Human Plasmacytoid Dendritic Cell Function: Inhibition of IFN-α Secretion and Modulation of Immune Phenotype by Vasoactive Intestinal Peptide

Dorit Fabricius, M. Sue O'Dorisio, Sue Blackwell and Bernd Jahrsdörfer

*J Immunol* 2006; 177:5920-5927; doi: 10.4049/jimmunol.177.9.5920
http://www.jimmunol.org/content/177/9/5920

References

This article cites 36 articles, 20 of which you can access for free at: http://www.jimmunol.org/content/177/9/5920.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Human Plasmacytoid Dendritic Cell Function: Inhibition of IFN-α Secretion and Modulation of Immune Phenotype by Vasoactive Intestinal Peptide

Dorit Fabricius,*† M. Sue O’Dorisio,*‡ Sue Blackwell,†‡ and Bernd Jahrsdörfer*†‡

Plasmacytoid dendritic cells (PDC) are considered the main sentinels against viral infections and play a major role in immune tolerance. Vasoactive intestinal peptide (VIP) is a potent immunomodulator, whose role in PDC function is unknown. The present study was designed to investigate whether human PDC express VIP receptors and whether VIP has immunological effects on PDC. Using real-time RT-PCR and immunofluorescence, we demonstrated that VIP receptors VPAC1 and VPAC2 are expressed on PDC. After culturing PDC with VIP and CpG oligodeoxynucleotides for 48 h, expression of surface molecules with significance for PDC-T cell interactions as well as IFN-α secretion were quantified using FACS analysis and ELISA, respectively. For functional assays, CFSE-stained CD4+ T cells were cocultivated with differentially treated PDC. T cell proliferation and production of various cytokines were determined by FACS analysis and ELISA. VIP enhanced PDC expression of CD86, MHC II, and CCR7. In contrast, VIP inhibited PDC secretion of IFN-α and expression of Neuropilin-1 and MHC I. The potential of CpG oligodeoxynucleotide-activated PDC to induce proliferation of allogeneic CD4+ T cells was impaired when VIP was present during activation. Furthermore, pretreatment of PDC with VIP resulted in a decrease of the IFN-γ:IL-4 ratio in cocultured T cells, suggesting a modulation of the immune response toward Th2. Taken together, these results strongly suggest that VIP regulates the immunological function of human PDC. VIP may thus be involved in the modulation of immune responses to viral infections as well as in the maintenance of immune tolerance. The Journal of Immunology, 2006, 177: 5920–5927.

Vasoactive intestinal peptide (VIP), traditionally defined as a neuropeptide, is involved in the modulation of innate and adaptive immune responses (1–3). VIP can be considered a cytokine because it is produced and secreted by immune cells including lymphocytes and granulocytes in response to various immune signals (2), exhibits a broad spectrum of immunological functions, and exerts them, in a paracrine and/or autocrine way, through three different specific receptors, VPAC1, VPAC2, and PAC1 (4, 5). Although VIP has been classically considered an immunosuppressive agent, several studies suggest this peptide is rather a modulator of the homeostasis of the immune system (5, 6). Although the immunological effects of VIP have been studied in mice (7), its role in the human immune system is less clear.

Dendritic cells (DC) are a heterogeneous population of APCs, which are crucial for the initiation of a primary immune response (8). In addition to this classical description, DC play an important role in maintaining peripheral tolerance through the induction of regulatory T cells (Treg) (9). Two naturally occurring DC types have been described in peripheral blood so far, plasmacytoid DCs (PDC) and myeloid DCs (mDC). PDC represent only 0.2–0.8% of PBMC (10), and yet are considered to be primarily responsible for IFN-α secretion in response to viral infection and for the establishment of an adaptive immune response against the virus (10). Importantly, PDC have also been shown to be critically involved in the pathogenesis of autoimmune processes including lupus erythematosus and may therefore play a major role in the maintenance of immune tolerance in healthy individuals (11–13).

Because VIP receptors are found ubiquitously in human epithelial tissues (14), the main portals of potential microbial invasion, its effects on DC, which are also present at these sites in larger numbers, are of notable interest for the understanding of the immune modulatory functions of VIP. Recent studies point to the involvement of VIP in the regulation of DC using either in vitro-generated human monocyte-derived DC (15–17) or murine bone marrow-derived DC (15, 18, 19). Although VIP studies on the function of in vitro-generated DC are highly suggestive of in vivo regulation, the effects of VIP on human peripheral blood DC subsets including PDC have not yet been examined.

