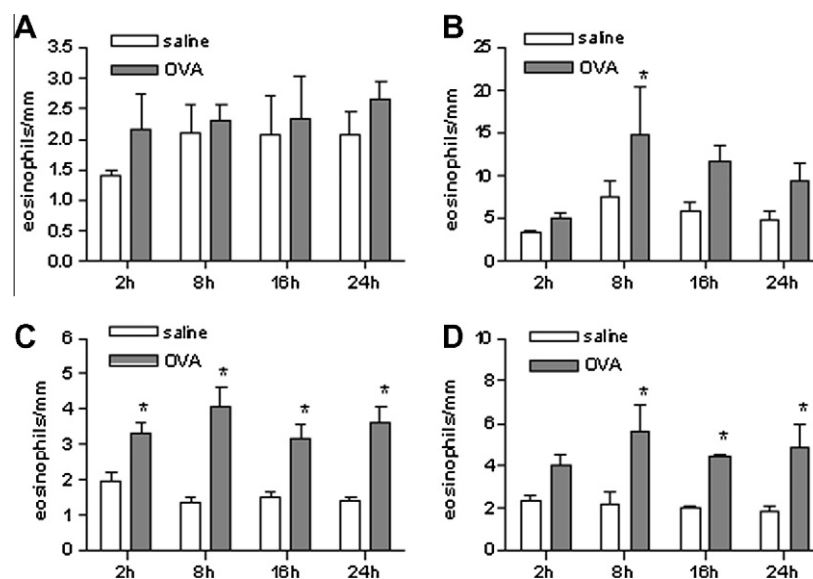


Fig. 3. Time course of eosinophilic infiltration in nasal tissues after allergen challenge. Eosinophil counts in the proximal (A) and distal septums (B), as well as in the maxilloturbinate (C) and the nasoturbinate (D) were assessed at the different time-points following challenge in May-Grunwald stained sections, by counting the total number of cells within the mucosa of the septum or mucosa of the turbinates. Data are expressed as mean eosinophils counts per mm of basal lamina ($\pm$ SEM); * $p < 0.05$, significantly different from respective group receiving saline ($n = 6$ per group).

Expression of βc returned to baseline values at 24 h following allergen challenge. The mRNA for CCR3 displayed a different expression profile upon allergen challenge. Overall, upregulation of CCR3 mRNA expression was not as dramatic and was delayed as compared to expression of βc . CCR3 expression started to increase in the distal septum at 8 h, and was maximal in the proximal (2-fold) and distal septums (2.1-fold), as well as in the NALT (2-fold, $p < 0.05$) 16 h following OVA challenge (Fig. 5). CCR3 levels were back to baseline at 24 h.

Gene expression for Th2 and Th1 cytokines involved in the nasal inflammatory response was also analyzed. Fig. 6 shows that IL-5 and IL-13 mRNA had distinct spatial and temporal modulation following allergen challenge. Induction of IL-5 mRNA levels was observed in the proximal septum and the naso- and maxilloturbinates, and peaked 8 h following allergen challenge, in a manner very similar to expression of its receptor, βc (see Fig. 5). Although allergen-induced modulation of IL-13 mRNA was observed in the same regions as IL-5, its temporal profile followed a biphasic curve. Analysis of

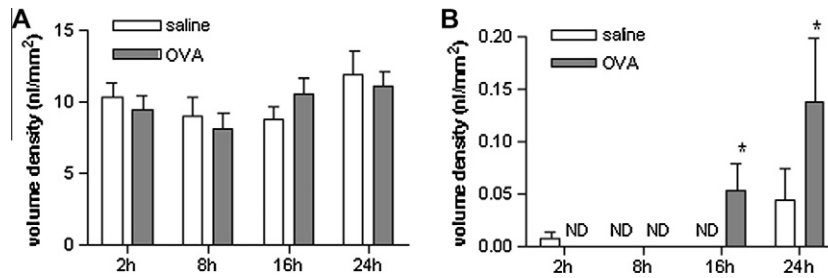


Fig. 4. Time course of intra-epithelial mucosubstance production after allergen challenge. Density of intra-epithelial mucosubstance was evaluated in (A) proximal and (B) distal septums. Data are expressed as mean volume density of intra-epithelial mucosubstances (nl per mm² of basal lamina $\pm$ SEM); $n = 5$ –6 per group.

