Allergic inflammation after allergen challenge – insights from the tissue

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To the Editor,

Limited data exist on the infiltration of eosinophils in direct response to allergen exposure in asthmatic patients. The experimental procedure of segmental allergen provocation (SAP) in mild asthmatic subjects is an extremely valuable study tool to investigate mechanisms of bronchial asthma in patients in general and in particular for the role of eosinophils. In this procedure, BAL and bronchial mucosa can be analysed simultaneously after the induction of allergic inflammation. Older studies yielded data on different time points after the challenge with contradictory results. Eosinophils were studied in mucosa and airway lumen of mild asthmatics undergoing segmental allergen provocation[1,2]. Here, eosinophils and their release products were investigated in thick sections of the bronchial biopsies. Subject data, methods and detailed results are given in the supplement. All subjects showed a clear eosinophilic response in the airway lumen and mucosa 24 hours after the challenge (Figure 1, S1). The data on neutrophils were inconclusive (Figure 1, S2). There was an increase of eosinophils in the mucosa and in the BAL. In the BAL the concentrations of IL-5 and ECP were elevated (supplement). In the mucosa ECP stained areas were found elevated as well as signs of eosinophil activation and degranulation (Figure 2). ECP staining was associated with cellular structures, small granules or dispersed over a large area beneath the epithelium. The degree of released mediators into the tissue is an important parameter investigating new drugs for asthma. In the presented study not cell associated, free ECP positive granules were seen at baseline but only to a small extent. However, after allergen challenge the ECP volume was significantly increased and only few distinct cells could be detected being positive for ECP. These results suggest that in human tissue eosinophils degranulate to a significant amount after a single allergen challenge and therefore release all their toxic content into the surrounding tissue. This is in contrast to animal models of asthma where eosinophilic degranulation after allergen challenge does not occur extensively [3,4]. In the present study, subjects with a strong IL-5 reaction in BAL showed a strong eosinophilic response in the lumen. However, BAL IL-5 levels did not correlate with eosinophil numbers in

the mucosa or volume of ECP positive surfaces in the mucosa (Figure S3). It is a long known fact that IL-5 levels in the BAL correlate highly with absolute numbers of eosinophils in the same compartment [5]. In the present study the allergic reaction is comparable to those of other studies. The missing correlation between IL-5 in the BAL and numbers of tissue eosinophils showed that the relationship between both compartments is not as simple as assumed. Here, the data for the volume of ECP positive surfaces may give an important hint. Interestingly, the volume of ECP positive surfaces was inversely correlated with TNF-α and IL-8. TNF-α enhances migration of eosinophils from mucosa to lumen shown in an in vitro cell culture model [6]. Therefore, one possible explanation is the more TNF-α is found in the BAL the less activated eosinophils are found in the mucosa. In conclusion, the findings in subjects with mild asthma are in alignment with other published results and suggest that 1) human tissue eosinophils release their granules in non-provoked state, and 2) toxic content of these cells is significantly released into the surrounding tissue after a single allergen challenge whereas the distribution and the degree of activation and degranulation of eosinophils differs widely between subjects. Many eosinophils in the airways indicate many eosinophils in the mucosa. Three-dimensional analysis in thick tissue sections using confocal microscopy is a valuable tool in the investigation of bronchial biopsies from patients suffering from bronchial asthma.

(Abbreviations: BAL=bronchoalveolar lavage, ECP=eosinophilic cation protein, IL=interleukin, MBP=major basic protein, NE=neutrophilic elastase, SAP=segmental allergen provocation, TNF=tumor necrosis factor)

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Figure legends

Figure 1: Displayed representative images show 3D-reconstructions of triple-immunostaining in 100 µm biopsy sections for $EG2^+$ surfaces as a marker for eosinophils (red surfaces), NE+ surfaces as marker for neutrophils (yellow surfaces) and cytokeratin (green surfaces) at baseline (A) and 24 hrs after segmental allergen provocation (B) of one subject; Settings for image acquiring: x25-water objective; argon laser (488 nm), helium/neon1 laser (543 nm) and helium/neon2 laser (633 nm) ; 90 optical slices with an interval of 1.1 µm; grid spacing: 50 µm; C-D: Depicted are the graphs of the quantification of volume of EG+ surfaces [µm/100000 µm3] (C) and volume of NE^+ surfaces [µm/100000 μm^3] (D) before (Baseline) and 24 hrs after segmental allergen provocation (Allergen). Each symbol represents one subject (for symbol legend refer to tab. 1). Per subject one 100 µm biopsy section comprised of 4 to 7 regions was analysed. The groups were compared using Wilcoxon matched-pairs signed rank test; **: $p < 0.01$, number of subjects: C: n = 10; D: n = 10; AL = Airway lumen; EG2 = antibody used to identify ECP; NE= neutrophil elastase; SAP = segmental allergen provocation.

