

Steroid Treatment Reduces Allergic Airway Inflammation and Does Not Alter the Increased Numbers of Dendritic Cells and Calcitonin Gene-Related Peptide-Expressing Neurons in Airway Sensory Ganglia

Duc Dung Le^a Ulrike Funck^a Sabine Wronski^{f,h} Sebastian Heck^a Thomas Tschernig^d
Markus Bischoff^e Martina Sester^b Christian Herr^c Robert Bals^c Tobias Welte^{g,h}
Armin Braun^{f,h} Quoc Thai Dinh^{a,c}

Departments of ^aInternal Medicine V – Experimental Pneumology and Allergology, ^bTransplant and Infection Immunology and ^cInternal Medicine V – Pneumology, Allergology and Respiratory Critical Care Medicine, and Institutes of ^dAnatomy and Cell Biology and ^eMedical Microbiology and Hygiene, Saarland University Faculty of Medicine, Homburg, ^fDepartment of Airway Immunology, Fraunhofer Institute for Toxicology and Experimental Medicine, ^gDepartment of Pneumology, Hannover Medical School, and ^hGerman Centre for Lung Research (DZL), Hannover, Germany

Key Words

Dendritic cells · Calcitonin gene-related peptide · Jugular-nodose ganglion complex · Neuroimmune interaction · Steroid treatment

Abstract

Objectives: Our previous data demonstrated that allergic airway inflammation induces migration of dendritic cells (DC) into airway sensory jugular and nodose ganglia (jugular-nodose ganglion complex; JNC). Here we investigated the effects of steroid treatment regarding the expression and migration of DC and calcitonin gene-related peptide (CGRP)-immunoreactive neurons of vagal sensory ganglia during allergic airway inflammation. **Methods:** A house dust mite (HDM) model for allergic airway inflammation was used. The mice received 0.3 mg fluticasone propionate per kilogram of body weight in the last 9 days. JNC slices were analyzed on MHC II, the neuronal marker PGP9.5, and the neuropeptide CGRP. **Results:** Allergic airway inflammation increased the numbers of DC and CGRP-expressing neurons in the JNC significantly in comparison to the controls (DC/

neurons: HDM $44.58 \pm 1.6\%$ vs. saline $33.29 \pm 1.6\%$, $p < 0.05$; CGRP-positive neurons/total neurons: HDM $30.65 \pm 1.9\%$ vs. saline $19.49 \pm 2.3\%$, $p < 0.05$). Steroid treatment did not have any effect on the numbers of DC and CGRP-expressing neurons in the JNC compared to HDM-treated mice. **Conclusions:** The present findings indicate an important role of DC and CGRP-containing neurons in the pathogenesis of allergic airway inflammation. However, steroid treatment did not have an effect on the population of DC and neurons displaying CGRP in the JNC, whereas steroid treatment was found to suppress allergic airway inflammation.

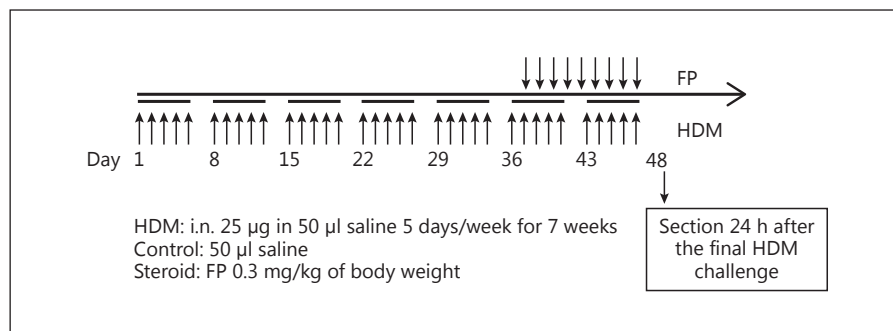
© 2015 S. Karger AG, Basel

Introduction

Allergic bronchial asthma is characterized by reversible airway obstruction, airway hyperresponsiveness (AHR), mucus hypersecretion, and chronic airway in-

D.D.L. and U.F. contributed equally to this work.

Fig. 1. Treatment protocol of the chronic HDM mouse model. The animals were systemically sensitized by intranasal application of HDM extract 5 days a week for 7 weeks. FP treatment was administered i.n. at 0.3 mg/kg of body weight in a volume of 50 μ l on the final 9 days. All analyses were carried out 24 h after the final challenge.



flammation with recruitment of various immune cells, including dendritic cells (DC) [1–3]. As antigen-presenting and phagocytic cells, DC are known to play a key role in the allergic immune response and to be localized in many organs like the skin, the mucosa of the intestines, and the lungs [2–5].

DC have been shown to have the capacity to activate T lymphocytes, induce immune tolerance, and maintain immune memory [3, 5–7]. Recently, the maturation and differentiation of DC have been described to be modulated by many cytokines of immune cells as well as neuropeptides such as the calcitonin gene-related peptide (CGRP) released by airway sensory nerve fibers [8–13].

CGRP consists of 37 amino acids [14] and is biosynthesized and released from sensory neurons innervating the airways in response to different stimuli including allergic airway inflammation [12, 14, 15]. CGRP released from airway nerve fibers has the capacity to act as a chemoattractant factor for different immune cells such as CD4⁺ T lymphocytes, CD8⁺ T lymphocytes [16, 17], eosinophils [18, 19], and DC [20] and to induce the proliferation of airway epithelial cells [21]. Besides, as a chemoattractant for inflammatory cells, CGRP plays an important role, with multiple effects in the pathogenesis of allergic airway inflammation [22, 23]. CGRP has been proposed to induce bronchoconstriction and vasodilation in airway diseases [24, 25].

