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Dendritic cells are able to produce IL-12p70 after uptake of apoptotic cells

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ABSTRACT

Dendritic cell derived IL-12p70 stimulates IFN-γ production in naïve T cells, thereby promoting Th1 responses, which counteracts induction of tolerance. Uptake of apoptotic cells by dendritic cells is generally considered to induce tolerance rather than immune activation and has been shown to specifically inhibit IL-12 production. However, we previously demonstrated that the activation state of apoptotic PBMC influence their immunogenic potential. Here we investigated whether dendritic cells that have engulfed apoptotic PBMC are able to produce IL-12p70 after a secondary signal. We show that dendritic cell ability to produce IL-12p70 after uptake of allogeneic apoptotic cells is dependent on the activation state of the apoptotic cells and subsequent CD40 ligation. CD40 ligation by a CD40L-transfected cell-line induced IL-12p70 in DC regardless of previous apoptotic cell uptake. Moreover, dendritic cells that were exposed to allogeneic activated apoptotic PBMC, but not to resting apoptotic PBMC, were able to produce IL-12p70 after co-culture with autologous T cells. These findings show that dendritic cells are able to produce IL-12p70 upon engulfment of apoptotic cells provided that a secondary activating signal such as CD40-ligand is delivered. In addition, resting apoptotic cell but not activated apoptotic cells reduced ongoing IL-12p70 production suggesting that the balance of activated and resting apoptotic lymphocytes influence the amount of IL-12p70 being produced.

Introduction

Uptake of apoptotic cells (AC) by dendritic cells (DC) is generally considered to generate tolerance or to be a null event rather than inducing immune activation (Savill et al. 2002; Stuart et al. 2002; Steinman et al. 2000; Luckashenak et al. 2008). If DC would alert the immune system upon every encounter with AC this would result in severe autoimmune responses. Therefore, under steady state conditions, uptake of AC generally induces tolerogenic and anti-inflammatory signals (Fadok et al. 1998, 2001; Stuart et al. 2002; Huynh et al. 2002). However, several studies have reported AC to induce immuno-stimulation both *in vitro* and *in vivo* (Albert et al. 1998; Feng et al. 2002; Spetz et al. 2002; Scheffer et al. 2003; Torchinsky et al. 2009). Features of AC suggested to predict their immunogenicity include containment of for example heat shock proteins (Kono and Rock 2008), of tumor antigen (Goldszmid et al. 2003) or exposure of calreticulin (Obeid et al. 2007). Recently, Gurung et al. (2009) demonstrated the involvement of CD154 expression on AC for the induction of immune responses in a model of delayed-type hypersensitivity reaction to a hapten in a mouse model. The type of signal inducing apoptosis has also been proposed to influence AC immunogenicity (Buttiglieri et al. 2003). We have earlier shown that the activation state of apoptotic PBMC determines their ability to stimulate DC (Johansson et al. 2007). Resting apoptotic PBMC (Non-act AC) were unable to generate a DC response while activated apoptotic PBMC (Act AC) induced DC up-regulation of co-stimulatory molecules, release of pro-inflammatory cytokines and proliferation of autologous T cells recognizing allo-antigen from engulfed apoptotic material. These T cells were also able to produce IFN-γ.

IL-12 is a heterodimeric cytokine consisting of the two subunits p40 and p35 together forming the biologically active IL-12p70 (Trinchieri 2003). This pro-inflammatory cytokine is secreted mostly by phagocytes and DC upon encounter with pathogens (Trinchieri 2003) and is further induced by a secondary signal delivered by T cells (Schulz et al. 2000; Fujii et al. 2004; Abdi et al. 2006). It acts on NK cells and T cells and supports IFN-γ production by these cells, which is a key component in the defence against intracellular pathogens (Trinchieri 2003; Walzer et al. 2005). In a paper by Kim et al. (2004) it was shown that IL-12p35 gene transcription is suppressed in macrophages following phagocytosis of AC. We here show that suppression of IL-12p70 after uptake of AC may occur in DC but can be reversed or surmounted depending on the activation status of AC and the type of subsequent stimulus. Low quantities of IL-12p70 can be detected in DC co-cultures with Act AC but not in cultures where DC are exposed to Non-act AC. Direct

Abbreviations: AC, apoptotic cells; Non-act AC, resting apoptotic PBMC; Act AC, activated apoptotic PBMC; DC, dendritic cells.

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CD40 ligation on DC upon exposure to either Non-act- or Act AC however leads to extensive production of IL-12p70. IL-12p70 production induced by combined IFN-γ and LPS stimulation is reduced in the presence of Non-act AC but unchanged or even enhanced in the presence of Act AC. Furthermore, we provide evidence that DC presenting allo-antigen to naïve autologous T cells after uptake of allogeneic Act AC are able to produce IL-12p70. The collective data from mice (Gurung et al. 2009) and from our human *in vitro* cultures further support the concept that there is a balancing act between resting and activated AC that participate in the induction of IL-12 and Th1 type of responses and that these regulatory mechanisms may operate in different species.

Materials and methods

Human cell isolation and differentiation

$CD14^+$ monocytes, immature DC ($CD1a^+/CD14^-/CD3^-/CD19^-/CD80^-/CD86^-$), T cells and PBMC were isolated and differentiated as previously described (Johansson et al. 2007; Smed-Sorensen et al. 2004).

