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Dose-dependent recruitment of CD25⁺ and CD26⁺ T cells in a novel F344 rat model of asthma

Thomas Skripuletz,¹ Andreas Schmiedl,¹ Jutta Schade,¹ Sammy Bedoui,^{1,2}
Thomas Glaab,³ Reinhard Pabst,¹ Stephan von Hörsten,^{1,4} and Michael Stephan¹

¹Department of Functional and Applied Anatomy, Medical School of Hannover, Germany; ²Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; ³Department of Respiratory Medicine, Medical School of Hannover, Germany; and ⁴Experimental Therapy, Franz-Penzoldt-Center, Friedrich-Alexander-University, Erlangen, Germany

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Skripuletz T, Schmiedl A, Schade J, Bedoui S, Glaab T, Pabst R, von Hörsten S, Stephan M. Dose-dependent recruitment of CD25⁺ and CD26⁺ T cells in a novel F344 rat model of asthma. *Am J Physiol Lung Cell Mol Physiol* 292: L1564–L1571, 2007. First published March 9, 2007; doi:10.1152/ajplung.00273.2006.—The ovalbumin (OVA)-induced airway inflammation in rats is a commonly used model to explore the pathobiology of asthma. However, its susceptibility varies greatly between rat strains, and presently Brown Norway (BN) rats are preferentially used. Since recruitment of T cells to the lungs depends on the CD26 (dipeptidyl peptidase IV, DPP-IV) expression, Fischer 344 strain (F344) rats are a highly relevant rat strain, in particular because CD26-deficient substrains are available. To establish a F344 rat model of asthma, we challenged F344 rats using different doses of aerosolized antigen (0%, 1%, 2.5%, 5%, and 7.5% OVA) and compared these effects with intratracheal instillation of OVA (1.5 mg/0.3 ml). Asthmoid responsiveness was determined by analysis of early airway responsiveness (EAR), antigen-specific IgE levels, as well as airway inflammation including the composition of T cell subpopulations in the bronchoalveolar lavage (BAL) and lung tissue with special respect to the T cell activation markers CD25 and CD26. Even low allergen doses caused allergen-specific EAR and increases of antigen-specific IgE levels. However, EAR and IgE levels did not increase dose dependently. Higher concentrations of OVA led to a dose-dependent increase of several immunological markers of allergic asthma including an influx of eosinophils, T cells, and dendritic cells. Interestingly, a dose-dependent increase of CD4⁺/CD25⁺/CD26⁺ T cells was found in the lungs. Summarizing, we established a novel F344 rat model of aerosolized OVA-induced asthma. Thereby, we found a dose-dependent recruitment of cellular markers of allergic asthma including the activated CD4⁺/CD25⁺/CD26⁺ T cell subpopulation, which has not been described in asthma yet.

ovalbumin; dipeptidyl-peptidase IV; animal; regulatory T cells

ALLERGIC ASTHMA IS ONE OF the most common diseases with its prevalence having increased dramatically in developed countries over the last two decades (35). Its pathogenesis involves a complex series of reactions within the airways that is associated with allergen-specific airway hyperresponsiveness (AHR) and inflammation. This inflammation is linked to immunological responses to allergenic proteins mediated by an activation of antigen-presenting cells (APC) and CD4⁺ T cells, predominantly, T helper type 2 (Th2)-like cytokines, increased IgE levels, and eosinophil influx characterize the resulting inflammatory cascade (20). However, although new segments

of underlying pathomechanisms are continuously discovered, the complex immunological interactions are not yet fully understood. Therefore, rodent models of asthma, especially the ovalbumin (OVA)-induced airway inflammation, have become helpful in exploring the underlying mechanisms. However, those models only partly mimic the reaction in humans with every model having pros and cons. In many respects, animal models are valuable to study the underlying asthmatic pathomechanisms. However, the clinical course of asthma in many models depends on the genetic background (11, 23, 29). Using rats, the genetically Th2-predisposed Brown Norway (BN) strain is most often used (30). It has been documented that BN rats can show granulomatous pneumonia spontaneously, partly depending on the provider, predisposing for asthma (6). Therefore, the Fischer 344 strain (F344) might be a relevant alternative. First, they demonstrate hyperreactive airways compared with other strains (12, 34). Second, they have not only a greater contractile response, but also a greater density of airway smooth muscles compared with Lewis rats (5, 12), for example. In addition, they enable further investigations on the impact of CD26 (dipeptidyl peptidase IV, DPP-IV) in allergic asthma, since CD26-deficient rat strains are available (13).

Therefore, we characterized the F344 rat strain in this model of allergic inflammation by using increasing doses of aerosolized OVA. The degree of allergic reaction was assessed by determination of early airway responsiveness (EAR), airway inflammation, as well as antigen-specific IgE levels. Moreover, we determined the composition of cells recruited to the lungs focusing on activated lymphocytes such as CD25⁺ (IL-2 receptor) and CD26⁺ T cell subpopulations. We (15) were recently able to show that increased expression of CD26 is associated with an increased recruitment of T cells to the lungs. Beside its peptidase activity (9), CD26 is known as an activation marker of T lymphocytes (21) and is also involved in the regulation of cell adhesion and interaction with the extracellular matrix (32, 33). Consequently, an important role for these cells in the pathogenesis of asthma has been suggested, e.g., even via an interaction with caveolin (22). Importantly, neither dose dependency nor specificity of the recruitment of CD26⁺ T cell subpopulations has been documented so far. We therefore also focused on the allergen-induced recruitment of CD26⁺ T cells.

Summarizing, we validated the F344 strain of the species *Rattus norvegicus* as a novel alternative model to study asthma.

Address for reprint requests and other correspondence: M. Stephan, Functional und Applied Anatomy -4120-, Medical School of Hannover, Carl-Neuberg-Str. 1, 30625 Hannover, Germany (e-mail: Stephan.Michael@mh-hannover.de).

