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Bisoprolol reverses epinephrine-mediated inhibition of cell emigration through increases in the expression of β-arrestin 2 and CCR7 and PI3K phosphorylation, in dendritic cells loaded with cholesterol☆

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ABSTRACT

The effect of bisoprolol on dendritic cell (DC) migration was investigated, including the analysis of protein expression, cytokine secretion and activation of the PI3K-pathway. The chemotactic cell numbers in cholesterol-loaded DCs treated with epinephrine were significantly decreased by 26.66 ± 6.29% (6 h), 35.67 ± 2.91% (12 h) and 29.33 ± 1.09% (24 h). This effect was significantly reversed by 46.00 ± 10.65% (6 h), 64.25 ± 6.77% (12 h) and 55.74 ± 5.51% (24 h) when bisoprolol and epinephrine were both present. In cholesterol-loaded DCs, treatment with epinephrine significantly increased AR-β1 protein expression by 56.99 ± 4.87%, but inhibited β-arrestin 2 and CCR7 protein expression by 30.51 ± 4.22% and 25.31 ± 0.04%, respectively. These effects were reversed by bisoprolol by 36.87 ± 4.40%, 41.47 ± 3.95% and 30.14 ± 0.54%, respectively. TNF-α and MMP9 levels were decreased by 68.33 ± 4.00% and 39.57 ± 9.21% in cholesterol-loaded DCs treated with epinephrine. In contrast, when bisoprolol and epinephrine were administered together, the secretion of these proteins was significantly increased by 233.81 ± 37.06 % and 76.66 ± 14.21%, respectively. Treatment with epinephrine decreased PI3K-phosphorylation by 31.88 ± 2.79%, 40.24 ± 5.69% and 30.93 ± 4.66% at 15, 30 and 60 min, respectively, whereas the effect of epinephrine on the expression of phosphorylated PI3K was reversed by 49.49 ± 12.12%, 70.93 ± 16.14% and 47.62 ± 6.00%, respectively, when cells were treated with both bisoprolol and epinephrine. Wortmannin inhibited the effects of bisoprolol on PI3K-phosphorylation (38.63 ± 6.12%), the expression of CCR7 (23.4 ± 2.72%), the secretion of TNF-α (69.46 ± 4.48%) and MMP9 (43.15 ± 4.63%), and the number of chemotactic cells (36.84 ± 5.22%). This is the first study to establish a signaling pathway, epinephrine-AR-β1-β-arrestin2-PI3K-MMP9/CCR7, which plays a critical role in the migration of DCs.

Introduction

Dendritic cells (DCs) are a specific type of leukocyte that are able to alert the immune system to the presence of antigens, infections and inflammatory mediators [1]. In recent years, the impact of DCs on the initiation and progression of atherosclerosis has been evaluated [2]. Foam cells can be induced by oxidized low density lipoprotein in human monocyte-derived DCs, which represents a new source of foam cells in atherogenesis [3]. Rapid regression of atherosclerosis involves foam cell migration from the plaque, decreased ApoB retention in the intima, suppression of hematopoietic stem cell proliferation and foam cell apoptosis [4,5]. Foam cell emigration is a very important process. In the early phase of atherosclerotic plaque formation, macrophage-derived foam cells emigrate from vessel endothelium into blood [6]. However, in the later phase of formation of atherosclerotic plaques, this process is blocked because of the fibrous cap, and instead, foam cells derived from DCs emigrate from the vessel adventitia into neighboring lymph nodes [7].

Abbreviations: DC, dendritic cell; PBMC, peripheral blood mononuclear cells; B, bisoprolol; E, epinephrine; Ch, cholesterol; rh GM-CSF, recombinant human granulocyte–macrophage colony-stimulating factor; rh IL-4, recombinant human interleukin-4; MCP-1, monocyte chemotactic protein-1; AR-β1, β1 Adrenergic Receptor; CCR7, cysteine cysteine chemokine receptor 7; TNF-α, tumor necrosis factor-α; MMP9, matrix metalloproteinase; IFN-γ, interferon-γ; IL-17, interleukin-17; PI3K, phosphatidylinositol 3-kinase; W, wortmannin.

☆ **Authors' contributions:** RZD and JPQ conceived the study. HY performed experiments and wrote the manuscript. SCC performed data analysis and discussed the results. YQT assisted with the experiments. All authors read and approved the final manuscript.

