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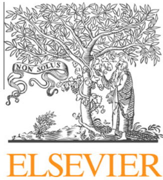

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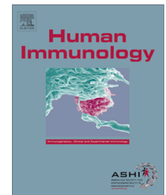

IL-27 improves migrational and antiviral potential of CB dendritic cells

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ABSTRACT

Interleukin (IL)-27 is known to be increased considerably in cord blood (CB) dendritic cells (DCs) after TLR ligation. Previously, we demonstrated that also basal IL-27 levels are higher in CB DCs. Here, we examined effects of IL-27 on monocyte derived dendritic cells (moDCs) to approach its particular role in the specialized immune system of the human neonate.

Exogenous IL-27 promotes IL-27 transcription in CB and adult blood (AB) moDCs. IL-27 acts on CB moDCs primarily by significantly augmenting IL-27 protein, secondarily by increasing transcription of CXCL10 among other chemokines, chemokine receptor CCR1, interferon stimulated genes, transcription factor IRF8 and genes involved in antigen presentation. Furthermore, CB moDCs respond to IL-27 with augmented IL-8 and Tumor necrosis factor (TNF)-α. The results suggest that IL-27 enhances migrational and antiviral properties of CB dendritic cells.

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1. Introduction

Human newborns are more susceptible to infectious diseases than adults, while vaccination is less effective. Yet, a specialized immune system is essential for the newborn to get along with the extraordinary amount of novel antigens in the postnatal environment, without the generation of disproportionate reactions. An aspect of this neonatal adaptation is the limited capacity of dendritic cells (DCs) to elicit Th1 (T helper 1) responses [1,2] compared to adults. This correlates with a shift towards Th2 responses following infections, arising from a reduced expression of Th1 master regulator IL-12 [3]. In contrast to that, the expression of the related IL-12 family cytokine IL-27, which also induces Th1 polarization, is augmented considerably in cord blood dendritic cells when TLR4 ligation in combination with IFNγ occurs [4]. We highlighted the particular role of IL-27 in the neonatal immune system compared to the adult by investigating CB and AB monocyte derived dendritic cells (moDCs).

IL-27, an IL-12 family cytokine, is a heterodimer composed of the IL-27p28 subunit and Epstein–Barr virus induced gene 3 (EBI-3) [5], and binds to a receptor consisting of WSX-1 and the gp130 subunit to induce signaling [6]. Based on observations in mice and human adults, IL-27 is considered to be critical for the early regulation of Th1 differentiation until it is overruled by IL-12, which also induces IFNγ production in naïve CD4+ T cells [7]. Furthermore, IL-27 is known to be a cytokine which elicits pleiotropic effects: In monocytes for example, IL-27 enhances LPS induced inflammatory effects [8]. However, it has also been reported that IL-27 induces IL-10 producing antiinflammatory Tr1 cells (Type 1 regulatory T cells) [9], and that it suppresses the differentiation of Th17 cells [10].

As we demonstrated recently that CB DCs express the IL-27 receptor [4], we intended to explore how IL-27 affects CB DCs. We determined mRNA levels of IL-27p28 and WSX-1 as well as IL-27 protein production in CB moDCs. Besides the fact that basal IL-27 levels are higher in cord blood moDCs, we could demonstrate that IL-27 induces its own production, most notably in CB moDCs. Furthermore, we show that IL-27 increases expression of genes involved in antigen presentation, chemokines and chemokine receptors as well as antiviral genes. Hence, IL-27 is able to expand the capacity of DCs to activate and recruit other immune cells and moreover to enhance antiviral capacities of DCs themselves.

Abbreviations: IL, interleukin; DCs, dendritic cells; TLR, toll like receptor; moDCs, monocyte derived dendritic cells; CB, cord blood; AB, adult blood; Th, T helper; TNF, tumor necrosis factor; IFN, interferon; IRF, interferon regulatory factor; ISGs, interferon stimulated genes; CD, cluster of differentiation; STAT, signal transducer and activator of transcription; HLA, human leukocyte antigen; PBMCs, peripheral blood mononuclear cells.

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2. Materials and methods

2.1. Isolation of CD14+ cells

Cord blood was acquired from caesarean sections of healthy full-term newborns with healthy mothers. An informed consent was obtained according to the local ethical committee's approval. The adult samples originate from leucapheresis obtained from healthy volunteer donors. PBMCs were isolated via Ficoll (PAA)-density centrifugation and cryopreserved in FCS (HyClone, Thermo Scientific) containing 10% DMSO. After thawing, cells were labeled with magnetic anti-CD14 microbeads (Miltenyi Biotech). Then CD14+ cells were enriched by positive selection. Routinely, a purity of the enriched fraction of more than 95% was assessed by flow cytometry.

2.2. Generation and stimulation of monocyte derived dendritic cells

CD14+ cells were cultured at a density of 1×10^6/ml in RPMI 1640 (10% heat-inactivated FCS, 2 mM L-glutamine, 1 mM sodium pyruvate, $1 \times$ MEM (minimal essential medium)-amino acids, 100 IU penicillin and 100 μg/ml streptomycin and 50 μM β-Mercaptoethanol) supplemented with IL-4 (20 ng/ml) and GM-CSF (100 ng/ml) in 24 well flat bottom plates (Nunc) at 37 °C, 5% CO_2. On day 3, half of the medium was replaced by fresh medium containing IL-4, GM-CSF and β-Mercaptoethanol.

Furthermore, we intended to mimic an immune challenging situation by adding exogenous stimuli to the moDCs. Therefore, these immature moDCs were stimulated with IL-27 (50 ng/ml) or LPS (100 ng/ml) for the last 24 h.