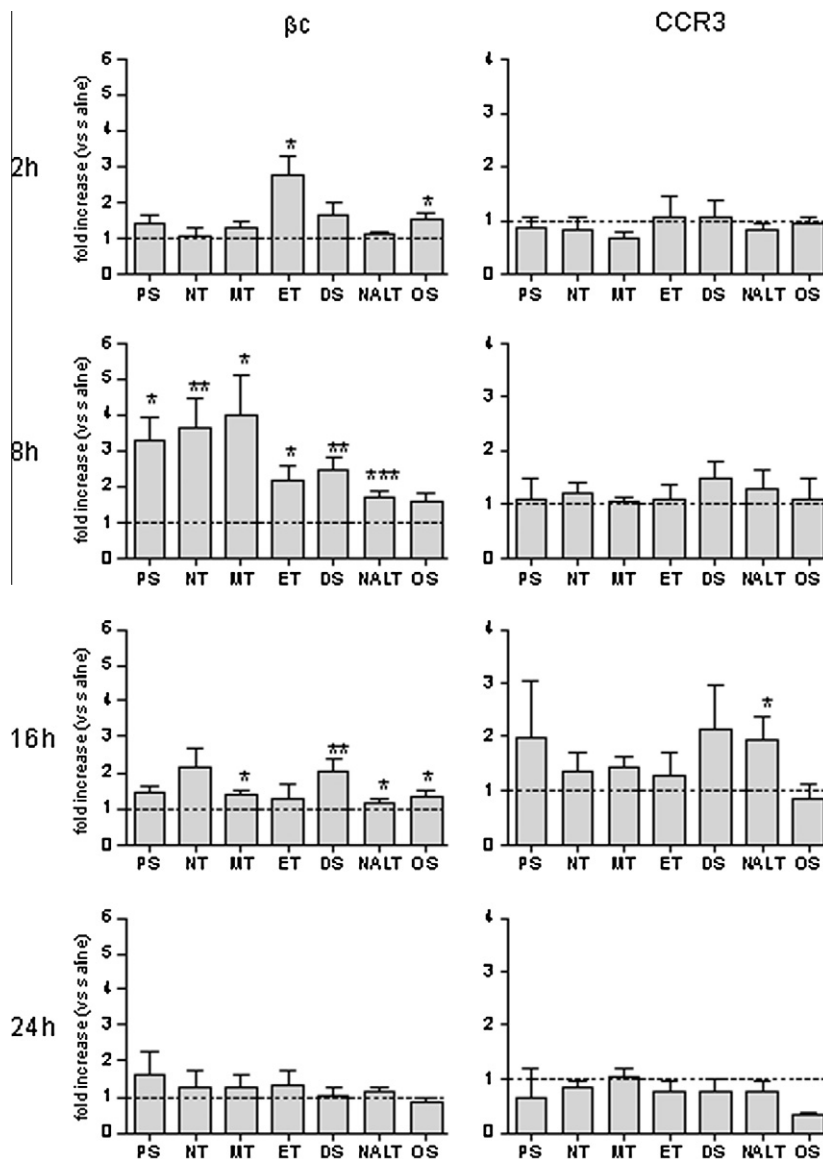


Fig. 5. Effect of allergen challenge on expression of βc and CCR3 mRNA in nasal tissues. Site-specific nasal epithelial and lymphoid tissues were microdissected at different time-points after allergen challenge. mRNA expression levels were quantified by QPCR analysis and normalized to Ppib, used as a reference gene. Data represents fold increase versus saline challenged ($\pm$ SEM). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, t -test.

