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IL-1β induces expression of costimulatory molecules and cytokines but not immune feedback regulators in dendritic cells

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ABSTRACT

Dendritic cells play an important role in the initiation of immune reactions. Due to their high capacity to prime T-cell responses, the activation of dendritic cells must be tightly controlled. Because Interleukin-1β (IL-1β) is a key player in autoinflammatory diseases, we compared the ability of IL-1β to activate human dendritic cells and induce immune-regulatory molecules versus the effects induced by pathogen-derived stimuli.

Upon activation with either IL-1β or microbial stimuli, monocyte-derived dendritic cells showed enhanced expression of costimulatory molecules, increased secretion of chemokines and cytokines, and the ability to activate T cells. In contrast, immune-feedback molecules, including PD-L1, IL-1RA, IL-10 and SOCS1, were exclusively upregulated in response to microbial stimuli, whereas IL-1β treatment had no inducing effect on them. Thus, the limited capacity of IL-1β to induce potential feedback inhibitors may support its key etiologic role in chronic inflammation and autoinflammatory responses.

1. Introduction

Interleukin-1β (IL-1β) is a key cytokine in inflammation and autoinflammation. Its dominant role in autoinflammatory diseases was clearly demonstrated by the use of IL-1 blockade to improve clinical outcomes [1]. IL-1β is mainly released by innate immune cells such as monocytes, macrophages and dendritic cells (DCs). Its expression can be triggered by microbes, but also by pro-inflammatory mediators, including IL-1β itself [2,3]. This indicates that IL-1β promotes the process of autoinflammation via a positive feedback loop.

DCs play a central role in host defense. They are at the interface between the innate and adaptive immune responses and are key regulators of T-cell differentiation. Upon stimulation with pathogen-associated factors or other inflammatory stimuli, DCs undergo a particular maturation process which renders them capable of inducing effector T-cell responses [4]. To be able to rapidly recognize and respond to invading microbes, DCs are equipped with germline-encoded pattern-recognition receptors (PRRs) such as the well-characterized family of Toll-like receptors (TLRs) [5]. Pathogen sensing by TLRs stimulates the maturation of DCs, which is characterized by upregulation of co-stimulatory molecules and release of pro-inflammatory mediators. In addition to PRR-mediated activation, DC maturation can also be induced by specific cytokines, including TNFα, IFNβ and IL-1β [6]. Activation of DCs with IL-1β promotes IL-23-mediated generation of IL-17-releasing T cells which, in the absence of proper homeostatic control, can result in autoimmune responses [7]. Accordingly, DCs of autoimmune patients show elevated production of IL-23 concurrent with increased IL-17 levels [8].

To avoid uncontrolled inflammation and to prevent an excessive T-cell response, DC function must be carefully regulated. In this regard, PD-L1 (Programmed cell death ligand 1) and PD-L2 provide critical negative signals via interaction with PD-1, thereby limiting T-cell activation [9]. In addition, anti-inflammatory mediators such as IL-10, IL-1RA and the cytosolic family of SOCS proteins have been shown to be potent suppressors of inflammation [10–13].

The present study compares IL-1β-driven DC activation versus activation induced by TLR ligands, and investigates the ability of IL-1β and microbial stimuli to induce the expression of negative regulatory molecules by monocyte-derived DCs (moDCs).

2. Materials and methods

All studies involving human cells were conducted in accordance with the guidelines of the World Medical Association's Declaration of

Abbreviations: APC, Antigen-presenting cell; DC, Dendritic cell; HCA, Heat-inactivated *Candida albicans* extract; IFN, Interferon; IL-1β, Interleukin-1β; IL-1RA, Interleukin-1 receptor antagonist; LDH, Lactate dehydrogenase; LPS, Lipopolysaccharide; PD-L, Programmed cell death ligand; PRR, Pattern recognition receptor; R848, Resiquimod; SOCS, Suppressor of cytokine signaling; TLR, Toll-like receptor; TNFα, Tumor necrosis factor-α; Treg, regulatory T cell