2.3. Analysis of signal transducer and activator of transcription 1 (STAT1) phosphorylation

Phosphorylation analysis of STAT1 was performed as previously done by Doganci et al. [11]. In brief, 2×10^5 immature moDCs were seeded into 96 round bottom well plates in RPMI supplemented with heat-inactivated FCS. Cells were left unstimulated or stimulated with IL-27 (50 ng/ml) or IL-27 in combination with LPS (10 μg/ml) or LPS (10 μg/ml) and IFNγ (100 ng/ml) for 5 min, 10 min, 15 min, 30 min, 60 min or 120 min at 37 °C, 5% CO_2. Afterwards, 2% paraformaldehyde (Sigma Aldrich) in PBS as cell fixation reagent was added for 10 min at 37 °C, 5% CO_2. Then the cells were permeabilized with ice cold methanol [12] for 30 min on ice. After that, Perm/Wash buffer (BD Biosciences) was added and the cells were stained with CD14-APC and anti-STAT1-pY701-PE (BD Biosciences) at room temperature for 30 min. A final washing step with Perm/Wash buffer (BD Biosciences) was performed before the samples were immediately analyzed on a BD™ LSRII. Data were analyzed by BD FACSDiva™ Software (BD Biosciences).

2.4. Gene expression analysis

Following cell culture, stimulated or control moDCs were harvested and total RNA was isolated (High Pure RNA-Isolation Kit™, Roche Applied Science). RNA was transcribed into cDNA (Omniscript™ Reverse Transcriptase Kit, Qiagen) with Random Primers (Promega) and to avoid loss of RNA, an RNAse Inhibitor (Ambion) was added. Relative quantification by Real Time PCR was performed using a LightCycler® (Roche Applied Science) with the LightCycler® TaqMan® (Roche Applied Science). Primers (MWG Eurofins) were designed at the Universal Probe Library Assay Design Center (Roche Applied Science) and used with the appropriate UPL probes (Roche Applied Science). The following primers were used: IL-27p28, WSX-1, IRF8, CXCL10. TBP (TATA-Box binding protein) was used as reference gene. As internal run control we used a calibrator for each run. More precisely, we used RNA/cDNA of PBMCs (stimulated with PHA) from one individual. Furthermore, we performed RT^2 Profiler PCR Arrays (Qiagen) according to the manufacturer's instructions with moDCs (with or without 50 ng/ml IL-27). In the arrays, ACTB (Actin B), GAPDH (Glycerinaldehyde 3 phosphatedehydrogenase), HPRT (Hypoxanthine guanine phosphoribyl transferase), B2M (Beta-2-Microglobulin), RPL13A (Ribosomal Protein L13a) were used by default as reference genes in the PCR Arrays. The fold regulation describes the increase of the respective gene expression induced (or reduced) by IL-27 compared to unstimulated control moDCs.

The RNA was isolated as described above. cDNA was obtained by RT^2 First Strand cDNA Kit (Qiagen). The array was analyzed using a LightCycler®480 (Roche Applied Science) system.

2.5. Flow cytometric analysis

For intracellular staining, protein transport inhibitor BD Golgi Plug™ (BD Biosciences) was added for the last 18 h of cell culture. Cells were harvested after 24 h of stimulation with 50 ng/ml IL-27. The following antibodies were used for surface staining: CD11c APC (BD Biosciences), CD11c Pacific Blue (R&D Systems), CD40 PE (BD Biosciences). Cells were permeabilized by BD Cytofix/Cytoperm™. For intracellular staining the following antibody was used: IL-27 FITC (R&D Systems). Flow cytometry was performed on BDBD™ LSRII and data were analyzed by BD FACSDiva™ Software (BD Biosciences).

2.6. Cytokine detection in cell culture supernatants

Supernatants were collected on day 4 of moDC cultures for detection of secreted cytokines.

IL-8 and TNFα were quantified using a BD™ Cytometric Bead Array (CBA) according to the manufacturer's instructions (Human Inflammation CBA Kit, BD Biosciences). Data were analyzed by FCAP Array™ Software.

CXCL10 was measured by ELISA (Human CXCL10/IP-10 DuoSet, R&D Systems) according to the manufacturer's instructions.

3. Results

3.1. IL-27 induced its own production

Real time PCR with moDCs was performed to assess relative levels of IL-27p28 mRNA. When the cells were stimulated with IL-27 (50 ng/ml) for 24 h, we observed an increase of IL-27p28 mRNA both in CB and in AB moDCs (Fig. 1 A).

The expression of WSX-1, a subunit of the IL-27 receptor, was higher in unstimulated CB moDCs than in unstimulated AB cells (Fig. 1 B). Interestingly, WSX-1 was slightly downregulated by IL-27. When the cells were stimulated with LPS, WSX-1 levels actually decreased significantly in CB moDCs, but not in AB moDCs.

The observations concerning IL-27 induction by exogenous IL-27 were confirmed by measuring intracellular IL-27 protein in moDCs stimulated for 24 hours. Cord blood moDCs exhibited a significantly higher basal expression of IL-27 protein (Fig. 1 C, D) than AB moDCs. Furthermore, CB cells showed significantly higher expression of intracellular IL-27 compared to unstimulated moDCs in CB in the presence of exogenous IL-27 (50 ng/ml). In AB moDCs, IL-27 also elevated IL-27 protein compared to the quite low basal production, but the increase was not significant (Fig. 1 C, D).

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