Figure 2: Images of a granule filled cell positive for EG2 (red) displayed in the fluorescent channel (A), transmitted light channel (B) and as a merge of both channels (C); white circle: outline of cell, white disrupted circle: cell nuclei; D: distribution of $EG2^+$ staining within a tissue volume of a 20 µm thickness; E: 3D-reconstruction of the stack image shown in D; white stars: $EG2^+$ staining/surfaces resembling cellular structure; white small circles: $EG2^+$ staining in free granule structures; thin white arrows: $EG2^+$ staining distributed within the tissue unbound; F – H: Images show 3D reconstruction of 100 µm biopsy sections of three different subjects 24 hrs after segmental allergen provocation. The distribution of $EG2^+$-staining (red surfaces) beneath the epithelial layer (green surfaces) is very diverse between the subjects. Settings for image acquiring A - E: x40-water objective; helium/neon1 laser (543 nm); grid: 10 µm; scale bar: 10 µm; F – H: x25-water objective; argon laser (488 nm) and helium/neon1 laser (543 nm); 90 optical slices with an interval of 1.1 µm; grid spacing: 50 µm; AL = airway lumen; EG2 = antibody used to identify ECP; SAP = segmental allergen provocation.

Figures

Figure 1

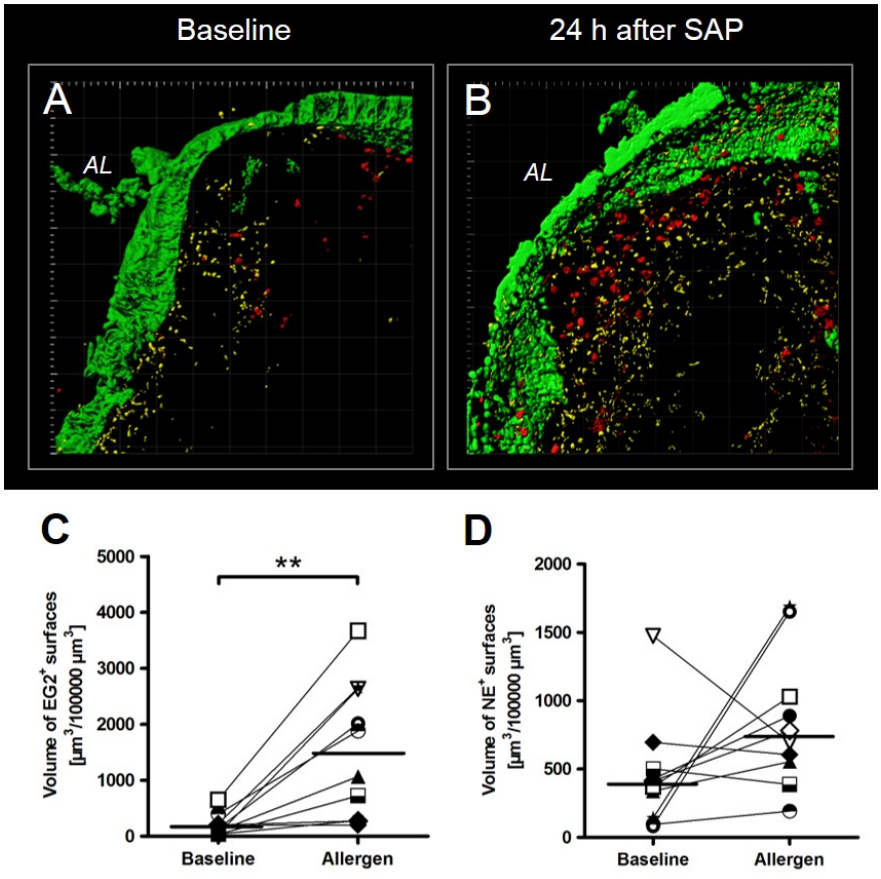

Figure 2