We therefore characterized VPAC1 and VPAC2 expression on human PDC. We then investigated whether or not VIP has an impact on phenotype and cytokine secretion of PDC and whether VIP treatment of PDC modulates their effect on T cells.

Materials and Methods

PDC precursor isolation and cell culture

This study was reviewed and approved by the University of Iowa Institutional Review Board. Buffy coats of 20 healthy subjects were received from the Blood Bank at the University of Iowa. PBMC were separated by Ficoll-Hypaque gradient centrifugation (Sigma-Aldrich) and RBC lysed with ACK (ammonium-chloride-potassium) (0.15 M NH₄Cl, 1 mM

---

*Department of Pediatrics, †Holden Comprehensive Cancer Center, and ‡Department of Internal Medicine, Carver College of Medicine, University of Iowa, Iowa City, IA 52242

Received for publication April 18, 2006. Accepted for publication August 1, 2006.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by the Carver Research Program of Excellence, the Children’s Miracle Network, and National Institutes of Health Grant RO1 CA82691 (to M.S.O.).

2 Address correspondence and reprint requests to Dr. M. Sue O’Dorisio, Pediatric Hematology/Oncology, 375 Newton Road, 4235 Medical Education Building Research Facility, University of Iowa Carver College of Medicine, Iowa City, IA 52242. E-mail address: sue-odorisio@uiowa.edu

3 Abbreviations used in this paper: VIP, vasoactive intestinal peptide; DC, dendritic cell; PDC, plasmacytoid DC; Treg, regulatory T cell; mDC, myeloid DC; ODN, oligodeoxynucleotide; C₅, cycle threshold; MFI, median fluorescence intensity; CFDA-SE, carboxyfluorescein diacetate succinimidyl ester.
of 0.1 mM EDTA (pH 7.3) lysing buffer. PDC precursors were isolated from PBMC with anti-BDCA-4 magnetic beads or the PDC isolation kit (Miltenyi Biotec). The purified fraction was >90% BDCA-2* and <5% CD5* or CD14*. For PCR and some additional experiments, a combined negative and positive selection kit was used (Diamond PDC isolation kit; Miltenyi Biotec), where the purified fraction was >98% BDCA-2*.

PDC were cultured for 2 days in AIM-V medium (Invitrogen Life Technologies), supplemented with 10 ng/mL recombinant human IL-3 (R&D Systems) in a flat-bottom 96-well plate (Corning) at a density of 1 x 10⁶ cells/well. Purity at day 2 was >99% BDCA-2* cells. PDC were treated with 1 μg/mL CpG oligodeoxynucleotide (ODN) (Coley Pharmaceutical Group) and 10⁻⁸ M VIP (Bachem) after testing various concentrations. VIP was dissolved in 0.05 M acetic acid, neutralized before use with 2 M KOH, and added to the media at a final concentration of 0.1 mM. Underlined nucleotides have phosphorothioate linkages. All others have phosphodiester linkages. The human lymphoblast cell line Molb-4b and the human colon carcinoma cell line HT-29 were cultured as described previously (20).

Immunofluorescence

The PDC precursor cell suspensions were fixed with 4% paraformaldehyde in PBS (pH 7.2) for 30 min at room temperature, spun down, and resuspended in PBS. A drop of suspended cells was applied to poly-L-lysine-coated coverslips. Before VPAC1 staining, protein blocking was performed with 10% normal goat serum for 30 min at room temperature. Cells were incubated in primary Ab mouse anti-human VIP (Pharosephotonics) diluted 1/200 for 30 min at room temperature. Cells were incubated in primary Ab goat anti-human VPAC2 Alexa Fluor 568 (Invitrogen Life Technologies) diluted 1/200 for 30 min at room temperature. Before VPAC2 staining, protein blocking was performed with 5% normal rabbit serum in PBS for 30 min at room temperature. PDC were incubated in primary Ab goat anti-human VPAC2 (Santa Cruz Biotechnology) diluted 1/50 for 1 h at room temperature. Cells were incubated in primary Ab rabbit anti-α-tubulin Alexa Fluor 568 (Invitrogen Life Technologies) diluted 1/200 for 30 min at room temperature. Labeling with mouse anti-human BDCA-2 FITC (BD Biosciences), dilution 1/11, was then performed to demonstrate specificity for DCs. The coverslips were then counterstained with To-Pro-3 (Invitrogen Life Technologies) for 5 min to stain nuclei. The coverslips were attached to slides with Vectashield (Vector Laboratories) mounting medium. The cells were examined and imaged using a Bio-Rad MRC1024 laser scanning confocal microscope.

