ANNEXIN A1 INHIBITS THE MATURATION OF DENDRITIC CELLS WHILE INCREASING THEIR TH1 SKewing PROPERTIES

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In peripheral tissues dendritic cells (DCs) act as sentinels and, upon recognition of foreign antigens, they migrate to proximal lymph nodes where they activate T lymphocytes specific for the antigen encountered. The inflammatory mediators influencing DC migration also induce transformation of DCs into cells capable of influencing T cells polarization. DCs are more than a simple “on/off” switch of the immune response; rather, subtypes of DCs exert more subtle forms of control by influencing the character of T-cell differentiation into T helper type 1, type 2, or non-polarized T-cell (Lanzavecchia and Sallusto, 2001). Studies carried out in our laboratory have shown that Annexin A1 (AnxA1) favours Th1 response in vivo and in vitro. Here we asked whether AnxA1 might influence DC maturation and activation.

DCs were obtained by culturing bone marrow cells from male C57/BL6 mice (6-8 weeks of age) with GM-CSF derived from X63-GMCSF cell supernatants for 12 days (Slade and Langhorn, 1989). Thereafter, DCs were pre-treated with hrAnx-A1 (300 nM) for 1h and then primed with lipopolysaccharide (LPS 10 pg/ml) for further 24h. Cytokine tumour necrosis factor (TNF)-α and interleukin (IL)-12 release was measured with specific ELISA (eBioscience, Middlesex, UK). The effect of hrAnxA1 on phagocytosis in DCs, a mechanism characteristic and distinctive of immature DCs vs mature DCs, was studied using FITC-dextran (1mg/ml, 1h; Sigma-Aldrich, Poole, UK). MHC class II, CD40, CD54, and CD86 expression on DC was monitored by FACS using specific monoclonal antibodies (eBioscience). Data were analysed by ANOVA and Bonferroni test.

Stimulation of DCs with LPS increased TNF-α and IL-12 production (pg/ml: 150±12 and 42±2 vs. 90±3 and 24±1 in control cells, respectively; n=3, P<0.05). Pre-treatment with hrAnxA1 inhibited TNF-α production by 78±7% (n=3; P<0.01) while increasing IL-12 release by 122±15% (n=3; P<0.01). The endocytic capacity of hrAnxA1 treated mature DCs was strongly increased (100±3%; n=3; P<0.01). Finally, analysis of MHC class II, CD40, CD54, and CD86 expression on DC was monitored by FACS using specific monoclonal antibodies (eBioscience). Data were analysed by ANOVA and Bonferroni test.

These results demonstrate that hrAnxA1 inhibits DC maturation and antigen up-take by decreasing the production of early cytokines involved in this process (such as TNF-α). The protein affects their Th1 skewing phenotype by increasing IL-12 release and T cell co-stimulatory molecule expression. Thus, AnxA1 might influence adaptive immunity by converting DCs from an antigen-capturing into a T cell–sensitizing mode.


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