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2.1. Generation of monocyte-derived dendritic cells (moDCs)

Immature moDCs (iDC) were generated from monocytes isolated from fresh buffy coats of healthy, anonymous donors (provided by the blood bank Salzburg, Austria) as described previously [14]. Because our national regulations do not require informed consent in the case of anonymous blood cells discarded after plasmapheresis (buffy coats), no additional approval by the local ethics committee was required. Briefly, density gradient centrifugation using Histopaque-1077 (Sigma) was performed to isolate peripheral blood mononuclear cells. To generate iDCs, adherent monocytes were cultured in DC medium [RPMI 1640 (Sigma), 10% fetal calf serum (PAA), 2 mM L-glutamine, 100U/ml penicillin/streptomycin (Sigma), 50 μM β-mercaptoethanol (Gibco Laboratories)], supplemented with 50 ng/ml GM-CSF and 50 ng/ml IL-4 (generous gift from Novartis) for 6 days. At day 3, cells were fed 1 vol of DC medium containing fresh cytokines at a final concentration of 50 ng/ml. After 6 days, cells were harvested and replated in DC medium without cytokines. After differentiation, cell purity was assessed by staining with α-CD1a BV421 (eBioscience) and α-CD14 PerCP-Cy5.5 (BD Bioscience). Cell viability was analyzed by flow cytometry using Fixable Viability Dye eFluor506 (eBioscience). Purity was calculated from the percentage of $CD1a^+$ $CD14^-$ cells among viable moDCs after doublet exclusion and ranged from 73.5% to 91.9%.

2.2. moDC stimulation

1×10^5 iDCs/ml were plated in DC medium and stimulated for 48 h with 30 ng/ml IL-1β (Immunotools), 5 μg/ml R848 (Alexis Biochemicals), 30 ng/ml *Escherichia coli* lipopolysaccharide (LPS) 055:B5 (Sigma), or 0.5×10^6 cells of heat-inactivated *Candida albicans* per 1×10^5 moDCs. Concentrations for LPS, R848, IL-1β and HCA were chosen based on previous studies [15–18].

2.3. $CD4^+$ T-cell isolation and co-culture with moDCs

1×10^5 iDC were cultivated for 24 h in 250 μl of T cell medium [IMDM (Sigma), 10% fetal calf serum (PAA), 2 mM L-glutamine, 100U/ml penicillin/streptomycin (Sigma)]. Cells were either left untreated or cultured in the presence of LPS (30 ng/ml), IL-1β (30 ng/ml) or HCA (0.5×10^6cells/ml). After 24 h, allogenic $CD4^+$ T cells were isolated from PBMCs using a $CD4^+$ T cell Isolation Kit Human (Miltenyi Biotec), according to the manufacturer's recommendations. Subsequently, T cells were added to moDCs at a ratio of 1:10 in 250 μl T cell medium supplemented with IL-2 (100U/ml). T cells stimulated with 10 μg/ml plate-bound α-CD3 (clone OKT3, eBioscience) and 1 μg/ml soluble α-CD28 (BD Pharmagen) and T cells treated with the respective DC stimuli served as controls. One day later, each well was supplemented with 50 U/ml IL-2. After 6 days of co-culture, supernatants were collected and tested for IFNγ release by ELISA (Peprotech). HLA-DR expression was assessed on live (Fixable Viability Dye eFluor780, eBioscience), $CD3^+$ (FITC, ImmunoTools), $CD4^+$ (PE, ImmunoTools) cells after doublet exclusion.

2.4. Analysis of moDC surface marker expression

Surface expression of DC-maturation/activation markers was analyzed after 48 h of stimulation, using a FACS Canto II flow cytometer (BD Biosciences) and the following antibodies from eBiosciences (CD40 FITC, CD86 PE), BD Biosciences (CD80 APC-Cy7, CD83 PE-Cy7, PD-L1 PE-Cy7) and Miltenyi Biotec (PD-L2 APC-Vio770).