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Furthermore, instilled OVA was compared with increasing aerosolized antigen doses, the latter application allowing an additional measurement of EAR. In addition to classic asthma parameters [e.g., eosinophils in bronchoalveolar lavage (BAL) and lung histology, plasma IgE levels], CD25⁺- and CD26⁺-activated T cell subsets were focused on.

METHODS

Animals and experiments. Inbred F344 rats from the Central Animal Facility Hannover (F344/Ztm) were maintained in colony rooms at the Central Animal Laboratory (Medical School Hannover, Germany). Food (Altromin Standard Diät 1320; Lage, Germany) and water were available ad libitum. Animals underwent routine cage maintenance once a week and were microbiologically monitored according to Federation of European Laboratory Animal Science Associations recommendations (27). Rats were bred at a ratio of 1:2, and male offspring was randomly assigned to the different groups, aiming at a group size of eight.

All research and animal care procedures were approved by the Review Board for the Care of Animal Subjects of the district government (Hannover, Germany) and performed according to international guidelines on the use of laboratory animals.

Sensitization and allergen challenge. All rats were sensitized 14 and 7 days before challenge, as previously described (15). In brief, sensitization was performed with 1 mg of OVA (lot 108H7018; Sigma, Taufkirchen, Germany) and 200 mg of Al(OH)₃ (Sigma) in 1 ml of 0.9% sterile, pyrogen-free NaCl applied subcutaneously into the hind limb. As second adjuvant, concentrated preparations of 5×10^9 heat-killed *Bordetella pertussis* bacilli (kindly provided by Chiron Behring, Marburg, Germany) in 0.4 ml of 0.9% NaCl were given intraperitoneally at the same time. At the age of 12 wk, animals were challenged with 1%, 2.5%, 5%, or 7.5% of aerosolized OVA. Aerosolizing 10% OVA was abandoned, since preliminary tests showed a lower recruitment of inflammatory cells to the lungs when compared with 7.5%. These effects (data not shown) were most likely due to an insufficient aerosolizing of the antigen. Sham controls were challenged with aerosolized, pyrogen-free 0.9% NaCl as a treatment control. As an internal application control, we performed standardized instillation protocol (1.5 mg of OVA in 0.3 ml of saline solution) as hitherto used in our group.

Measurement of pulmonary function. Assessment of EAR in response to aerosolized allergen challenge was performed using a modified head-out body plethysmograph system for rats (2-chamber head-out body plethysmography system model 855; HSE-Harvard, March-Hugstetten, Germany). The head of each animal protruded through a neck collar (24-mm inner diameter, dental latex dam; Roeko, Langenau, Germany) into the ventilated head-exposure chamber. Monitoring of respiratory function was performed as previously described (7). When animals and individual measurements settled down to a stable level, we defined a baseline level pre-challenge. Then, in each case, 2 ml of solution was aerosolized over a period of 10 min via a Pari LC Star nebulizer (Pari, Starnberg, Germany). Different OVA doses (1%, 2.5%, 5%, and 7.5%) as well as a pyrogen-free 0.9% NaCl as sham control were used.

The parameters tidal midexpiratory flow (EF₅₀), time of expiration, tidal volume, and respiratory rate were calculated for each breath and were averaged in 40-s segments with commercial software (HSE Pulmodyn 1.1.1.28). During airway constriction in rats, the main changes in the tidal flow signal occurred during the midexpiratory phase. We defined EF₅₀ (ml/s) as the tidal flow at the midpoint (50%) of expiratory tidal volume, using it as a measure of bronchoconstriction (7). The amount of the decline of EF₅₀ to OVA challenge was calculated as a percentage compared with the initially measured baseline levels.

Isolation of BAL and lung tissue leukocytes. The animals were dissected under isoflurane anesthesia 22 ± 0.5 h after challenge, as

previously described (15). Briefly, the animals were killed by aortic exsanguination. For BAL isolation, a cannula was inserted into the trachea in situ, and the lungs were lavaged four times with portions of 5-ml 0.9% NaCl solution for BAL. The recovery of fluid was >90% in all animals. For further analysis, both lungs were excised from the thorax. The trachea, main bronchi, and hilar lymph nodes were removed from the rest of the lung tissue. For fluorescence-activated cell sorting (FACS) analysis, lung leukocytes were extracted from the left lung using a mechanical disaggregation method (30). For histology, the right lung was fixed in 4% buffered paraformaldehyde and embedded in paraffin.

Cell counts and staining for eosinophils in the BAL. Leukocyte numbers were defined using staining with Türk solution (Merck, Darmstadt, Germany) in a Neubauer counting chamber. After a May-Grünwald/Giemsa staining (Riedel de Haen, Seelze, Germany), eosinophil granulocytes were identified under the light microscope at $\times 630$ magnification. At least 1,000 cells were differentiated on each slide.

Qualitative and quantitative histology of lung tissues. To further illustrate the usefulness of this model and the importance of CD26⁺ subpopulation of T cells, CD26⁺ cells (SE8) and T cells (TCR⁺) (R73) in the lung tissue (also in the peribronchial and perivascular space) were stained using commercially available antibodies (all from Serotec, Düsseldorf, Germany).

To investigate eosinophil infiltration in the lungs, three serial sections (4 μ m thick and 40 μ m apart) were evaluated in three rat lungs of each group. The sections were dehydrated, stained with Giemsa solution, and differentiated in 100% methanol for 6 min and 96% ethanol (250 ml + 16 beads glacial ethanoic acid) for 6.5 min.