Real-time RT-PCR

RNA was isolated using RNAlater (Ambion) followed by the RNeasy Kit (Qiagen) from purified PDC (>98% BDCA-2*, CD5*, CD14*) and control cell lines Molb-4b and HT-29. The lymphoblast cell line Molb-4b served as negative control for VPAC1 and positive control for VPAC2; the colon carcinoma cell line HT-29 were cultured as described previously (20). The RNA concentration was determined by absorbance at 260 nm and RNA stored at -80°C. RNA was reverse transcribed using SuperScript III RNase H- Reverse Transcriptase (Invitrogen Life Technologies). Generated cDNA was quantified in PCR conducted with the Platinum Quantitative PCR SuperMix-UDG kit (Invitrogen Life Technologies). Primers and probes were purchased from Integrated DNA Technologies. Primer and probe sequences were as follows: hVPAC1 5'-TCG TGG TTT TGC CTT GGC TAC-3', hVPAC2 5'-GGG AGG ATC GGG GGG GGG-3', control primer 2243, 5'-GGG GGA CGC TGC TGG GGG GGG-3'. Underlined nucleotides have phosphorothioate linkages. All others have phosphodiester linkages. The human lymphoblast cell line Molb-4b and the human colon carcinoma cell line HT-29 were cultured as described previously (20).

Statistical analysis

Statistical significance was assessed by Student's t-test and mixed linear regression analysis as appropriate. Differences were considered significant at a p value < 0.05 and highly significant at a p value < 0.01.

IFN-γ and IL-4 secretion

Pan T cells were isolated from healthy donors using a negative selection kit (Miltenyi Biotec) and coincubated at a 1:50 DC: T cell ratio with PDC, which had been pretreated for 2 days as described previously. Briefly, A with a final concentration of 1 μg/ml (Epichrome Technologies) and a final concentration of 10 M (PBL Biomedical Laboratories) for TGF-β1 (Cell Sciences) and IL-10 (eBioscience). Supernatants (100 μl) or standards were placed in 96-well precoated ELISA plates and incubated at 24°C for 1 h (IFN-γ) or 2 h (IL-10). After washing, plates were incubated with equal volumes of biotinylated anti-IFN-γ or anti-IL-10 secondary Ab for 1 h at 24°C. Plates were washed and incubated at 24°C for 1 h with HRP-conjugated avidin. For TGF-β detection, samples were incubated at 24°C for 4 h with HRP-conjugated anti-TGF-β1 Ab in a precoated plate. After addition of the substrate tetramethyl-benzidine, plates were read in an ELISA reader (Molecular Devices). The lower limit of IFN-α detection was 156 pg/ml of IL-10 detection 2 pg/ml; of TGF-β 23.76 pg/ml.

T cell/PDC cocultures and carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) staining

CD4+ T cells from healthy donors were isolated via positive selection (Miltenyi Biotec) and resuspended in PBS (1 x 10⁵ cells/ml) containing 5-(and 6-) CFDA-SE (MOLECULAR PROBES) at a final concentration of 1 μM, incubated at 37°C for 10 min, and washed three times. CD4+ T cells were then coincubated with allogeneic PDC pretreated with CPg ODN with and without VIP for 5 days at different ratios on a 96-well plate in AIM-V medium in a 200-μl volume, as described previously. Cells were harvested and analyzed by FACS. Data analysis was performed using FlowJo Software (Tree Star).

ELISAs

PDC were isolated as described previously and cultured in IL-3-containing (10 ng/ml) AIM-V medium in the presence of CpG ODN (1 μg/ml) and VIP (10⁻⁸ M) after testing various concentrations. After 2 days, supernatants were harvested, and IFN-α, TGF-β, or IL-10 concentrations were determined by a sandwich immunoassay using human ELISA kits for IFN-α MultiSubtype (PBL Biomedical Laboratories), for TGF-β1 (Cell Sciences) and IL-10 (eBioscience). Supernatants (100 μl) or standards were placed in 96-well precoated ELISA plates and incubated at 24°C for 1 h (IFN-α) or 2 h (IL-10). After washing, plates were incubated with equal volumes of biotinylated anti-IFN-α or anti-IL-10 secondary Ab for 1 h at 24°C. Plates were washed and incubated at 24°C for 1 h with HRP-conjugated avidin. For TGF-β detection, samples were incubated at 24°C for 4 h with HRP-conjugated anti-TGF-β1 Ab in a precoated plate. After addition of the substrate tetramethyl-benzidine, plates were read in an ELISA reader (Molecular Devices). The lower limit of IFN-α detection was 156 pg/ml of IL-10 detection 2 pg/ml; of TGF-β 23.76 pg/ml.
Results

