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Apoptotic cells enhance IL-10 and reduce IL-23 production in human dendritic cells treated with zymosan

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ABSTRACT

Contact of apoptotic cells (AC) with phagocytes tilts the balance of pro-inflammatory and anti-inflammatory cytokines. To address the cell- and stimulus-dependency of this mechanism, human monocyte-derived dendritic cells were treated with Jurkat AC in the presence and absence of different stimuli. AC reduced the production of IL-23 and enhanced the production of IL-10 elicited by zymosan, but they did not influence IL-12 p70 production nor did they modify the effect of LPS. Since formation of lipid bodies (LB) and PGE_2 production have been associated with IL-10 induction, the effect of PGE_2, the formation of LB, and the role of PPAR-γ were assessed. Exogenous PGE_2 enhanced IL-10 expression, but no evidence of PGE_2 production elicited by AC was obtained. Inhibition of PPAR-γ activity reduced the production of IL-10 both in the presence and in the absence of AC, but formation of LB in response to zymosan and AC was not observed. Notably, AC induced a transient nuclear translocation of both the CREB coactivator CRTC2/TORC2 and the homeodomain protein PBX1, which are involved in the CREB/HOX/PBX/MEIS transcription complex. These data show a selective effect of AC on the production of cytokines elicited by the fungal surrogate zymosan through the enhancement of CREB-dependent transcription.

1. Introduction

Removal of apoptotic cells (AC) has been recognized as a central mechanism controlling tissue damage during the inflammatory response (for review, see Elliott and Ravichandran, 2010). Early attempts to characterize the chemical mediators involved in this regulatory role focussed on TGF-β, platelet-activating factor, and PGE_2 (Fadok et al., 1998), but most recently, modulation of the IL-12 p70/IL-10 balance seems to be the most relevant mechanism (Kim et al., 2004). Most studies have focussed on the phagocytosis of AC by macrophages, but defining the array of cell-specific responses seems to be of paramount importance since the large array of receptors involved in AC recognition and the list of mediators that can be produced by the distinct types of cells may vary broadly. Dendritic cells are characterized by their capacity to uptake microbial products and their ability to secrete the cytokines involved in the polarization of Th cells into Th1, Th2, and Th17 type responses. This is mainly achieved by the production of IL-12 p70, IL-10, and IL-23, respectively, and depends on the nature of the stimuli. Combination of LPS and IFN-γ is the most potent stimulus for IL-12 p70 production (Gautier et al., 2005), whereas zymosan, a cell wall extract of *Saccharomyces cerevisiae*, induces a response characterized by the production of IL-10 and IL-23, and a low amount of IL-12 p70 (Brown et al., 2003; Dillon et al., 2006; Leibundgut-Landmann et al., 2007). This effect of zymosan is particularly interesting because it contains PAMPs different from those found in LPS and is widely used as a surrogate for the study of the response to fungi because it mimics the composition of the cell wall of *Candida*, *Aspergillus*, and *Pneumocystis* spp. (Thomas and Limper, 2004) due to its high content in β-glucans and α-mannans. In addition, mutations and polymorphisms in dectin-1, the receptor involved in β-glucan recognition have been associated with an increased risk for fungal infections (Ferwerda et al., 2009; Cunha et al., 2010). The rationale to study the immuno-modulating effect of AC on zymosan effect stems from several facts: (i) AC modulate

Abbreviations: AA, arachidonic acid; AC, apoptotic cells; CBP, CREB binding protein; COX-2, cyclooxygenase-2; CRE, cyclic AMP response element; CREB, CRE binding protein; CRTC2, CREB-regulated transcription coactivator; DC, dendritic cells; FBS, fetal bovine serum; HIHS, heat-inactivated human serum; LB, lipid bodies; MEIS, myeloid ecotropic viral integration site; MFI, geometric mean fluorescence intensity; NHS, normal human serum; PBX1, pre-B-cell leukemia homeobox 1; PGE_2, prostaglandin E_2; PKA, protein kinase A; TBP, TATA box-binding protein; TORC2, transducer of regulated CREB activity 2.

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the response to PAMPs and seem to play a role in the development of the compensatory anti-inflammatory response syndrome (CARS), where infection by fungi is frequently observed. (ii) Fungal patterns can bind receptors involved in the recognition of AC (Chung et al., 2000; Zhou et al., 2001; Ziegenfuss et al., 2008; Means et al., 2009). (iii) Zymosan mimics some of the reported effects of AC since it is a good inducer of IL-10 and a week inducer of IL-12 p70.