Histological evaluation was carried out using a light microscope (Eclipse 80i fluorescence microscope; Nikon, Düsseldorf, Germany). Eosinophils were counted by systematic uniform random sampling using a stereo investigator system (MicroBrightField, Williston, VT). Briefly, in each section, 300–400 test fields (size of the test frame: $100 \times 100 \mu$ m) were evaluated with a constant grid distance of 400 μ m between the test fields in the *x*- and *y*-axes. The eosinophils counted in lung parenchyma (alveoli + alveolar septa + vessels in septa) were given as cell number per square millimeter (cell density) using the following formula: $Q = \text{number} \times \text{grid size} (400 \times 400 \mu\text{m})/100 \times 100 \mu\text{m}$.

Area of interest: immunostaining of lymphocyte subsets and FACS analysis. Analyses were run on a FACScan flow cytometer (BD FACSCanto, Heidelberg, Germany), as previously described (26), using commercially available antibodies [R73 for T cells, Ox12 for B cells, W3/25 for CD4-positive cells, OX8 for CD8-positive cells, OX39 for IL-2 receptor (CD25)-positive cells, OX61 for CD26-positive cells, and RP1 for granulocytes; dendritic cells (DC) were identified as double positive for Ox6 (major histocompatibility complex class II) and Ox62 (DC marker in the rat); all from Serotec]. As an additional marker for T regulatory cells, Foxp3 was used (BioLegend, San Diego, CA). At least 50,000 events were counted in the previously defined mononuclear cell gate, and the percentage as well as the absolute amount were calculated for each determined cell subset.

IgE ELISA. Total IgE and OVA-specific IgE levels in plasma obtained from peripheral blood were determined by ELISA using previously described techniques (15). All antibodies were purchased from Serotec.

DPPIV enzymatic activity. To determine plasma and BAL DPPIV enzyme activity, a microplate-based fluorescence assay was applied. In brief, the assay consisted of 20 μ l of plasma sample, 100 μ l of H₂O, 100 μ l of HEPES buffer (pH 7.6), and 50 μ l of substrate [glycyl-prolyl-4-nitroaniline (GPPNA) $\times$ HCl]. The release of pNA from the substrate was monitored at 405 nm and 37°C using the PowerWave XS spectral photometer (BioTek Instruments, Bad Friedrichshall, Germany).

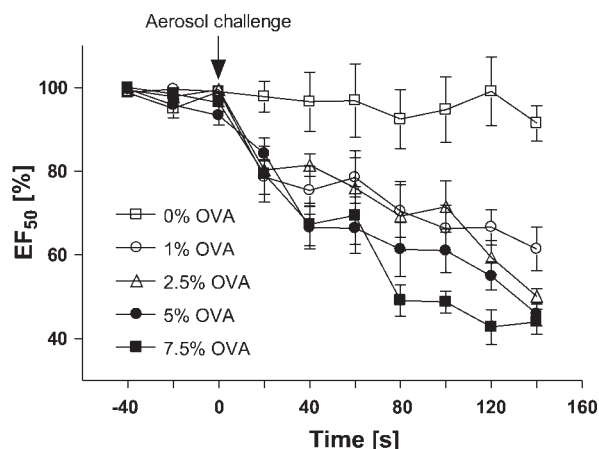


Fig. 1. Time-response course of early airway responsiveness (EAR) as indicated by a decline of tidal midexpiratory flow (EF₅₀) to different doses of aerosolized ovalbumin (OVA) (1%, 2.5%, 5%, and 7.5%). Saline-challenged rats (0% OVA) failed to show an EAR. EF₅₀ is expressed as percentage change compared with baseline, which was calculated for each rat after habituation to the plethysmograph. The minimal response was determined noninvasively. The decline of EF₅₀ is expressed as means $\pm$ SE.

Statistical analysis. Statistical analysis was performed using one-way analysis of variance (ANOVA) with the factor “treatment/dose of allergen” followed by the Fishers protected least significant difference test for post hoc comparison if appropriate. Analysis of EAR was carried out using two-way ANOVA for repeated measurements. All data are given as arithmetic means $\pm$ SE. *P* values of the different ANOVAs are given in RESULTS, while group comparisons derived from post hoc analysis are provided in the figures. In the latter case,

significant effects vs. the control animals are indicated by asterisks (**P* < 0.05; ***P* < 0.01; ****P* < 0.001).

RESULTS

Bronchoconstriction in response to OVA exposure. To investigate whether rats show different airway responsiveness to increasing doses of aerosolized antigen, the amount of the decline in EF₅₀ during OVA exposure was measured. A marked EAR was evident as determined by a significant decline in EF₅₀ in all the groups challenged with aerosolized doses of OVA relative to baseline (Fig. 1). This OVA-specific EAR was already detectable 20 s after starting the allergen administration. By contrast, control animals showed no EAR. ANOVA for repeated measurements revealed a significant interaction of both factors (*P* < 0.0001), indicating both a dose- and a time-specific effect for the various treatments.

Higher IgE levels in OVA-challenged rats compared with sham controls. To further characterize this new model of asthma, OVA-specific IgE plasma levels were determined 22 h after challenge. Whereas previous tests showed that rats, which were neither sensitized nor challenged with OVA, produced no OVA-specific IgE (unpublished data), we here found that even saline-challenged rats showed an antigen-specific titer due to the previous sensitizations (optical density: 1.74 ± 0.33). When analyzing possible treatment effects, significant differences (*P* = 0.001) due to increases of OVA-specific IgE levels compared with sham controls (optical density: instillation: 2.18 ± 0.07 ; 1% OVA = 2.4 ± 0.06 ; 2.5% OVA = 2.5 ± 0.09 ; 5% OVA = 2.5 ± 0.05 ; 7.5% OVA = 2.6 ± 0.1) were

