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Interleukin 35: Inhibitory regulator in monocyte-derived dendritic cell maturation and activation

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ABSTRACT

IL-35, a novel IL-12 family member, is a potent inhibitory cytokine predominantly produced by regulatory T and B lymphocytes that exerts optimal suppression in immune response. However, it remains unclear whether IL-35 plays an inhibitory role on human dendritic cells. In the present study, we focused on the possible immunosuppressive effect of IL-35 on the differentiation, maturation and function of monocyte-derived DCs (MoDCs). Addition of exogenous IL-35 was able to partially suppress MoDCs differentiation in vitro. Subsequently, LPS was used for the maturation of MoDCs and IL-35 was found to mainly restrain the maturation of MoDCs, characterized by the remarkable down-regulation of costimulatory molecules, CD83 and HLA-DR as well as a reduced production of pro-inflammatory cytokines (IL-12p70, IFN-γ, and TNF-α). Furthermore, IL-35-treated MoDCs exhibited strong inhibition in the proliferation of allogeneic $CD4^+$/$CD8^+$ T lymphocytes. Meanwhile, IL-35-treated MoDCs also suppressed the polarization of naïve $CD4^+$ T lymphocytes towards Th1 phenotype and impaired $CD8^+$ T cells allogeneic responses. And the foregoing suppression of MoDCs maturation and function by IL-35 might be due to the aberrant activation of STAT1/STAT3 and inhibition of p38 MAPK/NF-κB signaling pathway. Our results demonstrated for the first time that IL-35 played a critical role in modulating not only adaptive immune response, but also innate immune response. The inhibitory effect of IL-35 on MoDCs maturation and function may facilitate the development of promising therapeutic interventions in tumors and other diseases.

1. Introduction

Dendritic cells (DCs) are professional antigen presenting cells (APCs) that play a critical role in immune surveillance protecting against malignancy and infection [1]. Their functions directly control the results of immune response mediated by T helper (Th) cells and cytotoxic T lymphocytes (CTLs) [2–4]. The molecular signatures of human DC subsets located in tissues strongly suggests that in addition to classical DCs derived from dedicated precursors (the pre-DCs), monocyte-derived DCs (MoDCs) also exist in human tissues [5]. In their immature state, DCs are characterized by the strong ability to capture antigens coincident with the low expression of co-stimulatory molecules and cytokines [6]. On stimulation with microbial antigens or other damage-associated molecular signals, immature DCs (imDCs) undergo the process of maturation. This process includes the up-regulated expression of the co-stimulatory molecules (CD40, CD80 and CD86), the MHC class II molecules and inflammatory cytokines such as IL-12 and TNF-α, and a strong T-cell stimulatory ability [6–8]. However, a number of studies have shown that DCs can be arrested at the semimature status following coculture with regulatory T cells (Tregs), expressing low levels of co-stimulatory molecules, making them incapable of initiating T-cell proliferation [9]. The possible mechanisms of suppression by Tregs are being explored in mice and humans, which mainly through the secretion of inhibitory cytokines (IL-10 and TGF-β) and cell-to-cell contact [10,11]. Collison et al have discovered that Tregs can also secret IL-35 which contributes to their suppressive functions [12].

IL-35 is a novel dimeric protein composed of Epstein-Barr virus-induced gene 3 (EBI3) and IL-12p35 subunits, signaling through a unique heterodimer of receptor chains gp130 and IL-12Rβ2 or the homodimers of each chain in target cells [13,14]. IL-35 belongs to the enigmatic IL-12 cytokine family including other three cytokines IL-12, IL-23, and IL-27, while the source of IL-35 is different from others [15]. Early reports have demonstrated that IL-35 is specifically expressed by