IL-13 mRNA levels showed a first peak of induction at 8 h and a second peak 24 h following OVA challenge (Fig. 6). mRNA levels for IL-4 were below the limit of detection of the assay (data not shown). Analysis of IL-4R α mRNA showed that induction of its expression

followed a pattern similar to the expression profile of βc (Fig. 5). Expression of Th1 cytokines was also found to be modulated by allergen challenge in nasal tissues. Expression levels of TNF- α were rapidly and significantly upregulated in almost all the nasal epithe-

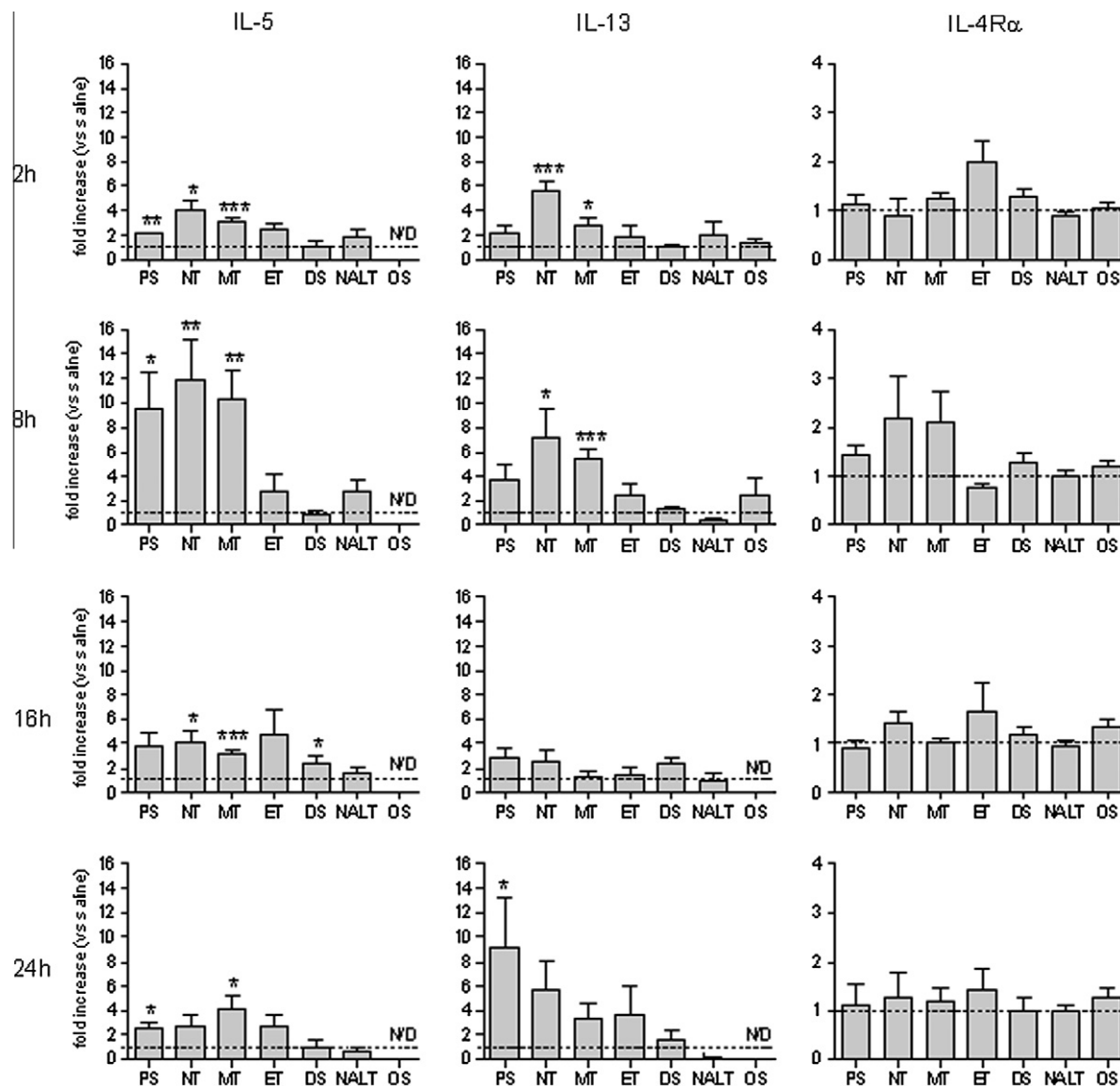


Fig. 6. Effect of allergen challenge on expression of IL-5, IL-13 and IL-4Rα mRNA in nasal tissues. Site-specific nasal epithelial and lymphoid tissues were microdissected at different time-points after allergen challenge. mRNA expression levels were quantified by QPCR analysis and normalized to Ppib, used as a reference gene. Data represents fold increase versus saline challenged ($\pm$ SEM). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, t -test. N/D, not determined.

lial tissues analyzed 2 h post-challenge, whereas IFN- γ peak expression was observed at the 8 h time-point in the maxilloturbinate (Fig. 7).