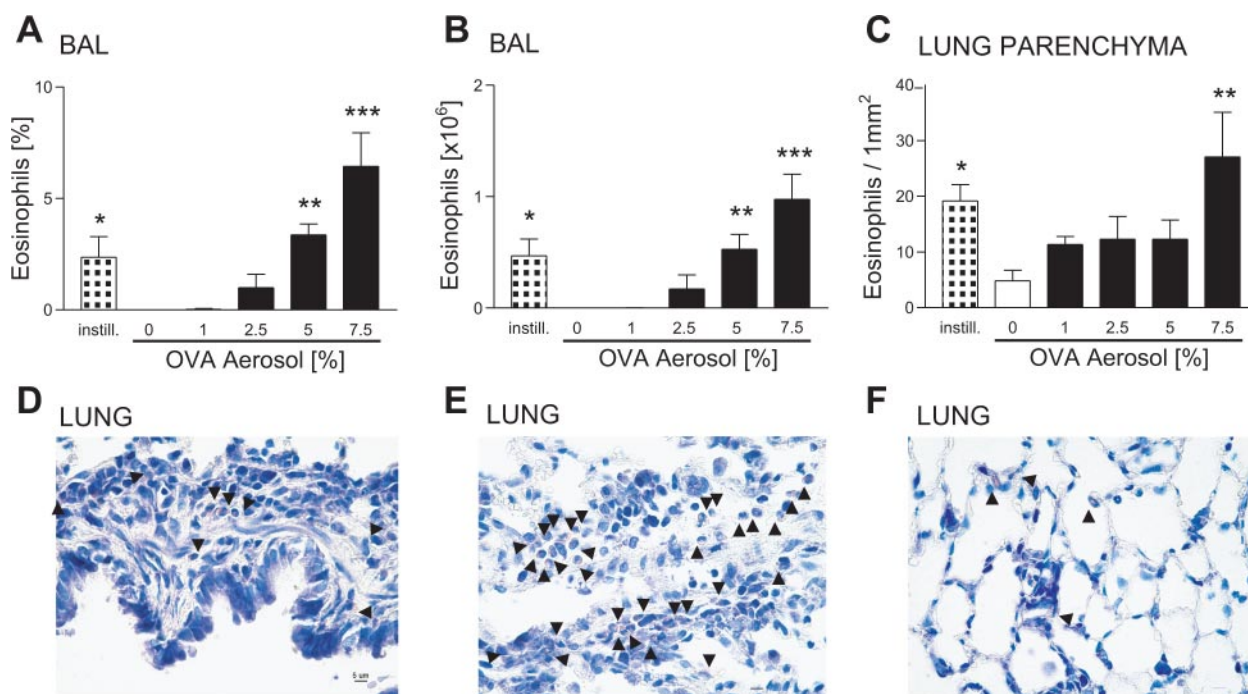


Fig. 2. Shown are the percentage (A) and absolute numbers (B) of eosinophil granulocytes in the bronchoalveolar lavage (BAL) and absolute numbers of eosinophils in 1 mm² of the lung parenchyma (C) 22 h after OVA challenge. Histological lung sections were stained with Giemsa solution. In addition to the different OVA challenge application types, which were instillation (instill.) and aerosolization (Aerosol), different OVA doses (0% = sham controls, 1%, 2.5%, 5%, and 7.5%) were aerosolized. Significant post hoc effects vs. sham controls are indicated by **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. After staining lung tissue sections of 5% OVA-challenged rats with Giemsa, compartmentalization of eosinophils in the lung tissue was obvious (D–F). Some eosinophils (arrowheads) were found in the lamina propria of bronchi and bronchioles (D). The perivascular space around vessels of different size was predominantly infiltrated (E), but eosinophils were also identified in the interalveolar septa (F).

found. However, total IgE levels showed no significant differences (data not shown).

Dose-dependent mobilization of eosinophils into the BAL and lung parenchyma. The recruitment of eosinophils into the BAL was significantly increased in the 5% and 7.5% groups (percentage, Fig. 2A, $P < 0.0001$; absolute numbers, Fig. 2B, $P = 0.0003$). Lower concentrations were not sufficient to mobilize higher amounts of eosinophils compared with sham controls; in fact, there were no eosinophils detectable in the BAL following both aerosolization of saline and 1% of OVA. Similarly, eosinophils were elevated after 7.5% of aerosolized OVA and after instillation treatment with OVA in the lung parenchyma (Fig. 2C; $P = 0.04$).

Dose-dependent mobilization of T cells, $CD4^+$ T cells, $CD8^+$ T cells, and $CD4^+/CD25^+$ cells into the lungs. Since T cells are believed to play a crucial role in the pathogenesis of asthma, we determined the recruitment of different T cell subsets to the BAL and to the lungs following different doses of antigen. Analysis of the absolute cell numbers of TCR^+ , $CD4^+/TCR^+$, and $CD8^+/TCR^+$ cells 22 ± 0.5 h after OVA challenge revealed a significant increase of absolute TCR^+ cells (Fig. 3A; $P < 0.0005$), $CD4^+/TCR^+$ cells (Fig. 3B; $P = 0.0006$), and $CD8^+/TCR^+$ cells (Fig. 3C; $P < 0.0001$) after 5% and 7.5% of aerosolized OVA. Interestingly, we found no significant effects for the TCR^+ and $CD4^+/TCR^+$ cell counts following instillation challenge. For the lung tissue, no significant differences of TCR^+ , $CD4^+/TCR^+$, and $CD8^+/TCR^+$ cell counts were found (data not shown).

Activated $CD4^+/CD25^+$ T cells exhibited a dose-dependent increase in the BAL and lung tissue (Fig. 4, A–D). In contrast, in the lung parenchyma, no significant effects induced by OVA instillation were found.

