Lipoxin A4 and a Related Analogue Suppress the Maturation State and TLR-mediated Production of Inflammatory Cytokines by Primary Dendritic Cells

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Introduction: Control of inflammation is crucial to prevent damage during infection and tissue injury. Dendritic Cells (DCs) are tissue-resident myeloid cells that link innate and cognate immune/inflammatory responses. DCs secrete pro-inflammatory cytokines (e.g. IL-6, TNF) following ligation of toll-like receptors (TLRs) and present antigenic peptides to T-cells with co-stimulation via CD80/CD86. Lipoxins (LX) are eicosanoids with anti-inflammatory and pro-repair properties. In this study the in vitro effects of LXA4 and a synthetic LX analogue (Ta89) on primary murine DCs were studied.

Materials and Methods: Primary DCs were purified from spleen and kidneys of adult B6 mice by anti-CD11c magnetic column separation after collagenase digestion. DCs were cultured with varying concentrations of LXA4, Ta89 or vehicle for 24hr then washed and exposed to ligands for TLR4 (LPS) and/or TLR2 (LTA) for further 24hr. In some experiments, LX and TLR ligands were applied simultaneously. DC-secreted cytokines (IL-6, TNF, IL-10) were measured by ELISA of culture supernatants. Analysis of surface expression of maturation markers (MHC II, CD80, CD86, CD40) was carried out by multi-colour flow cytometry.

Results: Dose-dependent inhibition by LXA4 of TLR4/2-stimulated IL-6 (Figure 1) and TNF secretion and CD40 up-regulation by splenic DCs was observed with maximal effect at 100ng/ml. Modest inhibition of IL-10 occurred with a paradoxical increase seen at 1000ng/ml. Inhibition of TLR4/2-stimulated IL-6 and TNF secretion by splenic DCs was also observed following pre-incubation with the LX analogue Ta89 with maximal effect seen at 10ng/ml. Simultaneous exposure to TLR4/2 ligands and Ta89 also resulted in cytokine inhibition but at a 10-fold higher conc. of Ta89 (100 ng/ml). Pre-incubation of splenic DCs with 10ng/ml Ta89 resulted in reduced surface staining for CD80 and CD86 with selective effect on mature (CD80hi/CD86hi) DCs (Figure 2). Similar results were obtained for IL-6 secretion and surface expression of CD80 and CD86 by primary DCs purified from mouse kidney in the presence of 10 ng/ml Ta89.

Figure 1: Dose dependent inhibition of TLR4/2-induced splenic DC secretion of IL-6 by LXA4.

Figure 2: Reduced CD80 and CD86 expression by splenic DCs with LX analogue Ta89 10ng/ml.

Discussion and Conclusions: The results demonstrate an inhibitory effect of LXA4 and a LX analogue on pro-inflammatory and pro-immunogenic properties of primary DCs. Cytokine responses mediated through TLRs were suppressed suggesting a potential therapeutic role in tissue injury. Similar inhibitory effects were observed for tissue-resident DCs from kidney – an organ highly susceptible to ischemia-reperfusion injury. The capacity of LX analogues to suppress DCs represents an attractive therapeutic prospect for prevention or resolution of acute inflammation and for re-establishment of tissue homeostasis.

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