Expression of Muc5AC mRNA occurred late after OVA challenge and reached 15-fold increase at 24 h in the distal septum (Fig. 8). Muc5AC mRNA induction followed that of mucus proteins in the epithelium of the distal septum (Fig. 4B). Muc5AC mRNA was only slightly induced in the proximal septum at 24 h, in accordance with the lack of increase in mucosubstances in response to OVA in the proximal septum (see Fig. 4A). The late expression of Muc5AC mRNA also correlated with the late IL-13 mRNA response (Fig. 6). IL-13 is a known inducer of mucin secretion [43,44], which is in accordance with the timely regulation of both genes observed here.

4. Discussion

The nose is a very complex organ with a lining surface constituted of structurally and functionally distinct epithelia involved in smell, air conditioning and host defense [45]. The majority of the nonolfactory nasal epithelium is ciliated respiratory epithelium

(RE). Mucous secreting goblet cells are predominantly located in this region and recent animal studies have indicated that the RE may be a focal point in the allergic response [42,46,47]. In BN rats used as AR models, the nasal response is characterized by a conspicuous eosinophilic infiltrate, principally restricted to the RE in both the proximal and distal nasal passages [42]. In rodents and in humans, tissue eosinophilia has been associated with increased expression of CCR3 and its ligands eotaxin, eotaxin-2, RANTES, MCP-3 and MCP-4 [48,49]. Moreover, cDNA microarray analysis of nasal mucosa from AR patients has recently shown that many of the upregulated genes encode for chemokines and chemokine receptors involved in Th2-type responses, of which CCL11 (eotaxin) and CCR3 were identified [50]. The influx of eosinophils after allergen provocation in AR patients was also found to be associated with mRNA expression of IL-5 as well [51,52]. Data from the present study are in accordance with those observations, and show timely recruitment of eosinophils in the distal septum and maxilloturbinate following intranasal allergen challenge, with maximal recruitment by 8 h post-provocation. Notably, β c and CCR3, which are expressed by eosinophils, also had increased mRNA expression levels at 8 h in these same tissues, although for