**VPAC1 and VPAC2 are expressed on human PDC precursors**

Recently, we demonstrated that VPAC1 and VPAC2 receptors are expressed on human monocytes and T cells (3). The present study was designed to delineate the significance of VIP for PDC. Therefore, we first investigated whether or not PDC express the VIP receptors VPAC1 or VPAC2. To test the expression of the respective receptor proteins, isolated PDC were fixed in paraformaldehyde, stained with Abs to human VPAC1 and VPAC2, and subsequently analyzed by confocal microscopy. As illustrated in Fig. 1, PDC precursors show expression of both VPAC1 and VPAC2 in a pattern characteristic of membrane-bound proteins. Human cell lines HT-29 and Molt-4b served as negative control for VPAC1 and positive control for VPAC2, the colon carcinoma cell line HT-29 served as positive control for VPAC1 and negative control for VPAC2. HT-29 and Molt-4b were stained with anti-VPAC1 (red stain in G, negative in H) or anti-VPAC2 (red stain in J, negative in I). To-Pro-3 (blue) was used to stain nuclei. Representative results from one of three independent experiments are shown.

To confirm results obtained with immunofluorescence, PDC were highly purified and total RNA immediately extracted for quantitative analysis of VPAC1 and VPAC2 transcripts by real-time RT-PCR. As shown in Fig. 2, PDC precursors express RNA for both VPAC1 and VPAC2. RNA extracted from cell lines HT-29 and Molt-4b served as control. These data demonstrate that VPAC1 and VPAC2 are coexpressed in freshly isolated PDC precursors, as has been shown for human T cells and monocytes (5).

**VIP inhibits PDC secretion of IFN-α**

A main feature of PDC is their strong secretion of IFN-α in response to viral stimuli as well as to synthetic CpG ODN, which is the reason why they were formerly referred to as natural IFN-α-producing cells (10). We therefore investigated the effect of VIP on IFN-α production in response to class A and B CpG ODN. PDC were incubated in IL-3-containing AIM-V medium for 48 h in the presence or absence of VIP and/or CpG ODN, and the culture supernatants were subsequently harvested. The supernatants were then tested for IFN-α levels using ELISA. VIP inhibited PDC secretion of IFN-α in a dose-dependent manner with a maximum decrease at 10^{-6} M. Using this optimum dose, VIP decreased IFN-α secretion in four donors in the range of 20 and 90% for class...
B CpG ODN and in the range of 18 and 55% class A CpG ODN. The average decrease was 29% in the ODN class B-stimulated samples and 31% in ODN class A-stimulated samples (Fig. 3).

**VIP modulates human PDC phenotype**

The observation that PDC express receptors for VIP prompted us to test the effects of VIP on PDC phenotype. Human primary PDC were matured in vitro via the IL-3-dependent pathway in the presence or absence of VIP and CpG ODN. CpG ODN are synthetic ODNs containing CpG motifs, which mimic viral or bacterial DNA and are potent PDC activators (22). To determine the VIP-dose response, PDC were incubated for 48 h with increasing concentrations of VIP in combination with CpG ODN (Fig. 4C). The vehicle consisted of acetic acid, Tris buffer, and medium alone and had no effect (data not shown). Expression of CD86, BDCA-4, MHC class I, and MHC class II was determined by FACS analysis. The impact of VIP on PDC was most prominent at a concentration of $10^{-6}$ M, which was subsequently used in all experiments. PDC viability was not affected at this concentration (data not shown).

As has been shown by others (23), CpG ODN class B strongly up-regulate CD80 and CD86 in PDC. VIP enhanced this effect for the costimulatory molecule CD86 in a dose-dependent manner, whereas it had no effect on CpG ODN-mediated up-regulation of CD80 (Fig. 4, A and B). Expression of Ag-presenting molecules MHC class I and II is up-regulated by CpG ODN in mDC (24), and as shown in Fig. 4, this is in part true in human PDC. However, VIP on PDC oppositely regulated MHC class I and MHC class II. Although VIP down-modulated MHC class I expression, it up-regulated MHC class II expression. Both effects were dose-dependent and were observed in the presence and the absence of CpG ODN. Fig. 4 also demonstrates dose-dependent down-regulation of BDCA-4 expression by VIP, both in the presence and the absence of CpG ODN. The chemokine receptor CCR7, previously shown to be up-regulated by CpG ODN (25), is also up-regulated in the presence of VIP, but VIP does not further stimulate CpG ODN induction of CCR7 (Fig. 4). Likewise, in two experiments, we observed a tendency of CD83 up-regulation, a DC activation marker, which together with CCR7 expression characterizes mature DC (26). CD83 is up-regulated by VIP in the absence, but not further enhanced in the presence of CpG ODN (Fig. 4).