The effect of AC on cytokine production has been associated with different mechanisms. On the one hand, about a dozen of receptors have been involved in the recognition of AC, some of them requiring the cooperation of opsonins such as complement system components or bridging proteins as growth arrest-specific protein 6, protein S (Anderson et al., 2003), and milk fat globule-EGF factor 8 protein (Hanayama et al., 2002). The activation of those receptors induces a set of responses that ultimately impinge on the activation of different transcription factors, coactivators, and corepressors that regulate the transcription of cytokines. Among these transcription factors, NF-κB plays a central role in the production of IL-12 p70, TNF-α, and several chemokines, whereas CREB is mainly involved in *il10* transcriptional activation (Platzer et al., 1999; Martin et al., 2005; Hu et al., 2006; Ananieva et al., 2008; Alvarez et al., 2009; Kelly et al., 2010; Mellett et al., 2011). Transcription factors such as PPAR-γ and LXR have been involved in the response to apoptotic cell burden, although in many cases they elicit indirect effects by acting as transrepressors (A-Gonzalez et al., 2009; Jennewein et al., 2008). The purpose of this study has been addressing the effect of AC on the production of the cytokines involved in the polarization of the immune response by human monocyte-derived dendritic cells (DC). We have observed an enhancement of IL-10 production by AC and a decrease of the amount of IL-23 elicited by the fungal surrogate zymosan. By contrast, the response to LPS and combination of LPS and IFN-γ was unaffected. Attempts to address the role of complement factors and serum opsonins disclosed an enhancing effect of normal human serum (NHS) that persisted after heat inactivation, thus suggesting the involvement of opsonins and/or endogenous PPAR-γ activators. Although exogenous PGE_2 enhanced the effect of AC, we did not find any evidence of the involvement of endogenous PGE_2 nor formation of lipid bodies (LB). Since we consistently observed a transient nuclear translocation of the CREB coactivator CRTC2/TORC2 and the homeodomain protein PBX1, which is an element of the CREB/HOX/PBX/MEIS transcription complex that displays higher-order interactions with CBP and TORC coactivators, our data disclose a selective effect of AC on the production of IL-10 by the fungal surrogate zymosan that is best explained by an effect on CREB-dependent transcription.

2. Materials and methods

2.1. Cells and materials

Mononuclear cells were collected from buffy coats of healthy donors and differentiated into DC as reported (Valera et al., 2008). In short, adhered monocytes were incubated in the presence of GM-CSF and IL-4 for 5 days, including the addition of fresh cytokines at day 2. Experiments were conducted without removing the medium, except in the experiments of arachidonic acid (AA) release, where the medium was replaced by delipidated BSA and in the experiments devoted to TGF-β1 assay (see below). Macrophages were obtained from monocytes by culture for 14 days in medium supplemented with 10% heat-inactivated human serum. Ethical Committee approval was received for the studies and informed consent of all participating subjects was obtained. The experimental setting included culture in the presence of 10% FBS unless otherwise stated. Jurkat T cells were incubated with 10 μM camptothecin for 5 h to induce apoptosis (Morris and Geller, 1996). AC were detected by Annexin V-FITC/PI staining (Becton Dickinson) and analyzed using a Gallios Flow Cytometer (Beckman Coulter). Viability of DC at the end of the experiments was confirmed by Annexin V-staining of cells expressing the mannose receptor. PMN were obtained by centrifugation on Ficoll-Hypaque and apoptosis was obtained by incubation at 37 °C for 24 h. Necrosis was induced by freeze-thawing. Zymosan, mannan from *S. cerevisiae*, and H89 were from Sigma Chemical Co. (St. Louis, MO). IL-12 p70 was assayed with reagents from Thermo Scientific Pierce (Rockford, IL) and IL-23 with reagents from R&D systems (Minneapolis, MN). IL-10 was assayed with Biotrack ELISA systems from Amersham Biosciences. Bodipy® was from Invitrogen Ltd. (Paisley, UK). TGF-β1 was assayed with reagents from R&D in conditioned medium where serum was substituted by BSA as per the manufacturer's instructions. In short, the cell medium containing 10% serum was substituted by medium containing 200 μg/ml crystalline BSA by several changes over 12 h, and the conditioned medium containing BSA was treated by acidification and neutralization before the assay. Opsonization of zymosan was carried out by incubating in a shaking bath 10 mg of boiled zymosan in 1 ml of fresh human serum for 20 min at 37 °C. At the end of this period, zymosan particles were intensively washed in phosphate buffered saline to remove unbound materials.

2.2. [^{3}H]AA release and PGE_2 assay

Radioactive labeling of DC with [^{3}H]AA was performed by incubation of cells for 5 h in the presence of 0.25 μCi/ml [^{3}H]AA in 0.25% essentially fatty acid-free BSA (Fernández et al., 2003). After labeling, DC were washed with phosphate buffered saline and allowed to equilibrate at 37 °C in medium containing 1% BSA before the addition of stimuli or vehicle. The release of [^{3}H]AA into the culture medium was measured by scintillation counting and expressed as percent of total incorporated [^{3}H]AA. PGE_2 were assayed in DC supernatants with Biotrack ELISA systems (Amersham Biosciences) according to the manufacturer's instructions. The detection limit of the assay is 2.5 pg/ml.

2.3. Immunoblots

Proteins were separated by electrophoresis in SDS/PAGE and transferred to nitrocellulose membranes. The membranes were used for immunodetection of COX-2 (sc-1745), CRTC2/TORC2 (sc-46272), PPAR-γ (sc-7196), and PBX1 (sc-889) with Ab from Santa Cruz Biotechnology Inc., Santa Cruz, CA. For immunoblots directed to assay nuclear proteins, the nuclear extracts were obtained using a nuclear extract kit (Active Motif, Carlsbad, CA). β-Actin and TBP were used as load control. Coimmunoprecipitation experiments were carried out using the Nuclear Complex Co-IP kit of Active Motif as reported (Alvarez et al., 2009).

2.4. Assay of LB formation

Cells stimulated under different conditions were incubated with 1 μM Bodipy® for 30 min at 37 °C and then fixed with 1% formaldehyde (Mattos et al., 2010). The induction of LB was measured at FL1 channel and was expressed as geometric mean fluorescence intensity (MFI). In the case of DC incubated with Jurkat AC, the analysis was conducted with two-color flow cytometric acquisition using anti-mannose receptor Ab to distinguish DC from Jurkat cells, which do not express this receptor. The analysis was performed on a Gallios Flow Cytometer using Kaluza software for quantitative data analysis. At least 10,000 cells were analyzed per sample.