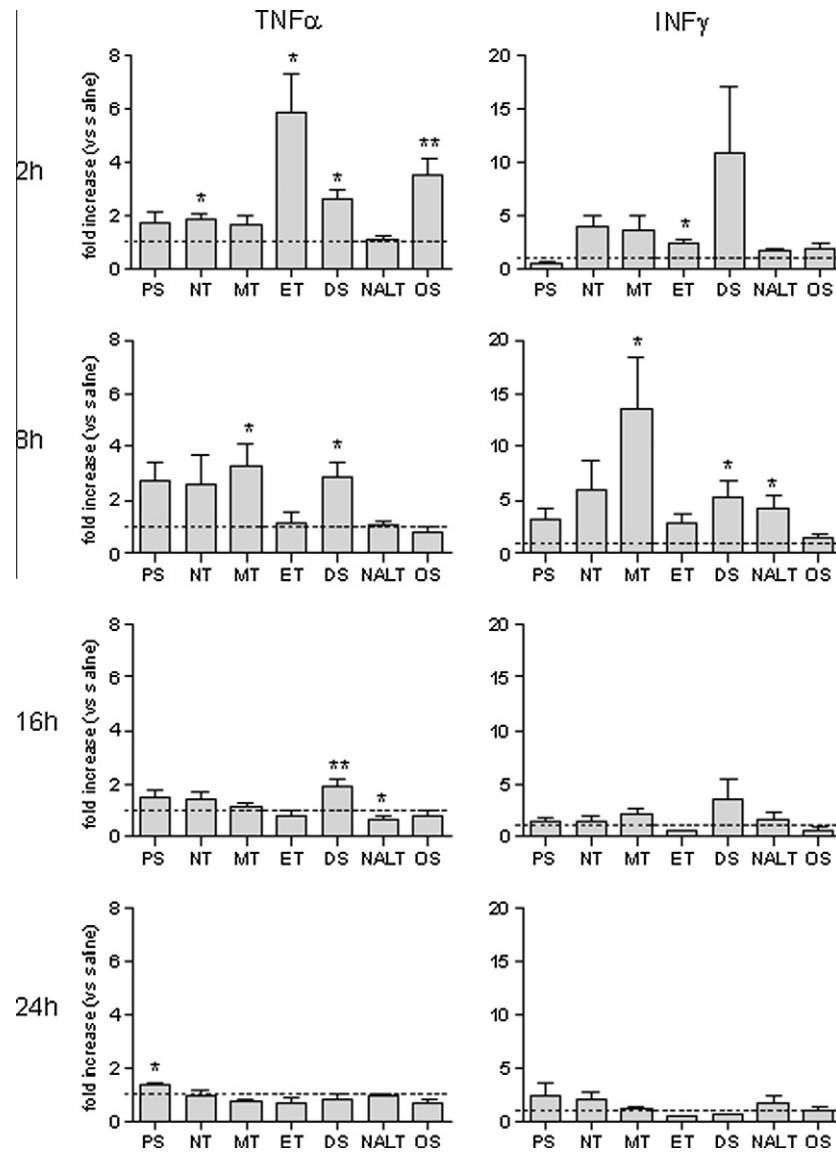


Fig. 7. Effect of allergen challenge on expression of TNF- α and IFN- γ mRNA in nasal tissues. Site-specific nasal epithelial and lymphoid tissues were microdissected at different time-points after allergen challenge. mRNA expression levels were quantified by QPCR analysis and normalized to Ppib, used as a reference gene. Data represents fold increase versus saline challenged ($\pm$ SEM). * $p < 0.05$; ** $p < 0.01$, t -test.

CCR3 in distal septum the peak of expression was delayed (16 h). This latter observation suggests that at 16 h, the recruited eosinophils further enhance their CCR3 expression. Alternatively, cell types other than eosinophils, such as epithelial and endothelial cells in the distal septum might upregulate their CCR3 expression upon allergen challenge [31]. We have previously shown that β c and CCR3 mRNA levels are well correlated with protein levels in the lungs of BN rats using a very similar protocol for OVA sensitization and challenge. Indeed, inhaled OVA challenge was found to increase both mRNA and protein detection of β c [38] and CCR3 [34] as compared to non-challenged animals in lung tissue. In addition, in a previous clinical study in allergen-challenged mild asthmatics, mRNA for β c and CCR3 in sputum-derived cells was used as a biomarker that reliably correlated with eosinophil recruitment and drug efficacy [39]. Given the similar recruitment of eosinophils in the lungs [34,38] and the nose (Figs. 2 and 3) of rats following OVA sensitization and challenge, it is likely that expression of β c and CCR3 proteins is induced in the same nasal regions as for the mRNA in response to nasal OVA challenge. Interest-

ingly, β c and IL-4R α mRNA were upregulated in the ethmoid (2 h), as well as in the proximal septum, naso- and maxilloturbinates (8 h), suggesting that Th2 cytokines-responsive cells present in those regions also play a role in the early reaction following nasal allergen challenge. As well, mRNA for IL-5 and IL-13, ligands for β c and IL-4R α , respectively, were also found to be upregulated in the same regions. IL-13 in particular has been shown to regulate mucin production in nasal epithelium in allergic rodent models [43,44]. Although we did not observe significant mucus accumulation in the proximal septum or turbinates, we have previously shown in BN rats that multiple exposures to allergen are required to elicit robust mucus responses in these epithelium [42].

Current therapeutic approaches for the treatment of AR include nasal corticosteroids, anti-histamines and anti-leukotrienes (see [6] for a review). Although AR symptoms are relatively well controlled by topical corticosteroids, long term use can lead to side effects in some patients [6]. A variety of immunomodulators are being developed and tested for the treatment of AR. Among them are anti-IgE mAbs, Th2 cytokines blockers, anti-IL-4, anti-IL-5 and