Fluticasone propionate (FP) has been commonly used for therapy of bronchial asthma and chronic obstructive pulmonary disease (COPD). Treatment with FP has been demonstrated to reduce the expression of sensory neuropeptides such as substance P and CGRP [26, 27] and to inhibit the migration of DC [28].

In our previous study, DC were found to be localized in the jugular-nodose ganglion complex (JNC) under physiologic conditions, and their number was signifi-

cantly increased during allergic airway inflammation by migration from outside into the ganglia [4]. However, the impact of FP on neuropeptide expression and on the migration of DC in airway sensory ganglia has been not explored so far.

The present study, therefore, aimed to investigate the anti-inflammatory effects of steroid treatment on the presence and the distribution of DC and CGRP-immunoreactive neurons in vagal sensory jugular-nodose ganglia under allergic airway inflammation.

Materials and Methods

Animals

Female wild-type BALB/c mice (6–8 weeks old) were purchased from Charles River. The animals were held in regular 12-hour dark/light cycles at a room temperature of 22°C and received laboratory food and tap water ad libitum. The animals were acclimatized for at least 2 weeks prior to the study. All animal experiments were performed in strict concordance with the German animal protection law and were approved by the appropriate governmental authority (No. 10/0207).

House Dust Mite Mouse Model

The application of house dust mites (HDM) to induce chronic allergic airway inflammation has been reported previously [4]. Shortly, BALB/c mice ($n = 6$) were exposed consecutively 5 days a week for a total of 7 weeks to intranasal (i.n.) instillation of HDM extract (catalog No. XPB82D3A2.5; Greer Inc.) at a dose of 25 μ g protein in 50 μ l saline. The control groups ($n = 6$) were treated i.n. with 50 μ l saline. Analyses were performed 24 h after the last allergen challenge (fig. 1).

Steroid Treatment

To assess steroid efficacy in HDM-treated animals, one group ($n = 6$) was treated i.n. with FP at 0.3 mg/kg of body weight in a volume of 50 μ l 1 h before HDM application daily during the final 9 days before necropsy. The animals in the nontreated groups received 50 μ l of vehicle i.n. instead. The body weights of the steroid-treated animals were monitored daily.

Table 1. Antibodies for immunofluorescence staining

Antibody	Source	Dilution
<i>Primary purified antibodies</i>		
Rabbit polyclonal antibody against mouse, human PGP9.5	Abcam, Cambridge, UK	1:200
Rat monoclonal antibody against mouse I-A/I-E	BioLegend, San Diego, Calif., USA	1:200
Goat polyclonal antibody against mouse CGRP	Acris, Herford, Germany	1:400
<i>Isotype control purified antibodies</i>		
Rabbit IgG	Dianova, Hamburg, Germany	1:5,000
Rat IgG 2b kappa	BioLegend	1:200
Goat IgG	R&D Systems	1:300
<i>Secondary antibodies</i>		
DyLight 488-conjugated donkey anti-rabbit IgG antibody	Jackson ImmunoResearch Inc., Baltimore, Md., USA	1:400
Cy3-conjugated donkey anti-rat IgG antibody	Jackson ImmunoResearch	1:500
Cy3-conjugated donkey anti-rabbit IgG antibody	Jackson ImmunoResearch	1:400
DyLight 488-conjugated donkey anti-goat IgG antibody	Jackson ImmunoResearch	1:400
DyLight 649-conjugated donkey anti-rabbit IgG antibody	Jackson ImmunoResearch	1:400

Ganglion Preparation

JNC ganglia were fixed immediately in Zamboni solution (Morphisto Evolutionsforschung und Anwendung GmbH, Frankfurt am Main, Germany) for 6 h, rinsed overnight in 0.1 M phosphate-buffered saline (PBS), and cryoprotected for 24 h with 30% sucrose in 0.1 M PBS. All of the above described steps were performed at 4°C. The ganglia were embedded in optimal cutting temperature medium and frozen in liquid nitrogen. Serial 8-μm sections of the ganglia were prepared using a cryostat (Leica CM 1900; Bensheim, Germany), placed on APES (3-aminopropyltriethoxysilane)-coated glass slides, dried at room temperature for 30 min, and then stored in the freezer at -80°C.

Immunofluorescence Staining

The immunofluorescence staining has been described previously [4]. Briefly, every fourth section from serial sections of JNC was dried at room temperature for 15 min and then rehydrated for 5 min in PBS. To reduce nonspecific antibody binding, the sections were incubated for 15 min at room temperature in 5% normal serum of the host species of the secondary antibody diluted in PBS. The sections were incubated with primary antibodies against PGP9.5, MHC II, CGRP, or the appropriate isotype control antibodies (table 1) for 1 h at room temperature and then overnight at 4°C. After rinsing with 0.1 M PBS twice, the sections were incubated with secondary fluorescein-conjugated antibodies (table 1) for 2 h at room temperature; for counterstaining, the sections were then incubated with 100 μl DAPI (0.5 μg/ml; Carl Roth, Germany) for 15 min at room temperature. Finally the sections were washed twice with 0.1 M PBS and once with double distilled water, mounted with the fluorescent mounting medium Prolong Gold (Invitrogen), and covered with coverslips.

For quantitative analysis of MHC II-immunoreactive cells, CGRP-immunoreactive neurons, and PGP9.5-immunoreactive neurons, 16 serial ganglion sections per mouse were stained and visualized with an epifluorescence microscope (Axioskop 2 Plus; Carl Zeiss, Jena, Germany), and cell counting was performed using

the software ImageJ (National Institutes of Health). The evaluations of MHC II-immunoreactive cells and CGRP-immunoreactive neurons were normalized by the total neuron number and expressed as percentages.

