Impact of valproic acid on dendritic cells function

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ABSTRACT

Objective: Recent data suggested that histone deacetylase (HDAC) inhibitors possessed potent anti-inflammatory and immunomodulatory properties both *in vitro* and *in vivo*. This study assayed the ability of the HDAC inhibitor, valproic acid (VPA), to influence the differentiation and functional properties of dendritic cells (DCs) generated from circulating peripheral blood monocytes.

Methods and results: Culture of monocytes in the presence of 0.5 mM of VPA did not impair DC differentiation. However, on the phenotypic level, in mature DCs, CD40, CD80 and CD86 were downregulated in the presence of VPA, compared to mature DCs generated in the absence of VPA. VPA led also to a significant down-regulation of CD83 and HLA-DR expression on mature DCs. Moreover, VPA treatment significantly inhibited IL-10 and IL-12p70 production by mature DCs. IL-10 and IL-12p70 altered secretion was observed whether DCs were matured with LPS alone or with LPS and IFN-gamma. In an allogeneic mixed lymphocyte reaction, the proportion of IFN-gamma+CD4+ T cells was decreased (26% *vs.* 51%, *p* = 0.005) when T cells were stimulated with DCs exposed to VPA. Also, CD8+ T cells stimulated with DCs treated with VPA, exhibited a significant decrease of Granzyme B expression.

Conclusion: These results suggest that HDAC inhibition by VPA alters essential human DC functions, highlighting the need for monitoring of immune functions in cancer patients receiving HDAC inhibitors, but also making these drugs attractive therapies in inflammatory, and autoimmune diseases.

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Introduction

Cellular survival, differentiation and gene expression are regulated by the opposing activities of histone deacetylase (HDAC) and histone acetyltransferase enzymes. Histone acetylation by histone acetyltransferases is associated with activation of transcription through relaxed chromatin structure, whereas deacetylation by HDACs induces a more condensed or inactive chromatin state, leading to gene repression. Although histones are the most studied acetylated protein substrates, HDACs are also responsible for modifying the activity of diverse types of nonhistone proteins, including signal transduction mediators and transcription factors (Atadja 2009; Drummond et al. 2005; Minucci and Pelicci 2006).

In the recent years, a group of structurally diverse HDAC inhibitors, such as sodium butyrate, suberoylanilide hydroxamic acid, trichostatin A, MS-275 and apicidin have been tested for cancer treatment (Drummond et al. 2005). However, more recent data suggested that HDAC inhibitors possessed potent anti-inflammatory and immunomodulatory properties both *in vitro* and *in vivo* (Bode et al. 2007; Camelo et al. 2005; Choi et al. 2005; Leoni et al. 2002; Reddy et al. 2008). The HDAC inhibitor valproic acid (VPA), derived from valeric acid, is currently the most widely used HDAC inhibitor in the routine clinic. VPA was shown to exert some antitumor effect in an array of cancers in preclinical studies, *in vitro* and *in vivo*, by modulating multiple pathways including cell cycle arrest, apoptosis, angiogenesis, differentiation, and senescence (Duenas-Gonzalez et al. 2008). Improvement was reported in many patients with different types of cancer following VPA treatment, with favorable responses in patients with myelodysplastic or myeloproliferative syndromes (Kuendgen et al. 2004), in patients with acute myeloid leukemia (Bug et al. 2005), and in patients with myelofibrosis (Inoue et al. 2005).

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Although the role of HDAC inhibition by VPA has been extensively studied in malignant cells, its effects on normal human immune function is not yet well established. Dendritic cells (DCs) are the most potent antigen-presenting cells *in vitro* and *in vivo*. They play a key role in the initiation of the immune response and are considered to be promising targets for immunotherapy (Palucka et al. 2008). The *in vitro* model of monocyte-derived DCs generated in the presence of granulocyte macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4 (Sallusto and Lanzavecchia 1994) represents an attractive model for studying the physiology of DCs and for identifying factors that might affect the differentiation, maturation and function of DCs. In this perspective, data on the effects of HDAC inhibition on DC-mediated antigen-specific immune responses are still sparse (Brogdon et al. 2007; Reddy et al. 2008; Song et al. 2011). In this study, we assayed the ability of VPA to influence the differentiation and functional properties of DC generated from circulating peripheral blood monocytes.

Materials and methods

Reagents

VPA was purchased from Sigma (St Quentin Fallavier, France) and was dissolved in RPMI-1640 (Sigma) at 50 mM and kept at −20 °C until further use. The drug was diluted in culture medium and used at various concentrations as indicated hereinafter.