Dose-dependent recruitment of $CD26^+$ as well as $CD4^+/CD25^+/CD26^+$ cells. Having recently reported that the recruitment of T cells is CD26-dependent (15), we now focused on $CD26^+$ cells in this model of asthma. It was found that 99% of all T cells were CD26-positive in the thymus. Following a 5% OVA challenge, T cells in the periphery expressed CD26 to a lower extent. Since 86% of all T cells express CD26 in the lymph node, 75% in the spleen, and 88% in the blood, we additionally focused on T cells in the BAL and lung of rats challenged with increasing doses of OVA.

In the BAL, not only the percentage of $CD26^+$ cells (Fig. 5A; $P = 0.0002$), but also the absolute number of $CD26^+$ cells (Fig. 5B; $P = 0.0001$) were significantly increased. This was not the case for the percentage of $CD26^+$ cells in the lung tissue (data not shown) but was again found for absolute numbers in the lung parenchyma ($P = 0.02$). This effect was due to a significant increase of these cells following 7.5% of OVA (data not shown).

It was even more interesting that significant, clearly dose-dependent effects of OVA challenge on relative ($P = 0.002$) and absolute numbers ($P < 0.0001$) of a subpopulation of activated, triple positive $CD4^+/CD25^+/CD26^+$ cells in the BAL (Fig. 5, C and D) were also observed. In the lung tissue, the percentage increase of this subpopulation of activated T cells was significantly induced by 7.5% of OVA ($P = 0.0002$; data not shown). However, the absolute amount of these cells in the lung parenchyma was even significantly increased following both 5% and 7.5% of aerosolized OVA ($P < 0.0001$; data not shown).

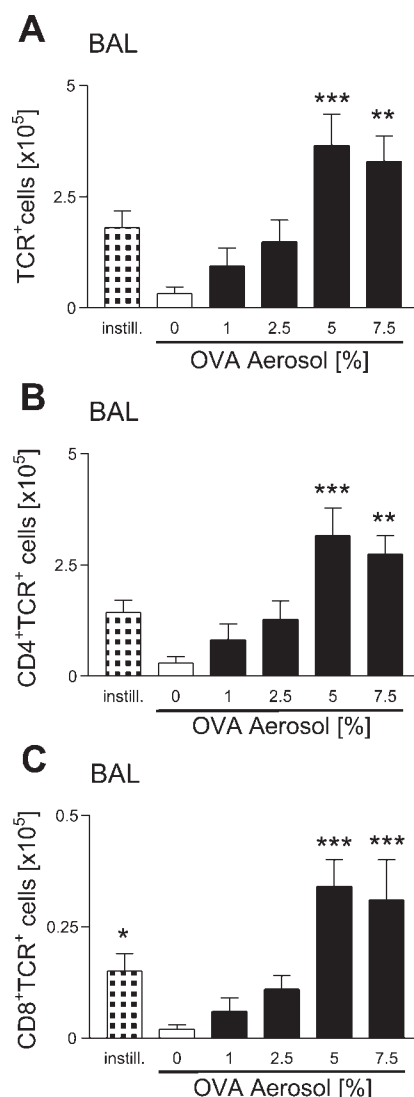


Fig. 3. Shown are the absolute numbers of regulatory T cells (TCR^+) (A), $CD4^+/TCR^+$ cells (B), and $CD8^+/TCR^+$ cells (C) in the BAL as detected by flow cytometry. In addition to the different OVA challenge application types, instillation or aerosolization, different OVA doses (0% = sham controls, 1%, 2.5%, 5%, and 7.5%) were aerosolized. Significant post hoc effects vs. sham controls are indicated by * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

DPPIV enzymatic activity in the peripheral blood and in the BAL showed no significant differences (data not shown).

CD26 expressed on a majority of regulatory T cells. To further characterize the $CD4^+/CD25^+/CD26^+$ cells with regard to regulatory functions, experiments were performed investigating the percentage composition of Foxp3 expression on these cells in the blood, the lungs, the BAL fluid, the mediastinal lymph node, and the spleen in OVA-challenged animals (5% OVA) compared with sham controls (0% OVA) (Fig. 6A). Here, it was found that a subpopulation of these triple positive cells were T regulatory cells.

Furthermore, the distribution of $CD4^+/CD25^+/Foxp3^+$ T regulatory cells was investigated within the different compartments mentioned above (Fig. 6B). Most of these cells expressed CD26 on their surface (Fig. 6C).