Confocal images were acquired by LSM 510 META (Carl Zeiss). The Images were processed using Imaris 4.5.2 (Bitplane, Zurich, Switzerland).

Lung Histology

Cryosections of optimal cutting temperature medium-embedded lungs (10 μm) were prepared using a cryostat (Leica CM 1900) and then fixed in cold acetone for 10 min. Lung cryosections were taken for assessment of airway inflammation by using hematoxylin and eosin (H&E) staining and periodic acid Schiff (PAS) staining.

Statistical Analysis

Data are given as means ± SEM. The data were analyzed using GraphPad Prism 5.0 (GraphPad Software Inc., La Jolla, Calif., USA). Comparisons between groups were analyzed by ANOVA with Bonferroni adjustment. p values <0.05 were considered statistically significant.

Results

Allergic Airway Inflammation after HDM and Steroid Treatment

Histological analysis with H&E and PAS staining of the lung tissues showed massive infiltration of mononuclear cells (fig. 2, H&E) and a high level of mucus secretion (fig. 2, PAS) in HDM- and HDM+FP-treated mice. In contrast, the lung sections of control mice did not con-

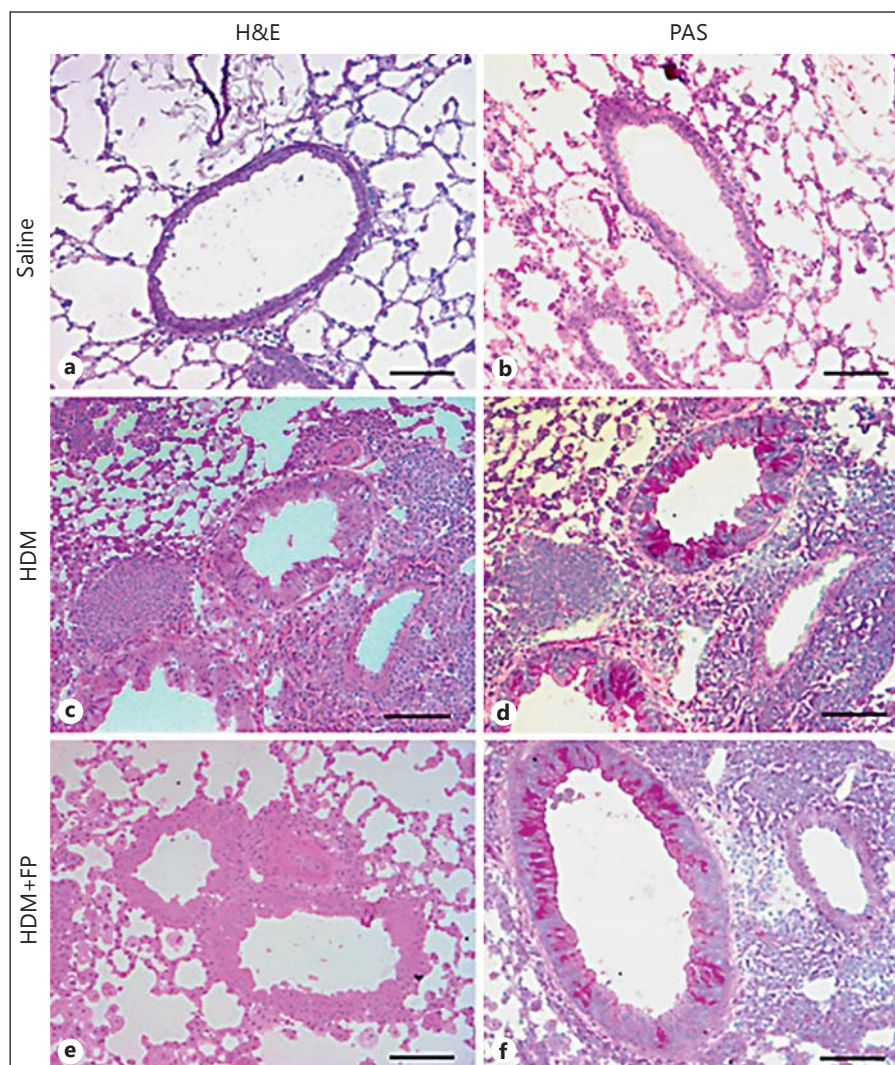


Fig. 2. Effects of chronic HDM and steroid treatments in the lung. Photomicrographs of H&E- (left) and PAS-stained (right) lung sections. The lung sections of control mice (**a, b**) did not contain aggregated inflammatory cells or mucus secretion, whereas lung sections from HDM (**c, d**) and HDM+FP (**e, f**) mice showed infiltration of mononuclear immune cells and a highly mucus secretion in the airways. Scale bars = 100 μ m.

tain inflammatory cell aggregates (fig. 2a) and no mucus secretion was detected (fig. 2b). Chronic allergic airway inflammation induced by HDM treatment was indicated by the recruitment of eosinophils, neutrophils, lymphocytes, and macrophages to the lung and the increased lung resistance in response to methacholine. Steroid treatment reduced the number of eosinophils in BAL and the AHR as previously reported [29].

Steroid Treatment Did Not Alter the DC in the JNC

To identify sensory neurons and DC in the JNC, the ganglia sections were analyzed with immunofluorescence for expression of the pan-neuronal marker PGP9.5 and the DC marker MHC II. Under physiological and allergic airway conditions, DC have been found to be widely dis-

tributed over the whole JNC, and they were located between the neurons and nerve fibers (fig. 3).