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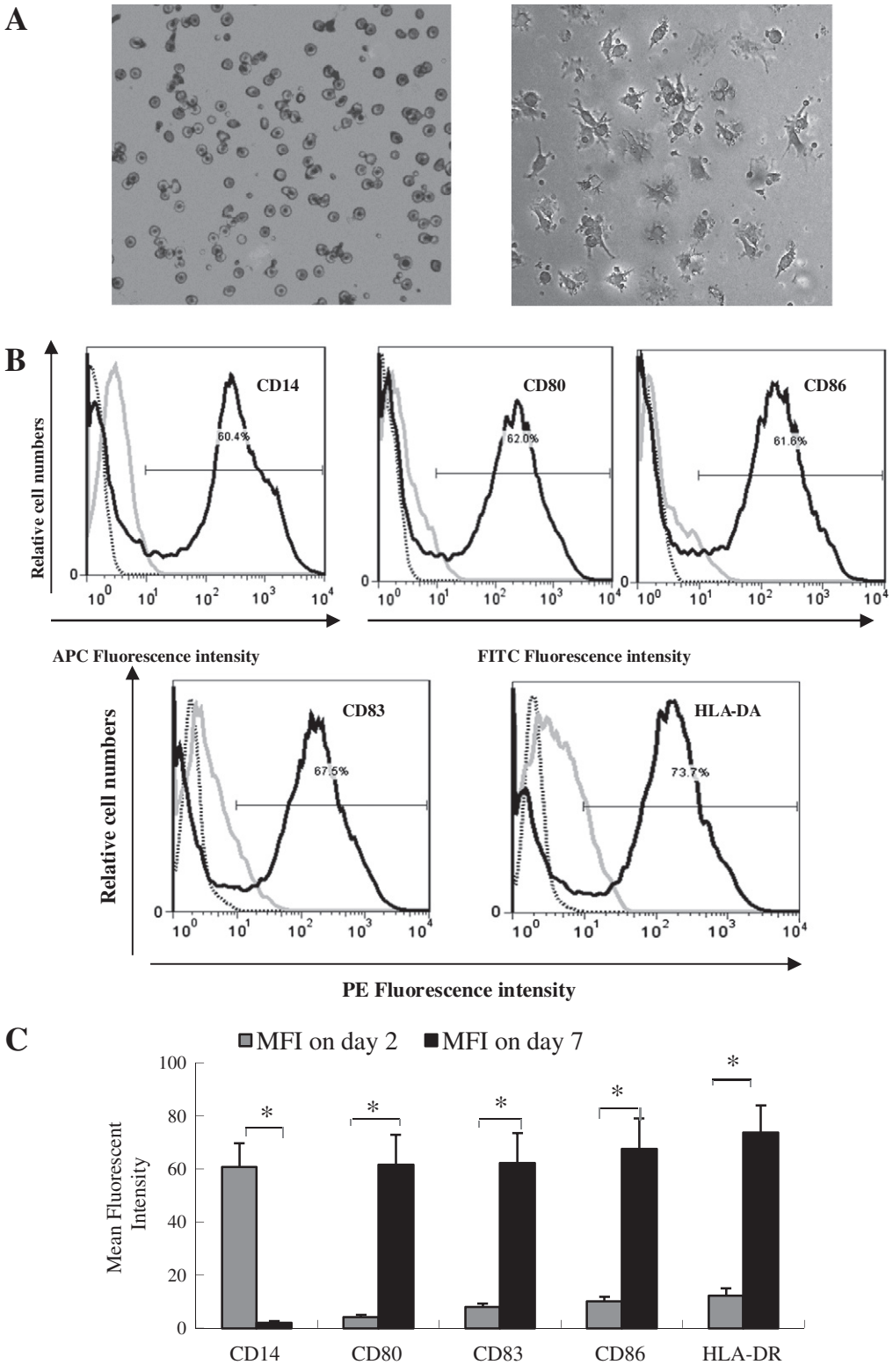

Fig. 1. Mature DCs express high levels of co-stimulatory molecules CD80, CD83, CD86, HLA-DR and low levels of CD14. (A) Light microscopy image of DC cultures on day 2 (left) and day 7 (right). (B) Typical histogram profiles showing the levels of co-stimulatory molecules after different periods of time. Cells are distributed according to the epitope: APC-labeled mouse anti-human CD14 antibody (upper left), FITC-labeled mouse anti-human CD80 antibody (upper middle), FITC-labeled mouse anti-human CD86 antibody (upper right), PE-labeled mouse anti-human CD83 (lower left), PE-Cy5™-labeled mouse anti-human HLA-D (lower right) and isotype controls (dotted-line), heavy grey line on day 2, solid line on day 7. (C) The expression of co-stimulatory molecules in DCs was analyzed by flow cytometry and expressed as mean fluorescence intensities (MFI) on day 2 (grey bar) or on day 7 (black bar). The expression levels of CD80, CD83, CD86, HLA-DR and CD14 are shown as the mean ± SEM fluorescent intensities from three separate experiments, The asterisk (*) symbol indicates a p-value of <0.05 when comparing MFI on day 2 vs. MFI on day 7 for each molecule.