Título: COOPERATION BETWEEN HUMAN DENDRITIC CELL SUBSETS: A ROLE FOR NOTCH

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Resumen: Cooperative events between DC subsets involve cell-cell contacts and soluble factors. Upon viral challenge, murine plasmacytoid DC (pDC) induces conventional DC (cDC) cooperation through CD40-CD40 ligand (CD40L) interactions and interleukin- (IL-) 15 secretion, whereas in humans the same effect is mediated by IFN-a. Conversely, during bacterial infections, pDC maturation may be induced by activated cDCs, although no mechanisms had been described so far. Here we investigated how pDCs are "conditioned" by cDCs. Blood-borne human DC subsets (cDCs and pDCs) were sorted from healthy donors. Interleukin-3 maintained pDCs were co-cultured with either lipopolysaccharide (LPS)-activated- or control-cDCs (cDCLPS, cDCCTRL). Then, the phenotype and cytokine/chemokine secretion of pDCs and cDCs cultured alone or in co-culture conditions were determined. cDCLPS-conditioned pDCs upregulated maturation markers such as CD25 and CD86. In addition, co-culture supernatants contained increased amounts of IL-6 and CCL19 compared to control conditions. Microarray and real time RT-PCR analyses on sorted conditioned pDCs showed the induction of several genes, including IL-6 and CCL19. Of particular interest was the upregulation of several Notch target genes.

Notch signaling is involved in multiple cellular processes. Recent data also supports their prominent role in the regulation of the immune response. So, we analyzed the expression and function of Notch receptors and ligands on both, cDCs and pDCs. The expression and modulation upon TLR activation of Notch molecules partially differed between cDCs and pDCs, but functional involvement of Notch pathway in both cell types was clearly

revealed by specific inhibition using DAPT. Beyond the induction of Notch target genes and modulation of maturation markers, Notch pathway was also involved in a differential secretion of some specific cytokines/chemokines by DC subsets. While Notch ligation induced IL-10 and CCL19 secretion in cDCs, Notch inhibition resulted in a diminished production of these proteins. Regarding pDCs, Notch activation induced TNF-a whereas Notch inhibition significantly abrogated the secretion of CCL19, CXCL9, CXCL10, and TNF-a. In addition, Notch modulation of DC subsets differentially affected Th polarization of allostimulated T cells. Further studies adding DAPT in the pDCs+cDCs co-cultures resulted in a blockade of the expression of Notch target genes in conditioned pDCs. Moreover, DAPT treatment also inhibited the maturation and hampered the secretion CCL19 (but no IL-6) on cDCLPS-conditioned pDCs. Our results suggest that Notch pathway may function as an additional mechanism controlling human DC responses and point to the implication of the Notch signaling pathway in the maturation of pDCs by LPS-activated cDCs, demonstrating a role for Notch molecules in the communication between human DC subsets. This control mechanism may ultimately contribute to define the local milieu promoted by these cells under the particular conditions of the immune response.