We wished to determine how the number of DC in the JNC changed during allergic airway inflammation and steroid treatment. The analysis of immunofluorescence staining showed that the number of DC in the JNC was significantly increased in HDM-treated animals (DC/neurons: HDM $44.58 \pm 1.6\%$, $n = 6$ vs. saline $33.29 \pm 1.6\%$, $n = 6$, $p < 0.05$; fig. 3j).

Steroid treatment had no effect on the migration of DC into the JNC in either group of HDM-treated mice (DC/neurons: HDM $44.58 \pm 1.6\%$, $n = 6$ vs. HDM+FP $43.69 \pm 3.0\%$, $n = 6$, $p > 0.05$; fig. 3j).

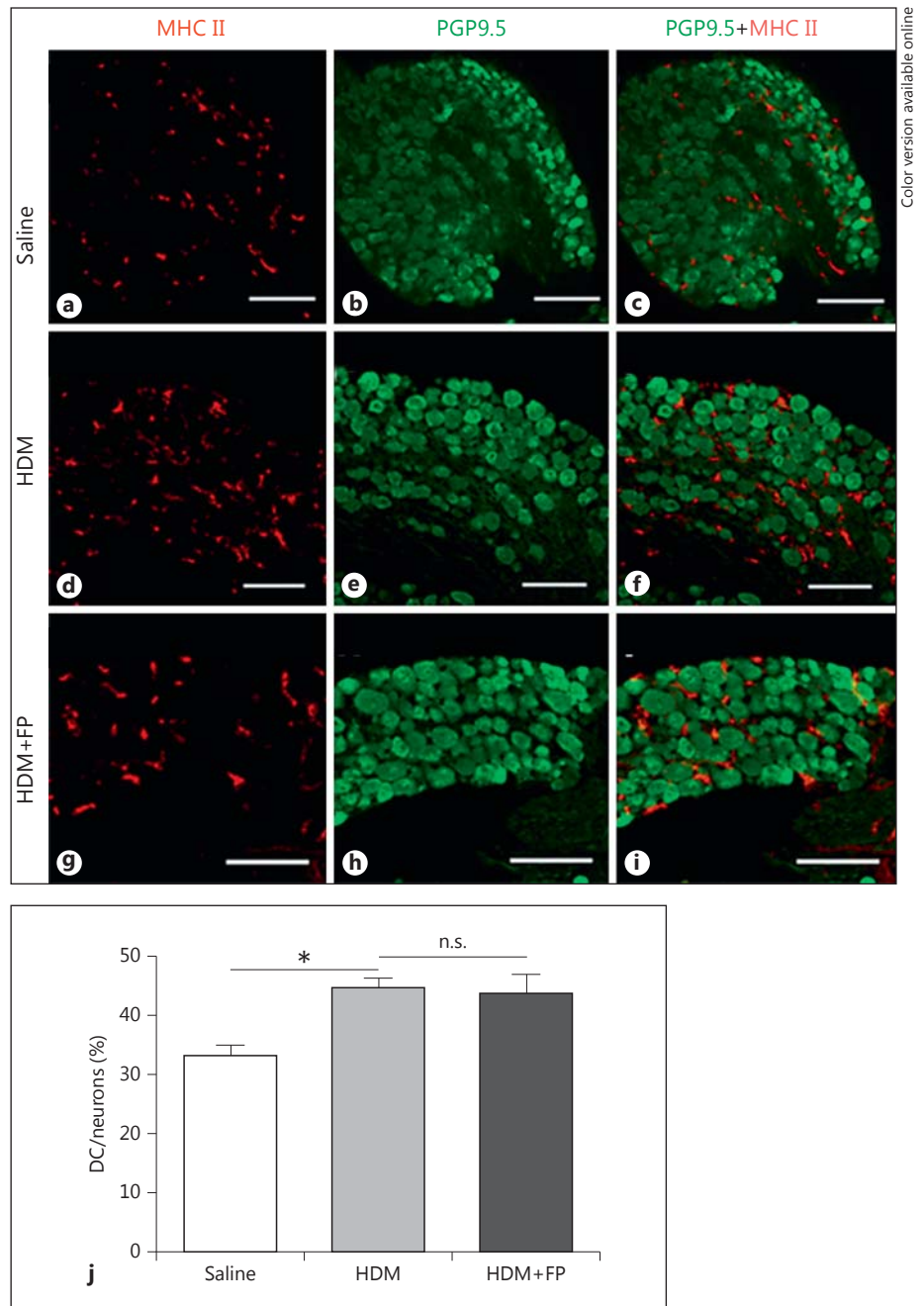


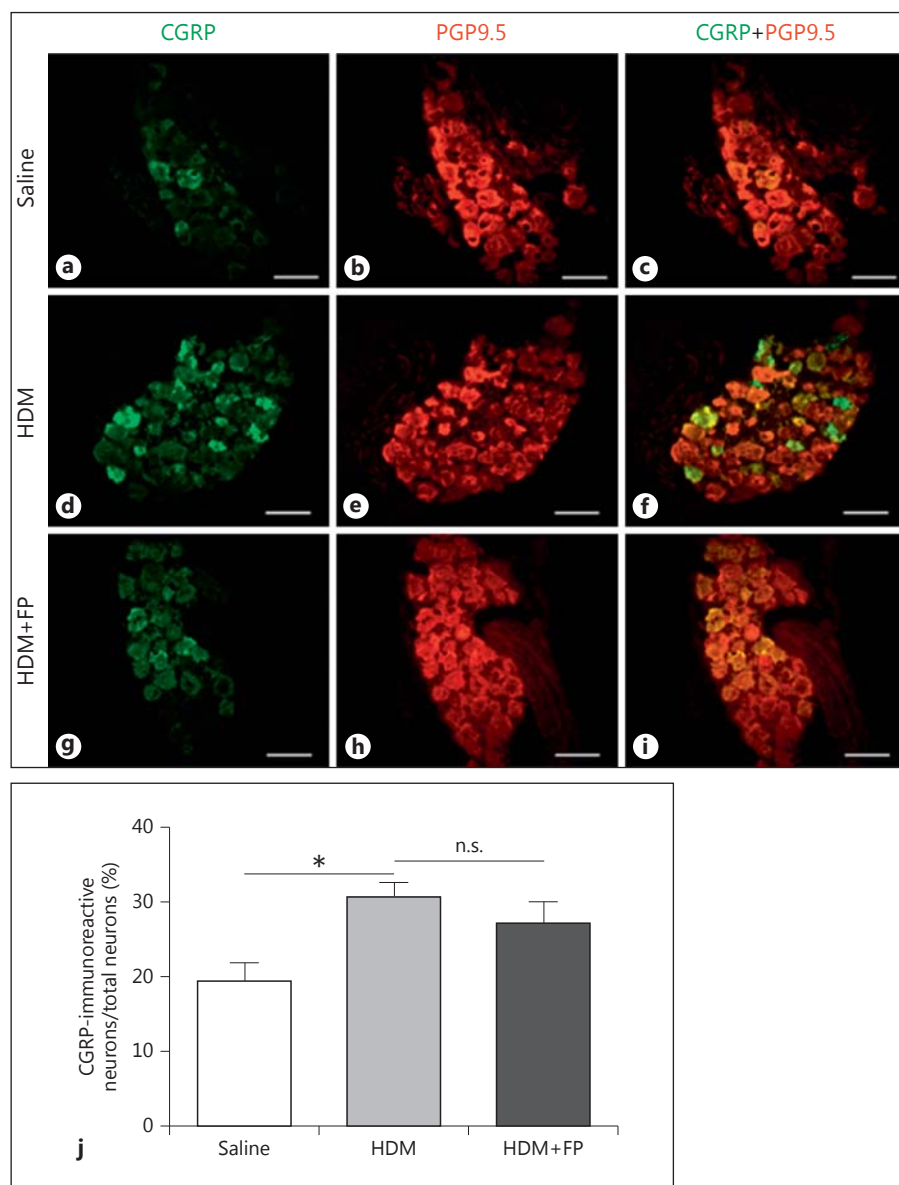
Fig. 3. Quantification of DC in the JNC. Sixteen ganglion sections per mouse ($n = 6$) were stained with MHC II antibody for DC and PGP9.5 for neurons, and the images were acquired using an epifluorescence microscope (Axioskop 2 Plus). The DC (MHC II), neurons (PGP9.5), and merged picture (PGP9.5+MHC II) of the JNC of the control (**a–c**), HDM- (**d–f**), HDM+FP-treated (**g–i**) groups are shown. **j** Quantification of DC in the JNC of all groups. Results are expressed as percentages of MHC II-positive cells in relation to PGP9.5-positive neurons. Data are given as means $\pm$ SEM. n.s. = Not significant. * $p \leq 0.05$. Scale bars = 100 μm .

CGRP-Expressing Neurons Were Not Affected by Steroid Treatment

Similar to our previous findings, the percentage of CGRP ir-neurons in relation to JNC-overall neurons was also found to be significantly increased by HDM-induced allergic airway inflammation (CGRP-positive neurons/total neurons: HDM 30.65 ± 1.9 , $n = 6\%$ vs. saline $19.49 \pm 2.3\%$, $n = 6$, $p < 0.05$; fig. 4j).