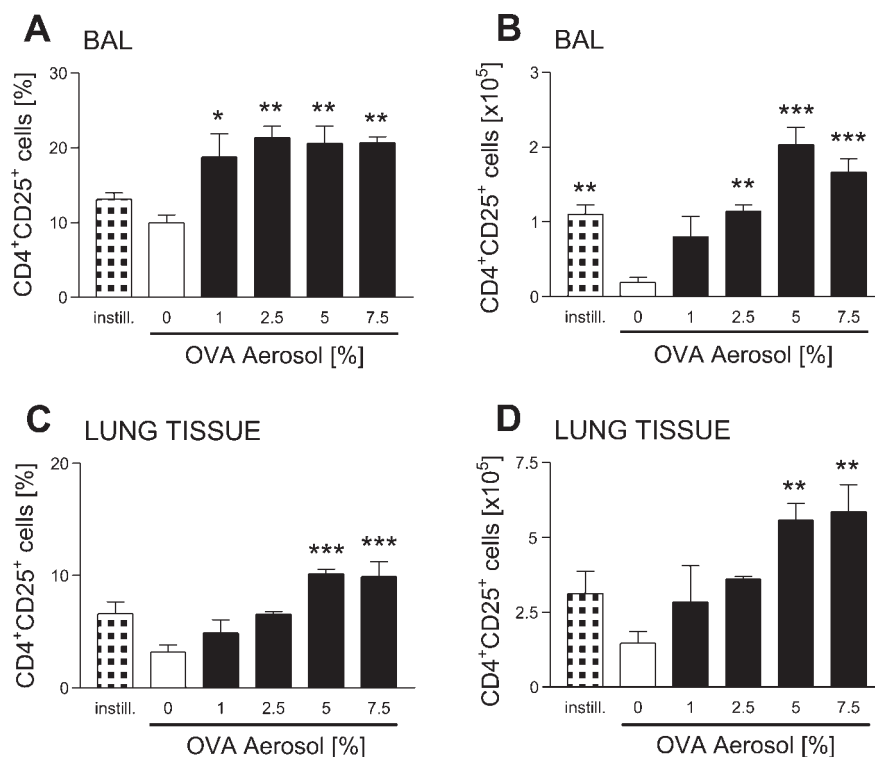


Fig. 4. Flow cytometry revealed significant effects on the percentage (A) and absolute number (B) of CD4⁺/CD25⁺ cells in the BAL and the percentage (C) and absolute number (D) of CD4⁺/CD25⁺ cells in the lung tissue. In addition to the different OVA challenge application types, instillation or aerosolization, different OVA doses (0% = sham controls, 1%, 2.5%, 5%, and 7.5%) were aerosolized. Significant post hoc effects vs. sham controls are indicated by **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

Dose-dependent increase of DC in the lung parenchyma. Local DC in the lung parenchyma were determined by FACS analyses. The percentage (Fig. 7A; *P* = 0.005) as well as absolute numbers (Fig. 7B; *P* = 0.004) of lung tissue DC were significantly increased after 5% and 7.5% of aerosolized OVA and after OVA instillation.

DISCUSSION

Here, we show for the first time that F344 rats represent an appropriate model to study asthma-like effects by demonstrating several dose-dependent effects, e.g., on leukocyte recruitment. Concerning the cellular composition of the inflammatory response, most notably we observed a clear dose-dependent recruitment of CD4⁺/CD25⁺/CD26⁺ T cells to the lungs, with a subpopulation of these cells being T regulatory cells as indicated by Foxp3 expression.

In this new rat model of allergic asthma, we show that the F344 strain of the species *R. norvegicus* provides a well-suited genetic background. Several investigators reported that no pulmonary inflammation was observed in F344 rats in contrast to BN rats (23, 29). Therefore, the BN rat is the most often used strain. A possible explanation for the lacking eosinophils in the F344 rats are chosen low OVA doses (0.5% and 1%) that were used albeit with a longer challenge time (23, 29). Confirming those results, we also found only small amounts of eosinophils after inhalation of 1% OVA. However, mobilization of inflammatory cells, e.g., eosinophil granulocytes to the BAL fluid, started after 5% OVA.

All aerosolized allergen doses were sufficient to induce an EAR. Interestingly, already low doses of OVA led to airway constriction. Similarly, IgE levels in the peripheral blood even responded to low doses of antigen. Increased allergen-specific IgE levels as well as airway eosinophilia represent important

features of allergic asthma in patients (10). However, the specific role of IgE is still controversial. Some studies reported changes in AHR and airway eosinophil infiltration that are associated with increased allergen-specific IgE levels (2, 31), whereas others did not (3, 8, 18).

Interestingly, we found a conspicuous dose-dependent increase of inflammatory markers. In contrast to the early allergic response, only higher concentrations (starting at 5% OVA) of the allergen led to an increase of eosinophil granulocytes, several T cell subpopulations, and DC. It is widely accepted that changes in recruitment and activation of eosinophils and T cells play a fundamental role in the pathogenesis of asthma (17, 36, 38). Eosinophils are recognized to secrete degradative enzymes, small-molecule lipid mediators of inflammation, and inflammatory cytokines that have significant effects on lung structure, including surfactant activity, leading to pulmonary dysfunction (16, 19). Analogous to the airway eosinophilia, we found only higher concentrations of the allergen sufficient to increase several T cell subpopulations, e.g., CD4⁺ T cells and CD8⁺ T cells. In particular, CD4⁺ T cells play a decisive role for allergic responses by secreting type 2 cytokines that again promote airway eosinophilia and immunoglobulin isotype switching to IgE (19, 20).

In addition, we demonstrated for the first time a dose-dependent increase of CD26-positive cells in the lungs. CD26 (apart from having several other functions) is known to be an activation marker of T lymphocytes (14, 37). So far, CD26-positive T cells have been primarily considered as Th1-like subsets (39). Interestingly, it has been recently reported that CD26 is not a helpful marker to differentiate between type 1 and type 2 T cell responses in humans suffering from atopic asthma (28). In line with this report, our data suggest that these cells may also fulfill a functional role in a classically Th2-like

