Background

Smokers have a two-fold increased risk to develop Crohn’s disease (CD). However, little is known about the mechanisms through which smoking affects CD pathogenesis. Especially Crohn’s ileitis seems to be negatively influenced by smoke exposure. Interestingly, the Peyer’s patches in the terminal ileum are the sites where the first CD lesions develop.

Methods

To investigate whether smoke exposure causes alterations in Peyer’s patches, we studied Peyer’s patches (PP) of C57BL/6 mice after exposure to air or cigarette smoke for 24 weeks. First, barrier function of the follicle-associated epithelium overlying PP was evaluated by means of immunohistochemistry for active caspase-3, a marker for apoptosis. Furthermore, immune cell numbers and differentiation were determined in the ileal PP by flow cytometry.

Results

Chronic smoke exposure is associated with increased apoptosis in the follicle-associated epithelium (FAE) covering PP. After smoke exposure, the apoptotic index in the FAE almost doubles (0.98 ± 0.09% after air exposure versus 1.76 ± 0.14% after smoke exposure, P < 0.001). Smoke-induced apoptosis is limited to the epithelium, and not found in the underlying tissue.

Figure 2: Smoke exposure induces apoptosis in follicle-associated epithelium (FAE) of Peyer’s patches (PP). (a) Immunohistochemistry for active caspase-3 in a PP of a smoke-exposed mouse. Inset details of an apoptotic epithelial cell. (b) Apoptotic index, defined as the percentage of apoptotic cells in the FAE. Data are represented as mean ± s.e.m., and are one representative of two independent experiments. ** P < 0.01; *** P < 0.001.

Figure 3: Cigarette smoke is associated with dendritic cell and T-cell accumulation in Peyer’s patches. (a) Total DC: 3.8 ± 0.3% versus 7.1 ± 0.5% after smoke exposure. (b) CD11b+ DC: 1.1 ± 0.1% versus 2.1 ± 0.1%. (c) Total T-cells: 10.9 ± 0.5% versus 14.8 ± 0.7%. Data are represented as mean ± s.e.m., and are one representative of two independent experiments. ** P < 0.01; *** P < 0.001.

Figure 4: Expression of chemokines CCL9 and CCL20 after smoke and air exposure. (a-b): Protein expression of CCL9 in Peyer’s patches is mainly localized in the FAE of Peyer’s patches and are known to attract CD11b+ DC towards the subepithelial dome through interaction with their respective receptors CCR1 and CCR6, both expressed by DC subsets. In contrast, expression of the chemokines CCL2 and CCL19, which are involved in recruitment of DC subsets towards other parts of Peyer’s patches, was not altered by smoke exposure.

Chemokine expression in PP was evaluated by RT-PCR and immunohistochemistry on cryosections.

Figure 5: Flowcytometric analysis of dendritic cell (DC) populations on the three most distal ileal Peyer’s patches. Gating strategy for total DC, defined as a CD11c highly positive and major histocompatibility class II (MHCII) positive cell population. Within this population, CD11c positive cells were identified as the CD11b+ DC subset.

Discussion

Our results demonstrate that chronic cigarette smoke exposure induces apoptosis in follicle-associated epithelium and is associated with immune cell accumulation in Peyer’s patches. Recruitment of DC (and in particular the CD11b+ DC subset) is probably induced by the upregulation of the chemokines CCL9 and CCL20 by the FAE. Furthermore, an increase in total T-cells and several T-cell subsets was observed. These results suggest that smoking causes alterations in the epithelial barrier of Peyer’s patches, leading to changes in the composition of the underlying immune cell population.

In combination with other predisposing factors, such as genetic factors, these alterations may lead to the development of intestinal inflammation distinctive for Crohn’s disease.

Funding

This work was supported by the Special Research Fund of Ghent University (101/17) and a Concerted Research Action of Ghent University (BOF 10/GOA/021). Stephanie Verschuere is supported by a Postdoctoral Grant from the Special Research Fund of Ghent University. Ken Bracke is a postdoctoral researcher of the Fund for Scientific Research (FWO) in Flanders.

Chemokine expression in PP was evaluated by RT-PCR and immunohistochemistry on cryosections.

Flow cytometry demonstrated significant increases in total dendritic cells (DC) (P < 0.01), and more in particular in the CD11b+ DC subset, which almost doubled (P < 0.001). Furthermore, shifts in T-cell populations were present. Total T-cells increased significantly (P < 0.001), and both the CD4+ and the CD8+ T-cell subset were higher after smoke exposure compared with air exposure. Also regulatory T-cells increased significantly in smoke-exposed mice.