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).

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 1.6 ± 0.3 towards 2.3 ± 0.5. (B) expression of Atg5 increases from 1.3 ± 0.2 towards 2.0 ± 0.1. (C) expression of Atg7 increases from 1.1 ± 0.1 towards 1.5 ± 0.1. N = 6. Data are represented as mean ± SEM. * p < 0.05

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).

Figure 1: TEM image of enterocytes of the follicle-associated epithelium of Peyer’s patches
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 increased 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.

Disclosure

This work was supported by the Special Research Fund of Ghent University (01J17507) and a Concerted Research Action of Ghent University (BOF 10/GOA/021). Stephanie Verschuere is supported by a doctoral grant from the Special Research Fund of Ghent University. Ken Bracke is a postdoctoral researcher of the Fund for Scientific Research (FWO) in Flanders. The authors have no conflicts of interest.