M1 MACROPHAGES ACTIVATE NOTCH SIGNALING PATHWAY IN EPITHELIAL CELLS: RELEVANCE IN CROHN’S DISEASE

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Introduction: Crohn’s disease (CD) is associated with impaired epithelial barrier function. The inflammatory microenvironment modulates the ability of epithelial cells to regenerate. Macrophages plasticity allows them to differentiate, depending on environmental factors, in different phenotypes.

Notch has recently emerged as a regulator of intestinal epithelial barrier function. It has been shown that Notch signalling pathway plays essential roles in proliferation of intestinal crypt cells and secretory cell differentiation.

Objective: To analyse the activation pattern of macrophages in the intestine of patients with CD and to determine the relevance of different phenotype macrophages in the activation of Notch signalling pathway in epithelial cells.

Patients and Methods: Both damaged and non-damaged mucosa from chronic patients with CD was obtained. Presence of macrophages, CD86+ macrophages (M1 phenotype) and CD206+ macrophages (M2 phenotype) was analyzed by immunohistochemistry. Quantification was performed counting positive cells in a total area of 0.152 mm² using an inverted microscope.

Macrophages derived from U937 cells were differentiated to M1 phenotype (LPS 0.1ng/ml + IFN-γ 20ng/ml, during 24h) or M2 phenotype (IL-4 20ng/ml, during 72h) and gene expression of Notch ligands (Dll4 and Jag1) was analyzed by real-time RT-PCR at different days. After differentiation, M1 and M2 macrophages were co-cultured with Caco cells during 24h. Protein levels of HES-1 were measured in epithelial cells by Western Blotting. Moreover, epithelial cell proliferation was analyzed at 24, 48 and 72h. Data were expressed as mean±SEM and performed in groups of n≥4.

Results: The number of macrophages in damaged mucosa of patients with CD (23.1±2.6) was higher than the number of macrophages in non-damaged mucosa (5±0.73). M2 macrophages were the main phenotype present in both damaged and non-damaged mucosa but the number of these cells was significantly higher in damaged (17.2±2.8) than in non-damaged mucosa (3.8±0.8). M1 macrophages were very low in the mucosa of patients with CD: 0.12±0.01 and 0.33±0.11, in non-damaged and damaged mucosa, respectively.

The mRNA expression of Notch ligands, Dll4 and Jag1, at time of differentiation (fold induction vs non-differentiated macrophages) was significantly higher in M1 macrophages (3.0±0.8) than in M2 macrophages (1.2±0.3). Furthermore, in M1 macrophages a time-dependent up-regulation was observed (4.3±1.1, 24h after differentiation and 13.7±5.1, 48h after differentiation). In contrast, M2
macrophages exhibited similar levels of expression of Dll4 and Jag1 along the time (0.8±0.3 and 1.8±0.6, 24h and 48h after differentiation, respectively).

In co-culture system M1 macrophages increased protein expression of HES-1 (1.5±0.1) while M2 macrophages failed to do it (1.1±0.1) compared with the expression in non-differentiated macrophages. In parallel, M1 macrophages increased epithelial proliferation at 24h (183±20%) and at 48h (270±7%) compared with the basal proliferation of epithelial cells (114±14% and 225±30%, respectively).

**Conclusion:** M1 macrophages increase the expression of several Notch ligands and modulate mechanisms of epithelial proliferation and differentiation in epithelial cells. The low presence of M1 macrophages in chronic patients with CD may difficult epithelial regeneration of the damaged mucosa.