Steroid treatment did not have any effect on the number of CGRP-expressing neurons in the JNC (CGRP-positive neurons/total neurons: HDM 30.65 ± 1.9 , $n = 6\%$ vs. HDM+FP $27.35 \pm 2.5\%$, $n = 6$, $p > 0.05$; fig. 4j).

The distribution of DC in correlation with CGRP-expressing neurons in the JNC during allergic airway inflammation and after steroid treatment was observed. DC were found to be distributed overall on the JNC



slices of all animals groups without any preferential localization to CGRP-positive neurons in all groups (fig. 5).

Discussion

DC represent a phenotypically heterogeneous malleable population with an important biological function in many immune responses such as allergic airway inflammation. There is increasing evidence showing the interactions of DC with airway nerves under allergic conditions.

Previous studies have shown that tachykinins and CGRP may have the capacity to regulate the migration of immune cells such as DC [16, 19, 20]. Recently, an increase in the number of DC and CGRP-expressing neurons in the JNC caused by HDM-induced allergic airway inflammation was found. These findings suggest that CGRP may contribute to the migration of immune cells such as DC into airway sensory ganglia and to enhance or suppress airway inflammation [4, 30]. In correspondence with our previous results, DC and CGRP-expressing neurons in the JNC were enhanced after treatment with HDM. DC were found to be distributed overall on the