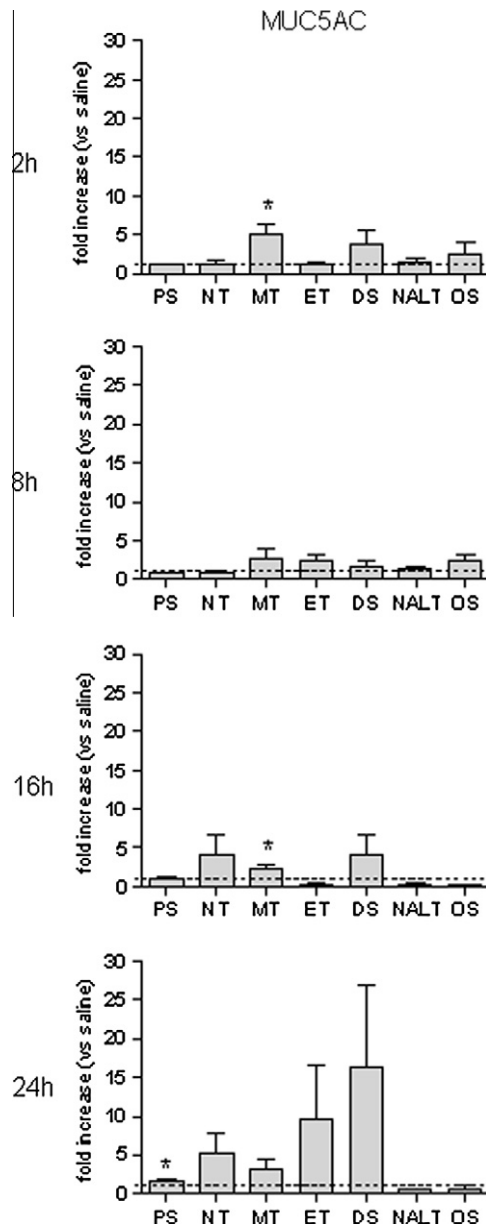


Fig. 8. Effect of allergen challenge on expression MUC5AC mRNA in nasal tissues. Site-specific nasal epithelial and lymphoid tissues were microdissected at different time-points after allergen challenge. mRNA expression levels were quantified by QPCR analysis and normalized to Ppib, used as a reference gene. Data represents fold increase versus saline challenged ($\pm$ SEM). * $p < 0.05$, t -test.

anti-IL-13 mAbs, or IL-4R α antagonists [53]. Blocking both the recruitment and survival/proliferation/differentiation of eosinophils through blockade of multiple cytokines and chemokine receptors might represent a new therapeutic strategy for allergic rhinitis. This idea is supported by a previous study, where a combination of blocking anti-CCR3 and anti-IL-5R α antibodies inhibited by 60% the *in vitro* transendothelial migration of eosinophils in response to an homogenate of nasal mucosa from AR patients [54].

TPI ASM8 is a product candidate that targets eosinophils for the treatment of asthma. This inhalable therapeutic is composed of two RNA-targeting antisense oligonucleotides (AON), one directed against β c and one directed against CCR3. In a recent proof-of-principle allergen challenge trial in mild atopic asthmatics, TPI ASM8 has shown protection against early- and late-stage allergic re-

sponse, with 46% reduction of sputum eosinophils as compared to placebo, and reduction in allergen-induced increase in CCR3 and β c mRNA expression in sputum cells [39]. Given that AR and asthma share multiple common pathophysiological features [1], TPI ASM8 could represent an interesting alternative approach for the treatment of allergic rhinitis, or as a dual therapy for asthmatic patients suffering from AR. Others have begun to explore the possibility of using nasal delivery of AONs as a therapy for AR [55,56]. Indeed, it has been reported that an FITC-tagged AON against IL-4, when incubated with nasal mucosa biopsies from AR patients *ex vivo*, was taken up by the tissue and caused a decrease in allergen-induced IL-4 mRNA and protein expression [55]. Although inhaled AONs for the treatment of asthma do not require specific formulation to reach their target cells in the lower airways [39], development of appropriate formulation might be required to retain the AON at target sites and increase contact time with the nasal epithelium where maximal β c and CCR3 occurs after allergen exposure [57].

Together, results from the present study shows that β c and CCR3, as well as other relevant chemokines and chemokine receptors involved in eosinophil recruitment and epithelial remodelling during AR, have distinct spatial and temporal expression patterns in the nasal epithelium in response to allergen in rats. Gaining better knowledge about the expression of key inflammatory mediators involved in eosinophilic inflammation is likely to be of benefit for future development of more effective and more specific immunomodulatory therapies for allergic rhinitis.

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