**VIP-primed PDC have a decreased potential to induce allogeneic CD4$^+$ T cell proliferation**

To investigate the effect of VIP on PDC-T cell interactions, CD4$^+$ T cells were isolated, stained with CFDA-SE, and coincubated with PDC, which had been cultured in IL-3-containing AIM-V medium in the presence of CpG ODN and/or VIP for 48 h. After 5 days, cocultures were harvested, and the percentage of proliferated T cells was determined by FACS analysis. Fig. 5A demonstrates that PDC matured in the presence of VIP, and CpG ODN have a reduced capability to induce allogeneic CD4$^+$ T cell proliferation as compared with PDC matured with CpG ODN alone. In experiments using six human PDC donors, the inhibitory effect of VIP pretreatment was apparent at PDC/T cell ratios of 1:50 and 1:500, whereas VIP alone had no significant effect on PDC stimulation of CD4$^+$ T cell proliferation (Fig. 5B).

**VIP-primed PDC shift IFN-γ:IL-4 ratio of allogeneic CD4$^+$ T cells toward IL-4**

Depending on the maturation signals they receive, PDC guide T cell differentiation into either IFN-γ or IL-4-producing cells (27). To examine the effect of VIP during PDC maturation, we isolated pan T cells and coincubated them for 48 h with PDC, which had been pretreated with VIP and/or CpG ODN for 48 h. PDC primed in the presence of VIP alone induced a shift of the IFN-γ:IL-4 ratio toward IL-4 in the responding CD4$^+$ T cell population as compared with unprimed PDC. This effect was mainly due to increased IL-4 production with unaltered IFN-γ. In contrast, PDC primed in the presence of CpG ODN generally induced higher levels of IL-4 and IFN-γ in the responding CD4$^+$ T cell population; VIP had no significant effect on this response (Fig. 6, A and B).

**VIP-primed PDC decrease TGF-β secretion of allogeneic CD4$^+$ T cells**

To further study the T cell phenotype in the presence of VIP-primed PDC, we coincubated isolated CD4$^+$ T cells for 48 h with PDC, which had been pretreated with VIP and/or CpG ODN for 48 h. Subsequently, we determined IL-10 and TGF-β contents in CD4$^+$ T cell/PDC coculture supernatants by ELISA. We found no significant impact on IL-10 secretion. However, TGF-β levels were significantly lower in the presence of VIP-primed PDC than in the presence of untreated PDC. This effect was absent when PDC had been pretreated with CpG ODN (Fig. 6C).

**Discussion**

PDCs represent one of the two currently known peripheral blood DC subsets. Depending on the conditions present when they are matured in vitro, they induce T cell activation or anergy. Consequently, PDC and DC are considered to be both the main sentinels...
for viral infections, and also critically involved in the induction and maintenance of immune tolerance (28). In vitro-generated monocyte-derived human DC as well as murine bone marrow-derived DC have recently been shown to be modulated by the neuropeptide VIP (15, 16, 19). The effects of VIP on freshly isolated human peripheral blood PDC have so far not been investigated. The present study is the first to delineate expression of VPAC1 and VPAC2 on human PDC and to characterize the biological response of PDC to VIP.

These results demonstrate transcriptional expression of VPAC1 and VPAC2 RNA as well as functional VPAC1 and VPAC2 receptor protein. VIP-induced inhibition of IFN-α secretion by PDC following stimulation with CpG ODN is potentially a clinically relevant observation. IFN-α is a pleiotropic cytokine with a broad spectrum of effects on cells of the immune system and also on tumor or virus-infected cells. The effects on the immune system include induction of cytotoxic activity in NK and T cells as well as maturation and differentiation of monocytes, macrophages, and DCs (29). IFN-α is considered a promising therapeutic agent because of its strong antiviral and antiproliferative activity. In contrast, IFN-α is pathophysiologically involved in the maintenance of autoimmune processes such as in systemic lupus erythematosus, where it induces monocytes to differentiate into DCs able to take up apoptotic cell debris and present it as self-Ag, thereby further enhancing and perpetuating the autoimmune response (11–13). Because of the variety of differential effects on the immune system, IFN-α synthesis and secretion requires tight control. Our study suggests that VIP may be one of the factors involved in this process, thereby contributing to the general regulation of the immune response.