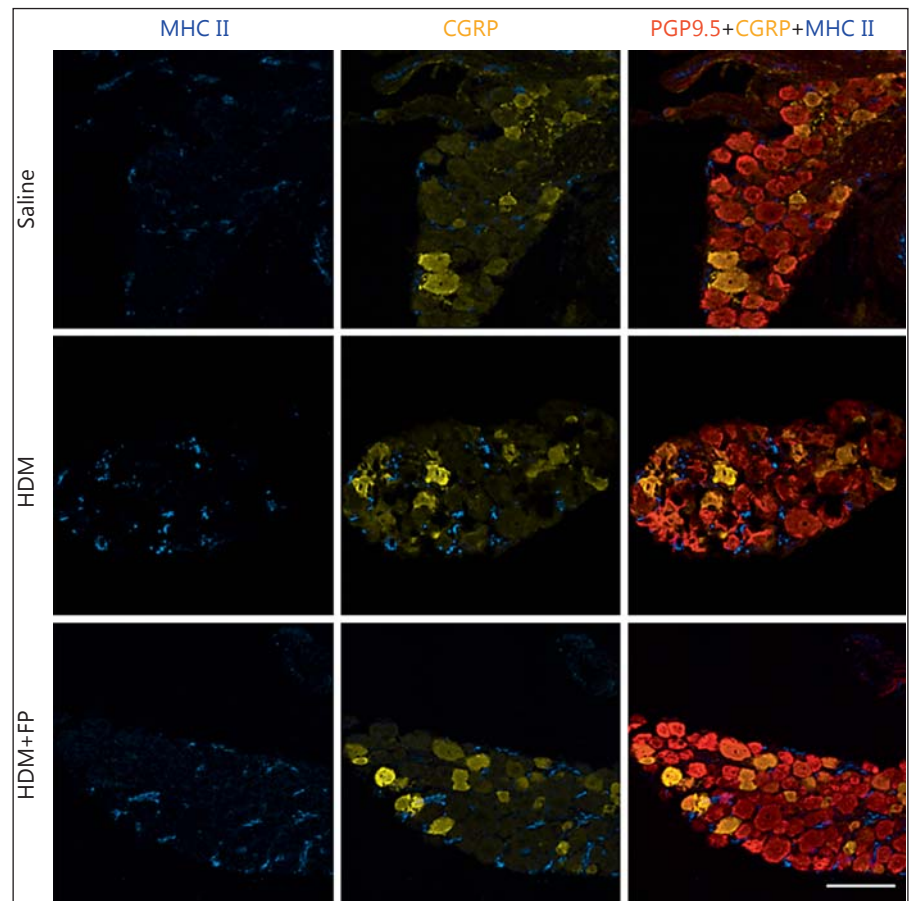


Fig. 5. Distribution of DC in correlation with CGRP-expressing neurons in the JNC. MHC II-positive cells distribute overall on the JNC slices of all animal groups without any preferred localization to CGRP-positive neurons. Images were acquired using a confocal microscope (LSM 510 META). Scale bar = 50 μ m.

JNC slices of all animals groups, without any preferential localization to CGRP-positive neurons. Supporting the present findings, neuropeptides such as CGRP have been found to be released from airway nerves to the lung during allergic and infectious airway inflammation [31, 32]. However, the functional role of intraganglionic DC and CGRP in allergic airway inflammation remains poorly understood.

Vagal sensory ganglia have been found not only to convey afferent information from the lung to the central nervous system but also to induce neurogenic inflammation and allergic airway inflammation by releasing numerous neuropeptides from peripheral nerve fibers [9, 11]. Neuropeptides such as CGRP and tachykinins released from airway nerves have the capacity to stimulate the proliferation and recruitment of immune cells to the airways [18–21]. On the other hand, immune cells have been shown to activate airway nerves via cytokines and neurotrophic factors such as neurotrophins (nerve growth factor and brain-derived neurotrophic factor)

[30, 33]. CGRP may act as a chemoattractant factor for the migration of DC into airway ganglia, leading to an increase in the intraganglionic DC population. This neuroimmune cross talk could somehow enhance allergic airway inflammation.

Intraganglionic DC in the JNC may also play a key role in the protection of neurons against viral and bacterial infection [34–37]. With respect to immune cells in ganglions, T cells and macrophages have been found in HSV-1-infected trigeminal ganglia [34].

To investigate the effect of steroids on intraganglionic DC and CGRP in sensory ganglia, FP was intranasally delivered on the final 9 days. FP has been commonly used for therapy of bronchial asthma and obstructive pulmonary disease. Treatment with FP has been demonstrated to improve the clinical response in allergic diseases via a decrease in tachykinins [38, 39]. As expected, in the present study, the treatment of FP was found to reduce allergic airway inflammation via a decrease in inflammatory cells like eosinophils in BALF and lung histology and to

reduce AHR [29]. However, FP did not inhibit the migration of DC or alter the number of CGRP-expressing neurons in airway sensory neurons. Although the previous study reported that FP treatment reduces the migration of plasmacytoid DC in the lungs, this effect does not occur with intraganglionic DC, which are also characterized by CD11c+CD11b- [4, 28]. The result indicates that the increase in intraganglionic DC occurs independently of processes inhibited by steroids. This finding is unexpected and surprising as systemic steroids have been established for the treatment of inflammation in neurological diseases. The blood-brain barrier seems to be no obstacle for systemic steroids. In contrast to the central nervous system, vagal sensory ganglia are enclosed by a capsule, so this may be one of the reasons for the survival and persistence of viruses like the herpesvirus in trigeminal ganglia. Otherwise, steroid treatment may not have an effect on airway nerves in terms of neuropeptide biosynthesis and release.

With respect to the steroid effect, the migration of intraganglionic DC could be effected by long-term treatment with FP and not short-term treatment within 9 days. The present findings demonstrate that the effects of FP on functions of intraganglionic DC remain unknown.