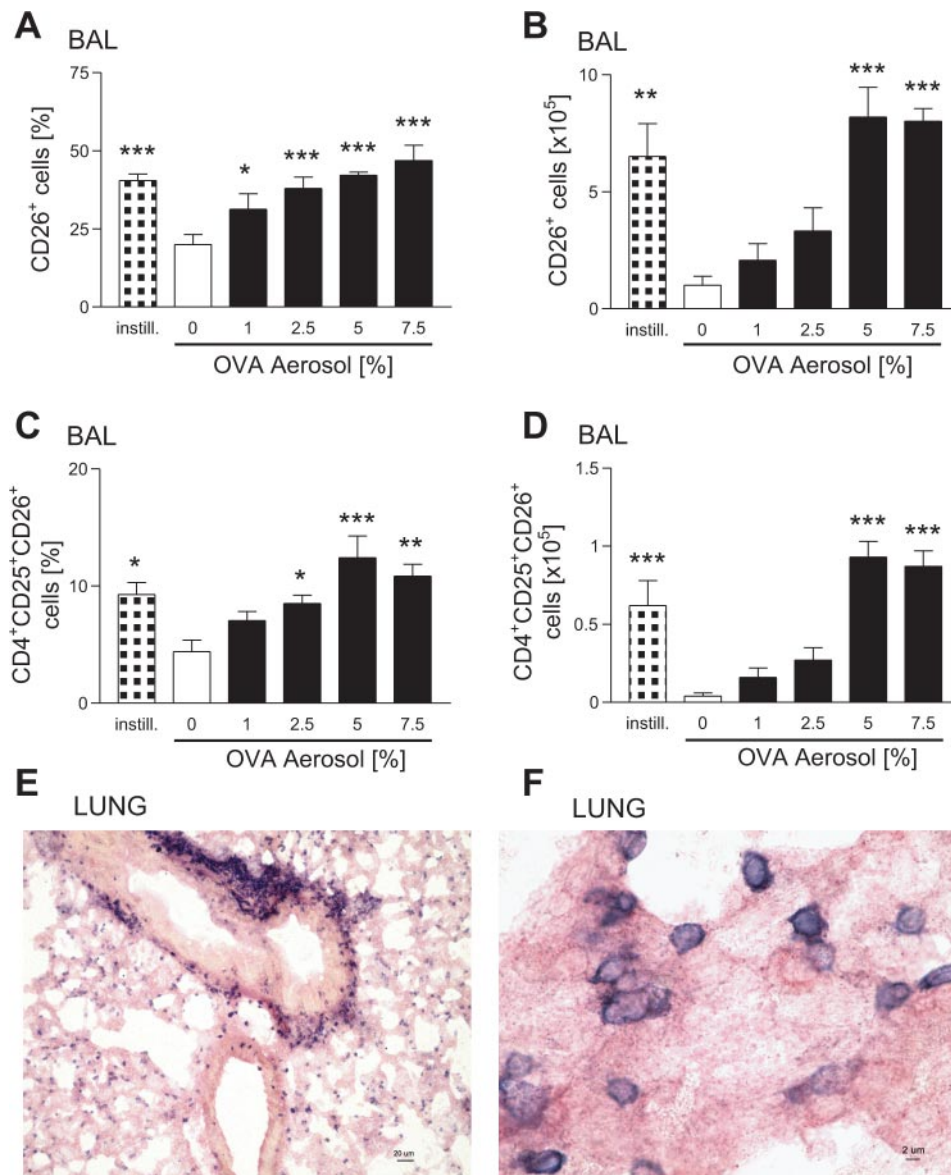


Fig. 5. Shown are the effects on the percentage (A) and absolute number (B) of CD26⁺ cells in the BAL and the percentage (C) and absolute number (D) of the triple positive CD4⁺/CD25⁺/CD26⁺ cells in the BAL. In addition to the different OVA challenge application types, instillation or aerosolization, different OVA doses (0% = sham controls, 1%, 2.5%, 5%, and 7.5%) were aerosolized. Significant post hoc effects vs. sham controls are indicated by **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. In CD26-positive F344 rats, an intensive staining of alveolar epithelium for CD26 was seen in lung tissue sections of 5% OVA-challenged rats (E and F). CD26⁺/TCR⁺ cells were found predominantly in the lamina propria of bronchi and bronchioli as well as in the perivascular space. Alveolar septa sporadically contained CD26⁺/TCR⁺ cells. CD26[−]/TCR⁺ cells were only occasionally found (~10%).

disease, as indicated in our model of allergic-like lung inflammation.

Apart from being involved in the Th1/Th2 balance and supposable even in T regulatory functions (1), CD26 may also mediate its effects via T cell adhesion and/or NH₂-terminal truncation of substrates such as CC chemokine receptor-3 (CCR3) ligands [e.g., eotaxin and regulated upon activation, normal T cell expressed, and, presumably, secreted (RANTES)]. Recently, we (15) were able to show that CD26 acts as an important factor for T cell recruitment as reduced T cell numbers were found in the airways of CD26-deficient rats. In general, these findings indicate an important role of CD26 via adhesion, ectopeptidase function, or T cell activation in the pathogenesis of asthma (22). Whereas we (15) reported that allergen exposure enhanced CD26⁺ T cell migration into the bronchoalveolar space, the present study demonstrates that the expression of CD26 increased even at low allergen doses and further increased in parallel to the allergen doses. This observation may suggest that CD26 represents a sensitive inflammatory marker within the immunological processes of asthma.

Within acute asthmatic processes, it is possible that CD26 exerts special functions with respect to subsets of CD4⁺/CD25⁺ cells. This is suggested by the identification of increased numbers of triple positive CD4⁺/CD25⁺/CD26⁺ cells in the lung parenchyma as well as in the BAL of allergen challenged rats. This T cell subpopulation has not yet been described in asthma, and its functional role is still unclear. As activated subtypes of T cells, CD4⁺/CD25⁺/CD26⁺ cells might represent an active state with increased cytokine production and/or potentially increased adhesion properties that may result in an increased potency of these T cells to leave the vessels and to enter the lung parenchyma. But most intriguing, apart from being an activation marker as indicated by a coexpression of CD26 and CD25 on some T cells, a considerable population of T regulatory cells also express CD26. This may be of additional clinical importance, since CD26 inhibitors, which have recently been established as a new class of drugs in diabetes mellitus therapy (4), might also target CD26⁺ T regulatory cells. Therefore, the potential impact of these compounds in allergic diseases such as asthma is worth inves-

tingating. Targeting CD26 with specific inhibitors might not only attenuate the characteristics of diabetes, but also affect asthmatic responsiveness or other allergic and inflammatory diseases.

Furthermore, CD26 is involved in upregulation of CD86 on APC, possibly mediated by CD26-caveolin-1 interaction, leading to a greater APC-T cell interaction and enhanced T cell proliferation (22). This latter interaction may be potentiated by a dose-dependent recruitment of DC and vice versa, creating a vicious circle.

