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# Chronic Smoke Exposure Is Associated With Increased Autophagy In Murine Peyer's Patches

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## Introduction

Cigarette smoke causes oxidative stress, leading to smoke-induced autophagy in several organs. Autophagy is a homeostatic process regulating the turnover of proteins and cytoplasmatic organelles.

However, recently it has also been associated with many autoimmune and inflammatory disorders, among which Crohn's disease. The purpose of the present study was to investigate whether cigarette smoke exposure is associated with increased autophagy in Peyer's patches and its epithelium.

## Aims & Methods

C57BL/6 mice were exposed to cigarette smoke or air. After 24 weeks, the animals were sacrificed and Peyer's patches were collected.

mRNA expression of three autophagy-related genes was determined by RT-PCR, and expressed relative to the expression of three reference genes (*Hprt*, *Gapdh* and *Tfrc*).

Transmission electron microscopy (TEM), which is considered to be the gold standard for detection of autophagy, was used to evaluate the presence of autophagic vesicles in the follicle-associated epithelium of Peyer's patches (Figure 1).

ImageJ software was used to measure cell areas and vesicle areas. A comparable number of cells were scored in Peyer's patches of air- and smoke-exposed animals. Only vesicles larger than 0.2 µm² were measured, to exclude lysosomes.

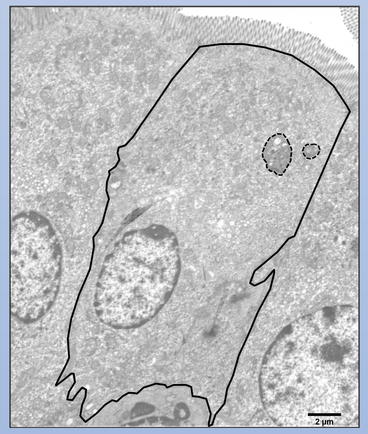


**Figure 1: TEM image of enterocytes of the follicle-associated epithelium of Peyer's patches**
Areas of epithelial cells (full line) and the autophagic vesicles enclosed within these cells (dotted line) were measured. Two different levels were studied per Peyer's patch, and 6 Peyer's patches per group (N = 6).

## Results

Expression of *Beclin-1*, a protein involved in the nucleation of autophagosomes, and of *Atg5* and *Atg7*, which both play a role in the autophagosome vesicle elongation and completion, increased after chronic smoke exposure (Figure 2A-C).

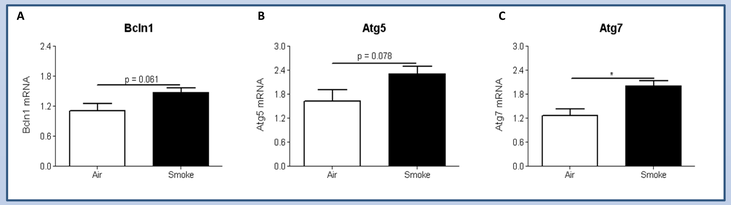


**Figure 2: mRNA expression of autophagy genes in Peyer's patches**
(A) Expression of *Beclin-1* increases from 1.1 ± 0.1 in air-exposed mice towards 1.5 ± 0.1 in smoke-exposed mice. (B) expression of *Atg5* increases from 1.6 ± 0.3 towards 2.3 ± 0.5. (C) expression of *Atg7* increases from 1.3 ± 0.2 towards 2.0 ± 0.1.
N = 6. Data are represented as mean ± SEM. * p < 0.05

TEM of the follicle-associated epithelium overlying Peyer's patches demonstrated that the mean area of autophagic vesicles per epithelial cell increased considerably from 1.1 µm² ± 0.4 µm² in the air group to 2.4 µm² ± 0.4 µm² in the smoke group (p < 0.05) (Figure3A-B).

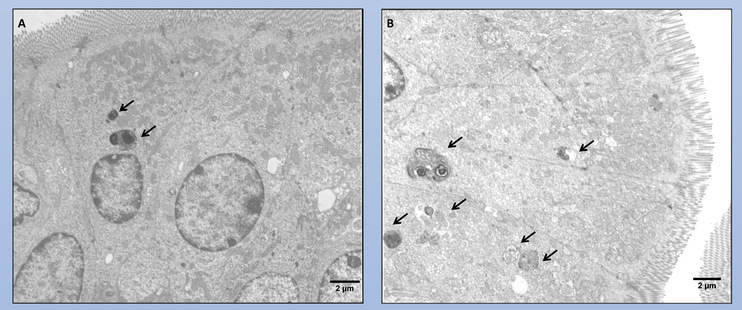


**Figure 3: Quantification of autophagic vesicles in the follicle-associated epithelium of Peyer's patches**
(A) TEM image of epithelium of an air-exposed mouse, showing the presence of a limited number of autophagosomes (arrows). (B) In contrast, epithelial cells of smoke-exposed animals contained a higher number of autophagic vesicles (arrows).

Epithelial cells had a significantly higher number of autophagic vesicles after smoke exposure compared with air-exposed animals. In contrast, the size of the vesicles did not differ between both groups (Figure 4A-B).

These findings indicate that the increased amount of autophagic vesicles in the follicle-associated epithelium after smoke exposure is due to the induction of vesicle formation, rather than to an increased vesicle size.

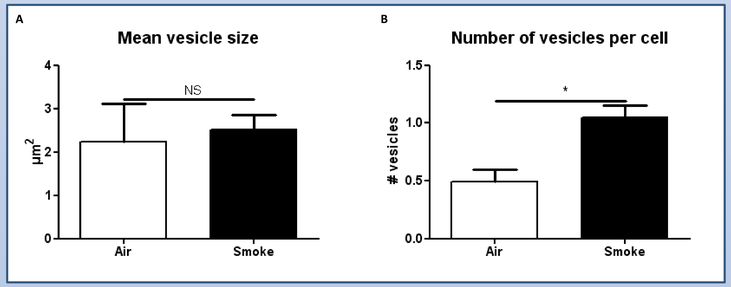


**Figure 4: Vesicle size and number per epithelial cell**
(A) The mean size of autophagic vesicles in epithelial cells of the follicle-associated epithelium did not differ between air- and smoke-exposed animals (2.2 ± 0.9 µm² versus 2.5 ± 0.93 µm²). (B) The number of autophagic vesicles per epithelial cell did increase significantly from 0.5 ± 0.1 vesicles per cell after air exposure towards 1.1 ± 0.1 after smoke exposure.
N = 6. Data are represented as mean ± SEM. NS: non-significant, * p < 0.05

## Conclusion

Here, we provide the first evidence that chronic exposure to cigarette smoke is associated with autophagy in murine Peyer's patches, and more in particular in the follicle-associated epithelium covering Peyer's patches.

Autophagy might act as a protective mechanism cleaning up the injured cells and cell organelles due to smoke-induced oxidative damage. In situations where the autophagic response is impaired, such as Crohn's disease, accumulation of damage due to oxidative stress can lead to an inflammatory response.

Our findings can help to understand the role of smoking in the pathogenesis of Crohn's disease.

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**Disclosure**

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