Unilateral vagotomy of allergic sensitized and challenged mice may help to investigate whether the increase in intraganglionic DC requires intact axons of the vagus nerve and if this is caused by a neurotrophic effect or a systemic response. However, vagotomy can lead to neuronal degeneration with alteration of neuropeptide biosynthesis and release and therefore may not reflect the real-life situation in mice. In this regard, further experiments need to be carried out in the future.

In conclusion, the present results demonstrated that the allergic airway inflammation induced by HDM led to an increase in the numbers of DC and CGRP-expressing neurons in sensory ganglia and that these numbers were affected by steroid treatment. These findings indicate that short-term steroid treatment did not have effects on migration of DC into the JNC, whereas steroid treatment was found to suppress allergic airway inflammation.

Acknowledgments

This project was supported by the GRK 1441 Research Training Group (Prof. T. Welte and Prof. Q.T. Dinh), by the German Research Foundation (DFG, DI 1429/2-1) and the Stiftung Bergmannshilfswerks Luisenthal foundation (Prof. Q.T. Dinh), and by SFB587 B4 and IMI UBIOPRED (Prof. A. Braun).

References

- ▶ 1 Barnes PJ: Pathophysiology of allergic inflammation. *Immunol Rev* 2011;242:31–50.
- ▶ 2 Gill MA: The role of dendritic cells in asthma. *J Allergy Clin Immunol* 2012;129:889–901.
- ▶ 3 Lambrecht BN: Dendritic cells and the regulation of the allergic immune response. *Allergy* 2005;60:271–282.
- ▶ 4 Le DD, Rochlitz S, Fischer A, Heck S, Tschernig T, Sester M, Bals R, Welte T, Braun A, Dinh QT: Allergic airway inflammation induces the migration of dendritic cells into airway sensory ganglia. *Respir Res* 2014;15:73.
- ▶ 5 Novak N, Haberkost J, Geiger E, Bieber T: Dendritic cells in allergy. *Allergy* 1999;54:792–803.
- ▶ 6 Lipscomb MF, Masten BJ: Dendritic cells: immune regulators in health and disease. *Physiol Rev* 2002;82:97–130.
- ▶ 7 Manicassamy S, Pulendran B: Dendritic cell control of tolerogenic responses. *Immunol Rev* 2011;241:206–227.
- ▶ 8 Dinh QT, Cryer A, Dinh S, Trevisani M, Georgiewa P, Chung F, Geppetti P, Hepp W, Klapp BF, Fischer A: Protease-activated receptor 2 expression in trigeminal neurons innervating the rat nasal mucosa. *Neuropeptides* 2005;39:461–466.
- ▶ 9 Rochlitz S, Veres TZ, Kuhne K, Prenzler F, Pilzner C, Knothe S, Winkler C, Lauenstein HD, Willart M, Hammad H, Muller M, Krug N, Lambrecht BN, Braun A: The neuropeptide calcitonin gene-related peptide affects allergic airway inflammation by modulating dendritic cell function. *Clin Exp Allergy* 2011;41:1609–1621.
- ▶ 10 Piedimonte G: Contribution of neuroimmune mechanisms to airway inflammation and remodeling during and after respiratory syncytial virus infection. *Pediatr Infect Dis J* 2003;22:S66–S74.
- ▶ 11 Barnes PJ: Neurogenic inflammation in the airways. *Respir Physiol* 2001;125:145–154.
- ▶ 12 Dinh QT, Mingomataj E, Quarcio D, Groneberg DA, Witt C, Klapp BF, Braun A, Fischer A: Allergic airway inflammation induces tachykinin peptides expression in vagal sensory neurons innervating mouse airways. *Clin Exp Allergy* 2005;35:820–825.
- ▶ 13 Martling CR, Matran R, Alving K, Hokfelt T, Lundberg JM: Innervation of lower airways and neuropeptide effects on bronchial and vascular tone in the pig. *Cell Tissue Res* 1990;260:223–233.
- ▶ 14 Groneberg DA, Quarcio D, Frossard N, Fischer A: Neurogenic mechanisms in bronchial inflammatory diseases. *Allergy* 2004;59:1139–1152.
- ▶ 15 Fischer A, McGregor GP, Saria A, Philippin B, Kummer W: Induction of tachykinin gene and peptide expression in guinea pig nodose primary afferent neurons by allergic airway inflammation. *J Clin Invest* 1996;98:2284–2291.
- ▶ 16 Foster CA, Mandak B, Kromer E, Rot A: Calcitonin gene-related peptide is chemotactic for human T lymphocytes. *Ann NY Acad Sci* 1992;657:397–404.
- ▶ 17 Lambrecht BN: Immunologists getting nervous: neuropeptides, dendritic cells and T cell activation. *Respir Res* 2001;2:133–138.
- ▶ 18 Manley HC, Haynes LW: Eosinophil chemotactic response to rat CGRP-1 is increased after exposure to trypsin or guinea pig lung particulate fraction. *Neuropeptides* 1989;13:29–34.
- ▶ 19 Davies D, Medeiros MS, Keen J, Turner AJ, Haynes LW: Eosinophil chemotactic peptide sequences in rat alpha-CGRP: activation of a novel trophic action by neutral endopeptidase 24.11. *Ann NY Acad Sci* 1992;657:405–411.