PBMC activation and apoptosis induction

PBMC were separated from healthy blood donors and were frozen directly or activated for 24 h with anti-human CD3 and CD28 mAbs (Johansson et al. 2007). Resting PBMC and activated PBMC were thawed and washed in complete medium before induction of apoptosis by γ-irradiation (150 Gy). In flow cytometry experiments PBMC were labelled with green fluorescent dye PKH67 (Sigma–Aldrich) before apoptosis induction. Irradiated cells were incubated at 37 °C for 12 h before addition to DC. Apoptosis was determined by flow cytometry analysis of AnnexinV/propidium iodide stained cells (Johansson et al. 2007).

DC/AC co-cultures

DC were co-cultured with allogeneic AC in a 1:2 ratio. After 7 h DC and DC/AC co-cultures were moved to wells containing CD40-ligand transfected (CD40L), or non-transfected murine L-cells (Smed-Sorensen et al. 2004). Alternatively autologous T cells were added to DC or DC/AC co-cultures. DC were treated with IFN-γ (50 ng/ml, Boerhinger-Ingelheim, Stockholm, Sweden) for 2 h before addition of AC and/or LPS (100 ng/ml, Sigma–Aldrich). Brefeldin A (10 μg/ml; Sigma–Aldrich) was added in cultures 6 h prior to intracellular staining and flow cytometry analysis. After totally 24 h of co-culture supernatants were collected and analysed for IL-12p70 by ELISA (R&D systems). Staining with DC surface markers (CD1a, HLA-DR) and intracellular staining with anti-human IL-12p70 was performed as previously described (Smed-Sorensen et al. 2004; Johansson et al. 2007). $CD1a^+$/HLA-DR^+/$PKH67^+$ cells were considered phagocytic DC in flow cytometry analysis and cytochalasin D was used as a negative control for AC uptake as previously described (Johansson et al. 2007).

Statistical analysis

The Wilcoxon rank-sum test was used to determine pair wise differences. $p < 0.05$ was considered significant (GraphPad Prism).

Results

DC exposed to AC prior to CD40L stimulation are able to produce IL-12p70

To determine whether DC have the ability to produce IL-12p70 after exposure to AC, we co-cultured DC with allogeneic Non-act AC or Act AC and co-cultures were then either maintained in the wells or transferred to wells containing L-cells or CD40L cells. Supernatants were collected after 24 h and analysed by ELISA. This time point was chosen based on initial kinetic analyses, where IL-12p70 production by CD40L stimulated DC peaked at 24 h (data not shown). DC co-cultured with L-cells or Non-act AC did not generate significant IL-12p70 production compared with medium control (Fig. 1). Co-culture of DC with Act AC resulted in a significant but low up-regulation (87 ± 13 pg) of IL-12p70 compared with medium control. Stimulation of DC with CD40L, regardless of initial co-culture with AC, resulted in substantial up-regulation of IL-12p70 (CD40L; 9918 ± 3090 pg/ml, Non-act AC + CD40L; 13,054 ± 4071 pg/ml, Act AC + CD40L 18,625 ± 5607 pg/ml). These results show that DC exposed to both Non-act AC and Act AC were able to produce IL-12-p70 after stimulation with CD40L-transfected cells. Direct CD40 ligation of DC resulted in high expression of IL-12p70 independent of prior co-stimulatory molecule- and pro-inflammatory cytokine expression by DC.

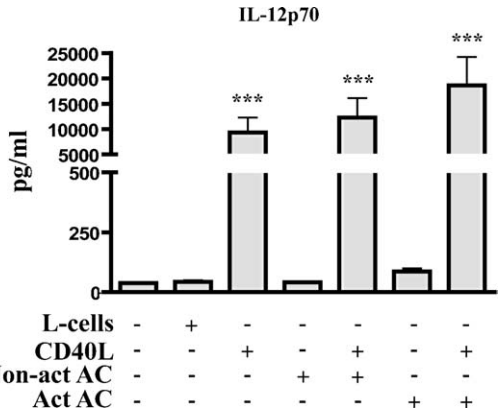

Fig. 1. DC exposed to AC and CD40L produce IL-12p70. DC were cultured with Non-act AC or Act AC for 7 h and were then moved to wells containing control L-cells or CD40L cells. Supernatants collected after 24 h were analysed for IL-12p70 content by ELISA. Results are expressed as mean value ± SEM ($n = 13$ in all groups except for L-cells where $n = 8$; $^{***}p \leq 0.001$ compared with medium control).

DC exposed to Act AC, but not to Non-act AC produce IL-12p70 after co-culture with autologous T cells

To analyse whether T cells are able to provide an IL-12p70 inducing signal upon contact with DC exposed to AC, resting autologous T cells were added to DC/AC co-cultures and supernatants were collected 24 h later (Fig. 2). DC co-cultured with autologous T cells did not produce IL-12p70. Allogeneic Non-act AC did not induce significant IL-12p70 production in DC regardless of T cell addition. However, co-culture with allogeneic Act AC followed by addition of T cells resulted in significant IL-12p70 production (279 ± 78 pg/ml) (Fig. 2). These data show that DC exposed to allogeneic Non-act AC were unable to interact with T cells in a manner that provided DC with a signal inducing IL-12p70. However, allogeneic Act AC programmed DC for interactions with T cells that led to IL-12p70 production.

IL-12p70 production by DC after IFN-γ/LPS stimulation is reduced in the presence of Non-act AC but not of Act AC

It was previously reported that the IL-12p35 gene is down-regulated in IFNγ/LPS stimulated macrophages after contact with apoptotic cells (Kim et al. 2004). Here, we addressed the question whether the activation state of the apoptotic cells prior to cell death play a role in the regulation of IL-12p70. LPS induced a low but sig-