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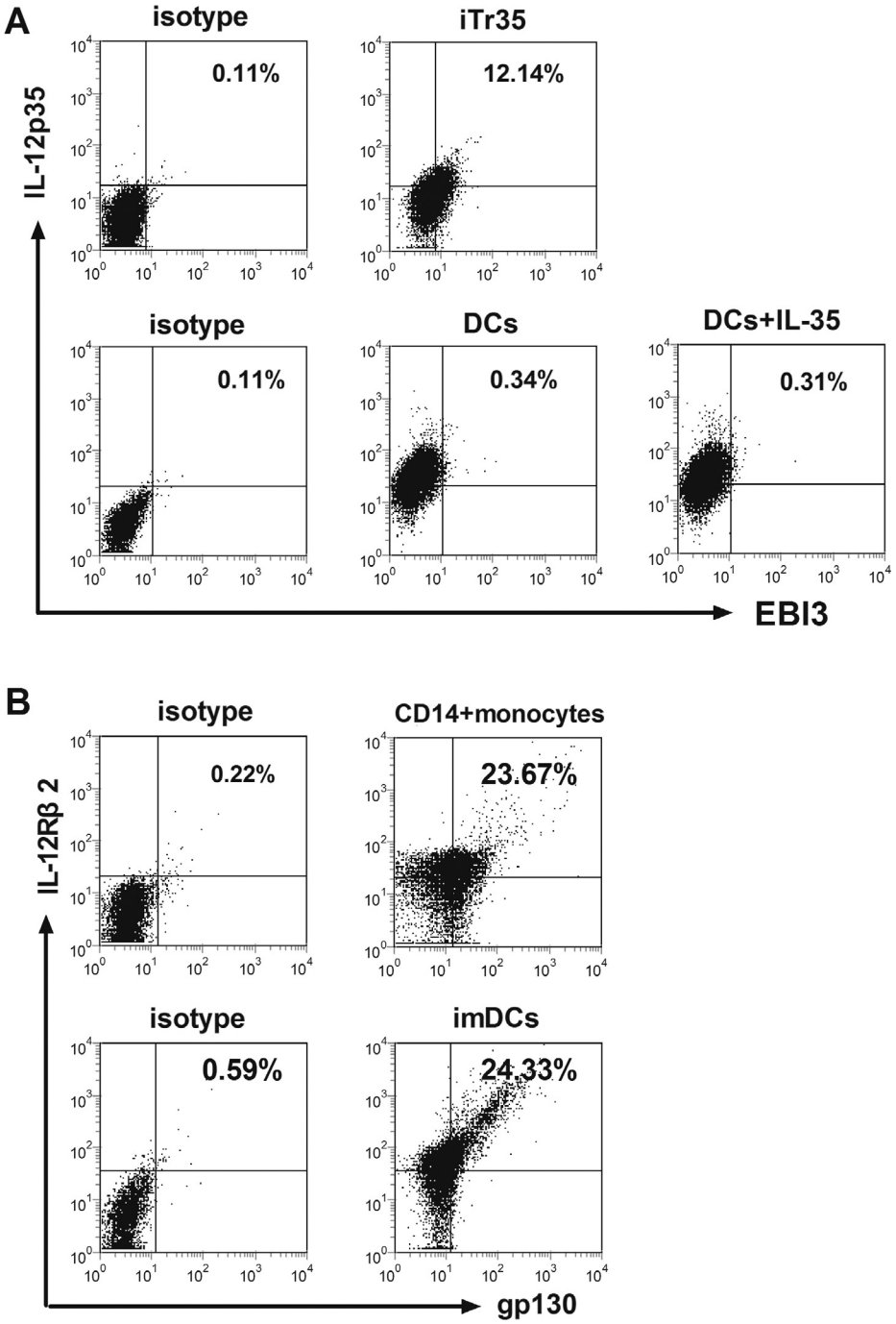

Fig. 1. IL-35 expression on monocytes and MoDCs. A. MoDCs were stimulated with LPS in the presence of IL-35 (25 ng/ml) or not for 48 h. The expression of IL-35 (IL-12p35 and EBI3) was examined by flow cytometry. iTr35 was chosen for the positive control. B. Flow cytometry analysis of IL-35 receptor (gp130 and IL-12Rβ2). $CD14^+$ monocytes and MoDCs were stained on their surfaces with designated antibodies. Isotype antibodies were used as negative controls. The results were representative of three independent experiments.

resting and activated $CD4^+Foxp3^+$ Tregs and is considered to be a crucial anti-inflammatory cytokine, which could suppress Th1, Th2 and Th17 cell-responses in a context-dependent manner [12,16–18]. Interestingly, the newest studies have shown that IL-35, rather than TGF-β or IL-10, is required in Tregs-mediated maximal immune suppression [12,19]. It is well known that TGF-β secretion and CTLA-4 expression on Tregs are necessary to inhibit immune responses by affecting the function of DCs to activate T cells [10,20]. However, to our knowledge, the role of IL-35 on the modulation of human DCs phenotypic and functional properties remains unknown. Our study aimed to extend limited knowledge on IL-35-induced impairment of DC maturation and function and related signal pathways and sought to gain further insight into dysfunction of IL-35-treated DCs on target T cells. To address these research questions, we firstly investigated the expression of IL-35 and its receptor on monocytes and MoDCs. Then we explored the effect of IL-35 on the differentiation and Lipopolysaccharide (LPS)-induced maturation of MoDCs. The functional impact of IL-35-treated MoDCs was investigated by examining their ability to promote T lymphocyte proliferation, skew naïve $CD4^+$ T cell polarization and elicit $CD8^+$ T cell alloreactive response. Further analysis of the underlying mechanisms indicated that the aberrant activation of STAT1/STAT3 and inhibition of p38 MAPK/NF-κB signaling pathway was involved in the suppression of LPS-induced maturation and function of MoDC by IL-35.