2.5. Multiplex immunoassay

After 48 h of stimulation, cytokine and chemokine production by activated moDCs was assessed using the Cytokine & Chemokine 34-Plex Human ProcartaPlex™ Panel 1A (Thermo Fischer), according to the manufacturer's recommendations. Analysis was performed on a Luminex MagPix (Luminex) instrument, and Procarta Plex Analyst Software (e-Bioscience) was used for data evaluation.

2.6. LDH assay

1×10^5 moDCs/ml were stimulated for 48 h as described before. Subsequently, cytotoxicity was assessed by determination of lactate dehydrogenase (LDH) levels in cell supernatants using the CytoTox 96® Non-Radioactive Cytotoxicity assay (Promega) according to the manufacturer's recommendations. As a control for maximum LDH release, cells were treated with 0.10% (v/v) Triton X-100. Analysis was performed using a microplate reader (Tecan).

2.7. Detection of SOCS and IL-1β mRNA expression by real-time PCR

Total RNA was isolated from 1×10^5 cells using TRI Reagent (Sigma) and reverse-transcribed with RevertAid H Minus M-MulV reverse transcriptase (Thermo Scientific) according to the manufacturer's instructions. RNA expression was quantified by performing quantitative real-time PCR (q-RT-PCR) on a Rotorgene 3000 (Corbett Research) with iQ SYBR Green Supermix (Bio-Rad) and the primers listed below. The relative mRNA content (x) was calculated using the formula $x = 2\text{-}\Delta ct$, where Δct describes the difference between the threshold cycles (ct) of the gene in question and the reference gene, large ribosomal protein P0 (RPLP0). The specificity of the PCRs was monitored by recording a melting curve for the PCR products. The following primer pairs were used for detection: SOCS1: sense 5′-TTGGAGGGAGCGGATGGGTG TAG-3′, antisense 5′-AGAGGTAGGAGGTGCGAGTTCAGGTC-3′ SOCS3: sense 5′-ATACTATACCTTCCTGTACCTGGGTGGATGGAGCG-3′, antisense 5′-TGAGTATGTGGCTTTCCTATGCTGGGTCCCTCT-3′, RPLP0: sense 5′- GGCACCATTGAAATCCTGAGTGATGTG-3′, antisense 5′-TTG CGGACACCCTCCAGGAAG-3′, IL-1β: sense 5′-GTACCTGAGCTCGCCAG TGA -3′, antisense 5′-TCGGAGATTCGT AGCTGGATG-3′.

2.8. Statistical analysis

Statistical analyses were performed using GraphPad Prism Software Version 6.01. P values for multiple groups were calculated via one-way ANOVA with a Tukey's post-hoc test. P-values < 0.05 were considered statistically significant ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$).

3. Results and discussion

3.1. DC-activation upon treatment with IL-1β, R848, LPS and HCA

IL-1β triggers a cascade of inflammatory chemokines and cytokines and is regarded as key player in auto-inflammation [19]. Furthermore, activation of DCs by IL-1β leads to enhanced expression of CD80, CD83 and CD86 and increased secretion of IL-23 and IL-6, which in turn stimulate the generation of IL-17-producing T helper cells [20]. This indicates that the IL-17-promoting activity of IL-1β is not exclusively based on T cells, but involves DCs as well [21].

To compare IL-1β-induced DC activation with microbial-related responses, we treated moDCs with IL-1β, heat-inactivated *C. albicans* extract, the TLR4 agonist LPS, and the TLR7/8 ligand R848 (Resiquimod), which mimic microbial and viral infection, respectively. As expected, LPS, a well-described activator of co-stimulatory molecules in moDCs [15], enhanced the levels of CD40, CD80, CD83 and CD86 and so did R848 and HCA (Fig. 1A). This latter observation is in line with previous findings, that the TLR7 ligand Imiquimod and the fungal pathogen *C. albicans* induced the expression of co-stimulatory molecules in human dermal DCs [22] and moDCs [23]. In contrast to