Cell separation and DC generation

Blood was obtained from healthy donors (Etablissement Français du Sang Pays de Loire) after informed consent. CD14+ monocytes were purified by centrifugal elutriation with backward flow (Beckman Avanti J20, Beckman Coulter, Brea, USA) performed within the "DTC Platform" dedicated to cell sorting (M. Grégoire, CHU Nantes). This elutriation permitted to separate red blood cells, lymphocytes and monocytes (CD14+). The purity of the CD14+ fraction was always >90% as assessed by flow cytometry. Purified monocytes were cultured in RPMI-1640 medium containing 2 mM L-glutamine, 100 UI/ml penicillin, 0.1 mg/ml streptomycin and 10% fetal calf serum (Sigma, St Quentin Fallavier, France). The CD14+ cells were seeded at 1×10^6 cells/ml in the presence of 1000 UI/ml GM-CSF (Cell Genix, Clermont l'Herault, France) and 200 UI/ml IL-4 (Cell Genix). VPA was added at the dose of 0.5 mM on day 0 and on day 5 after culture. On day 5, the medium was replenished with GM-CSF and IL-4 and final maturation of DC was induced by adding 1 μg/ml LPS (from *Escherichia coli*, serotype O26:B6; Sigma) or LPS plus 500 UI/ml of IFN-gamma (Hilkens et al. 1997) (AbCys, Paris, France). DCs were collected on day 6 for further analyses.

Flow cytometry analysis

The following monoclonal antibodies were used for flow cytometry: anti-CD80 FITC from BD Pharmingen (San Diego, CA, USA), anti-CD83 PerCpCy5.5, -CD86 PE, -HLA ABC APC and -HLA-DR Pacific Blue from BioLegend (San Diego, CA, USA) and isotypic control mouse IgG1, mouse IgG2a and mouse IgG2b from BD Pharmingen. FITC-conjugated anti-CD14, PE conjugated anti-CD40 and PECy5 conjugated anti-CD1a were from Beckman Coulter (Marseille, France). Samples were analyzed using a FACSCanto II flow cytometer (BD Biosciences, Le Pont de Claix, France). Data for at least 1×10^5 cells/sample were acquired and analyzed using FlowJo software (Tree Star).

Primary mixed lymphocyte reaction (MLR)

CD4+/CD45RA+ naive T cells were purified by negative selection of total lymphocytes using naive CD4+ T cell Isolation Kit human (Miltenyi Biotec, Bergisch Gladbach, Germany). This kit permits to eliminate positive CD8, CD14, CD16, CD19, CD36, CD45RO, CD56, CD123 cells. Purity was superior to 95% as controlled by FACS analysis. The naive CD8 T cells were purified using the Naive CD8+ T Cell Isolation Kit (Miltenyi). DC were cocultured with naive CD4+ or CD8+ T cells at various ratios (DC:T cells ratios: 1:10; 1:20; 1:40 and 1:80). Proliferation of T cells was monitored by measuring methyl-[3H]-thymidine incorporation during the last 16 h of a 5 day culture period. The cells were then harvested onto a glass fiber filter using a TOMTEC cell harvester. Thymidine incorporation was measured by liquid scintillation counting using a Beta plate counter.

Cytokine measurement by ELISA

Supernatants of DC cultures were harvested at day 6 after culture. IL-10, IL-12p70, IL-6, IL-23, IL-27 and TNF-α concentrations were measured using specific enzyme-linked immunosorbent assay (ELISA) sets purchased from BD Pharmingen (IL-10, IL-12p70, IL-6 and TNF-α), eBioscience (IL-23) and R&D system (IL-27) according to the manufacturer's instructions. IL-4 and INF-γ were measured after coculture using ELISA OptEIA from BD Bioscience.

Detection of intracellular cytokines

DCs generated in the presence or absence of VPA were cocultured with CD4+/CD45RA+ T cells or naive CD8 T cells for 7 days. Then T cells were stimulated with 25 ng/ml phorbol-12-myristate-13-acetate (PMA; Sigma), 1 μg/ml ionomycin (Sigma) and 10 μg/ml brefeldin A (Sigma) for 5 h. For intracellular cytokine production by CD4 T cells, anti-IL-4 PE, anti-IFN-g FITC and anti-CD4-APC antibodies were used according to the manufacturer's instructions. For CD8 T cells, we used anti-IFN-gamma PE (Miltenyi), anti-Granzyme B APC (Invitrogen, Cergy Pontoise, France) and anti-CD8 FITC (BD Pharmingen). Cells were collected, washed, fixed and permeabilized using PBS/0.1% BSA and 0.1% Saponine. Data for at least 2×10^4 cells/sample were acquired on a FACSCanto II (BD Bioscience) using DIVA software and analyzed using FlowJo software.

Statistical analysis

The significance of differences between the indicated values was assessed by Mann–Whitney test using GraphPad Prism software; a P value of 0.05 or less was considered significant.

Results

Effect of HDAC inhibition by VPA on DC survival and differentiation

Prior to any further experiment, we sought to investigate the impact of VPA on DC viability. Thus, we cultured purified CD14+ human monocytes in the presence of GM-CSF, IL-4 and various concentrations of VPA. Based on the generated cells viability and on the expression of CD1a, a specific marker of DC differentiation, and the monocytic marker CD14, a dose of 0.5 mM of VPA was chosen and used in all further experiments (data not shown). Culture of monocytes in the presence of 0.5 mM of VPA did not induce significant morphological changes (data not shown). At the phenotypic level, the presence of VPA at day 0 and day 5