Rodent models of asthma have a number of advantages but also several limitations (25), which can be subdivided into those specific for the species under investigation (mice, rats, guinea pigs) and others dealing with the induction and moni-

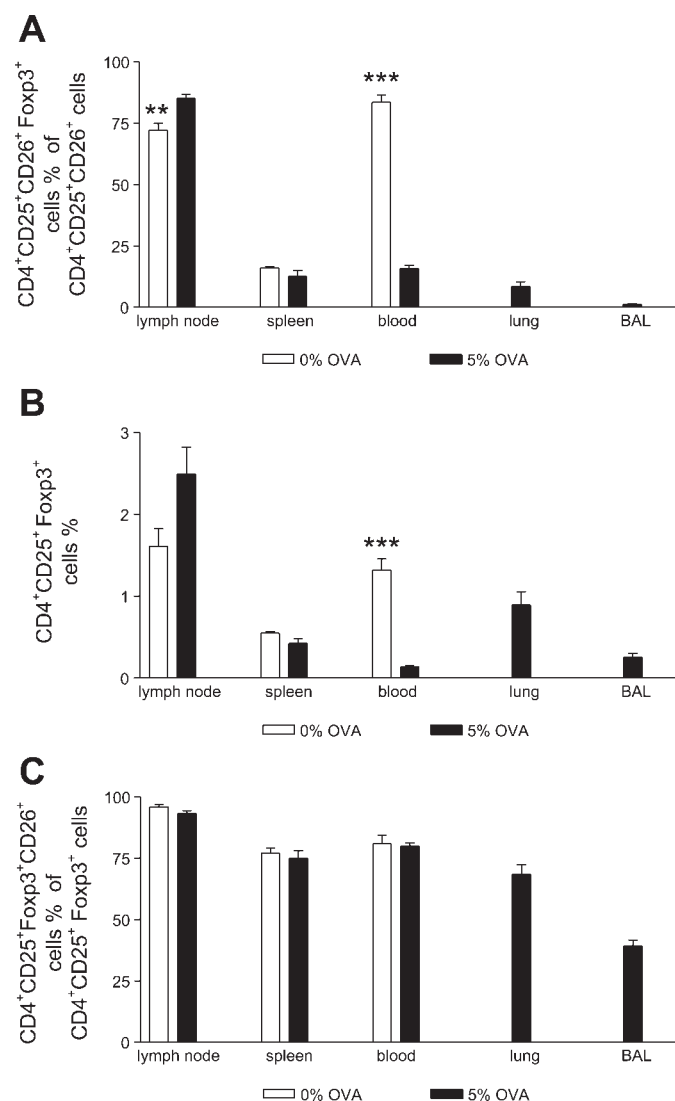


Fig. 6. Displayed is an overview of T regulatory cells and the T regulatory CD26⁺ subpopulation in different compartments comparing OVA-challenged (5%) rats with sham controls. The percentage composition of Foxp3 expression on CD4⁺/CD25⁺/CD26⁺ cells is displayed (A). Most of the CD4⁺/CD25⁺/Foxp3⁺ regulatory T cells (B) show a clear surface expression of CD26 (C). Significant post hoc effects vs. sham controls are indicated by ***P* < 0.01 and ****P* < 0.001. In the BAL fluid as well as in the lungs of rats receiving no OVA challenge, there were hardly any T regulatory cells detectable using flow cytometry.

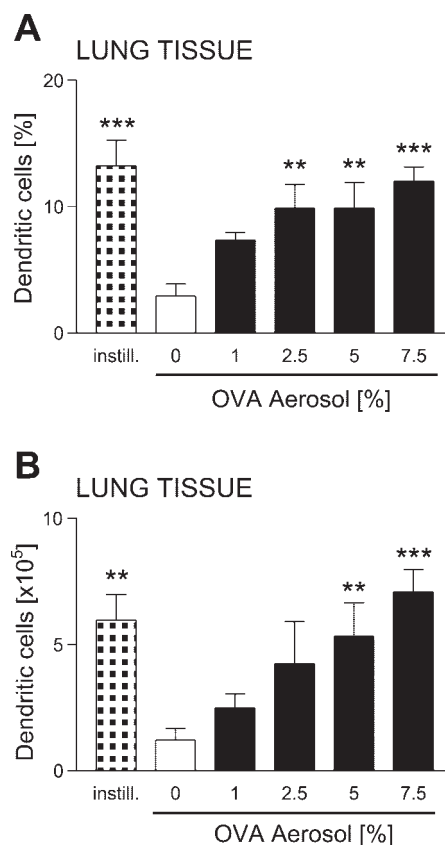


Fig. 7. Shown are the percentage (A) and absolute numbers (B) of dendritic cells (DC) characterized by flow cytometry as double positive for major histocompatibility complex class II and Ox62 (DC marker in the rat) in the lung parenchyma. In addition to the different OVA challenge application types, instillation or aerosolization, different OVA doses (0% = sham controls, 1%, 2.5%, 5%, and 7.5%) were aerosolized. Significant post hoc effects vs. sham controls are indicated by ***P* < 0.01 and ****P* < 0.001.

toring of the disease in the animal. The typical chronic character of asthma in humans is not present or at least not inducible in rodents. In OVA animal models, sensitization via injections is in strong contrast to the situation in human asthma, where it occurs via the respiratory tract (24).

In conclusion, the present work demonstrates that the F344 rat strain represents an important genetic background to study the pathology of asthma. In this new model, even low doses of antigen were sufficient to produce a marked EAR and led to an elevation of OVA-specific plasma IgE levels. However, in the cellular reaction to OVA, there is a clear dose dependency concerning the recruitment of eosinophils and several T cell subpopulations, especially of the triple positive CD4⁺/CD25⁺/CD26⁺ cells 22 h after allergen exposure. These cells represent an interesting, novel T cell subpopulation, which has not yet been described in asthma.

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