- ▶ 20 Dunzendorfer S, Kaser A, Meierhofer C, Tilg H, Wiedermann CJ: Cutting edge: peripheral neuropeptides attract immature and arrest mature blood-derived dendritic cells. *J Immunol* 2001;166:2167–2172.
- ▶ 21 Kawanami Y, Morimoto Y, Kim H, Nakamura T, Machida K, Kido T, Asonuma E, Yatera K, Yoshii C, Kido M: Calcitonin gene-related peptide stimulates proliferation of alveolar epithelial cells. *Respir Res* 2009;10:8.
- ▶ 22 Davies D, Spicer BA, Smith H, Haynes LW: Effect of neonatal capsaicin on peptide-containing primary afferent fibres, eosinophil distribution and hyperresponsiveness in rat lung tissue following experimentally induced eosinophilia. *Neuroimmunomodulation* 1994;1:308–314.
- ▶ 23 Kawamura N, Tamura H, Obana S, Wenner M, Ishikawa T, Nakata A, Yamamoto H: Differential effects of neuropeptides on cytokine production by mouse helper T cell subsets. *Neuroimmunomodulation* 1998;5:9–15.
- ▶ 24 Palmer JB, Cuss FM, Mulder PK, Ghatei MA, Springall DR, Cadieux A, Bloom SR, Polak JM, Barnes PJ: Calcitonin gene-related peptide is localised to human airway nerves and potently constricts human airway smooth muscle. *Br J Pharmacol* 1987;91:95–101.
- ▶ 25 DeWitt BJ, Cheng DY, Caminiti GN, Nossaman BD, Coy DH, Murphy WA, Kadowitz PJ: Comparison of responses to adrenomedullin and calcitonin gene-related peptide in the pulmonary vascular bed of the cat. *Eur J Pharmacol* 1994;257:303–306.
- ▶ 26 Schaper C, Gustavus B, Koch B, Ewert R, Felix SB, Kunkel G, Noga O, Glaser S: Effect of fluticasone on neuropeptides in nasal lavage in persistent allergic rhinitis. *J Investig Allergol Clin Immunol* 2010;20:214–221.
- ▶ 27 Han SH, An HJ, Song JY, Shin DE, Kwon YD, Shim JS, Lee SC: Effects of corticosteroid on the expressions of neuropeptide and cytokine mRNA and on tenocyte viability in lateral epicondylitis. *J Inflamm (Lond)* 2012;9:40.
- ▶ 28 Lommatzsch M, Kraeft U, Troebs L, Garbe K, Bier A, Stoll P, Klammt S, Kuepper M, Bratke K, Virchow JC: Fluticasone impact on airway dendritic cells in smokers: a randomized controlled trial. *Respir Res* 2013;14:114.
- ▶ 29 Rochlitz S, Hoymann HG, Muller M, Braun A: No exacerbation but impaired anti-viral mechanisms in a rhinovirus-chronic allergic asthma mouse model. *Clin Sci (Lond)* 2014;126:55–65.
- ▶ 30 Veres TZ, Rochlitz S, Shevchenko M, Fuchs B, Prenzler F, Nassenstein C, Fischer A, Welker L, Holz O, Muller M, Krug N, Braun A: Spatial interactions between dendritic cells and sensory nerves in allergic airway inflammation. *Am J Respir Cell Mol Biol* 2007;37:553–561.
- ▶ 31 Dakhama A, Kanehiro A, Makela MJ, Loader JE, Larsen GL, Gelfand EW: Regulation of airway hyperresponsiveness by calcitonin gene-related peptide in allergen sensitized and challenged mice. *Am J Respir Crit Care Med* 2002;165:1137–1144.
- ▶ 32 Tan Y, Yang T, Liu S, Liu H, Xiang Y, Qu F, Li H, Qin X: Infection with respiratory syncytial virus alters peptidergic innervation in the lower airways of guinea pigs. *Exp Physiol* 2008;93:1284–1291.
- ▶ 33 Braun A, Lommatzsch M, Mannsfeldt A, Neuhaus-Steinmetz U, Fischer A, Schnoy N, Lewin GR, Renz H: Cellular sources of enhanced brain-derived neurotrophic factor production in a mouse model of allergic inflammation. *Am J Respir Cell Mol Biol* 1999;21:537–546.
- ▶ 34 Theil D, Derfuss T, Paripovic I, Herberger S, Meinel E, Schueler O, Strupp M, Arbusow V, Brandt T: Latent herpesvirus infection in human trigeminal ganglia causes chronic immune response. *Am J Pathol* 2003;163:2179–2184.
- ▶ 35 Tsiang H, Ceccaldi PE, Lycke E: Rabies virus infection and transport in human sensory dorsal root ganglia neurons. *J Gen Virol* 1991;72:1191–1194.
- ▶ 36 Li XQ, Fu ZF, Alvarez R, Henderson C, Tripp RA: Respiratory syncytial virus (RSV) infects neuronal cells and processes that innervate the lung by a process involving RSV G protein. *J Virol* 2006;80:537–540.
- ▶ 37 Antinone SE, Smith GA: Retrograde axon transport of herpes simplex virus and pseudorabies virus: a live-cell comparative analysis. *J Virol* 2010;84:1504–1512.
- ▶ 38 Schaper C, Gustavus B, Koch B, Ewert R, Felix SB, Kunkel G, Noga O, Glaser S: Effect of fluticasone on neuropeptides in nasal lavage in persistent allergic rhinitis. *J Investig Allergol Clin Immunol* 2010;20:214–221.
- ▶ 39 Callebaut I, Vandewalle E, Hox V, Bobic S, Jorissen M, Stalmans I, De Vries A, Scadding G, Hellings PW: Nasal corticosteroid treatment reduces substance P levels in tear fluid in allergic rhinoconjunctivitis. *Ann Allergy Asthma Immunol* 2012;109:141–146.