In general, DC maturation is associated with strong up-regulation of costimulatory and Ag-presenting molecules (28, 30, 31). In PDC, this effect can be observed when the maturation occurs in the presence of CpG ODN (24). We found that VIP was able to further enhance this effect for CD86 and BDCA-4, MHC class I, and MHC class II, whereas not for CD80. Similar results were obtained from another group in immature murine bone marrow-derived DC, where VIP treatment up-regulated CD86 but not CD80 expression (32). The same group observed a lack of up-regulation of costimulatory molecules when treating LPS-matured human monocyte-derived DC or murine bone marrow-derived DC with VIP (17, 32, 33). In contrast to the enhancement of CpG ODN-induced MHC class II up-regulation on PDC, we observed that VIP inhibited CpG ODN-induced MHC class I up-regulation, suggesting that VIP promotes the interaction of PDC with CD4+ T cells rather than with CD8+ CTLs. Furthermore, we found that in MLRs with differentially primed PDC and allogeneic CD4+ T cells, VIP-primed PDC supported a Th2-weighted cytokine profile by inducing IL-4 rather than IFN-γ production by T cells. These data support the general perception of VIP as an immunomodulatory cytokine. We further show that VIP
VIP-treated PDC decrease the ratio of IFN-γ/IL-4 secreted by CD4⁺ T cells and reduce TGF-β secretion. PDCs were cultured in IL-3-containing (10 ng/ml) AIM-V medium in the presence of CpG ODN (1 μg/ml) and VIP (10⁻⁶ M) for 48 h. Allogeneic CD4⁺ T cells from healthy donors were isolated, stained with CFDA-SE, and coincubated with precultured PDC at different ratios as indicated. T cells in the absence of PDC or in the presence of anti-CD3/CD28 Abs served as negative and positive controls. After 5 days, cocultures were harvested, and the percentage of proliferated (i.e., CFDA-SElow) T cells was determined by FACS analysis. A, Histograms from one of six representative experiments are shown. Gray line represents T cell proliferation in response to PDC not stimulated with VIP, and red lines represent T cell proliferation in response to PDC stimulated with VIP. B, Line graphs represent average T cell response of six different donors. Error bars indicate SEM. *, p values <0.05 indicate significant differences at a PDC:T ratio 1:50 and 1:500.

VIP inhibits the potential of PDC to induce proliferation of allogeneic CD4⁺ T cells. Proliferation of allogeneic CD4⁺ T cells after 5-day coculture with PDCs preincubated with different stimuli for 48 h is shown. PDC were isolated as described and cultured in IL-3-containing (10 ng/ml) AIM-V medium in the presence of CpG ODN (1 μg/ml) and VIP (10⁻⁶ M) for 48 h. Allogeneic CD4⁺ T cells from healthy donors were isolated, stained with CFDA-SE, and coincubated with precultured PDC at different ratios as indicated. T cells in the absence of PDC or in the presence of anti-CD3/CD28 Abs served as negative and positive controls. After 5 days, cocultures were harvested, and the percentage of proliferated (i.e., CFDA-SElow) T cells was determined by FACS analysis. A, Histograms from one of six representative experiments are shown. Gray lines represent T cell proliferation in response to PDC not stimulated with VIP, and red lines represent T cell proliferation in response to PDC stimulated with VIP. B, Line graphs represent average T cell response of six different donors. Error bars indicate SEM. *, p values <0.05 indicate significant differences at a PDC:T ratio 1:50 and 1:500.

VIP-treated PDC decrease the ratio of IFN-γ/IL-4 secreted by CD4⁺ T cells and reduce TGF-β secretion. PDCs were cultured in IL-3-containing (10 ng/ml) AIM-V medium in the presence or absence of VIP (10⁻⁶ M) and CpG ODN (1 μg/ml) as described. Pan T cells were isolated by negative magnetic selection and coincubated with VIP or not for 48 h with differently pretreated PDC. Brefeldin A (10 μg/ml), PMA (50 ng/ml), and Ionomycin (1 μg/ml) were added during the last 4 h of culture. Counterstaining with anti-CD4 and intracellular staining with FITC-conjugated anti-IFN-γ and PE-conjugated anti-IL-4 Abs was performed as described. A, Zebra plots of one representative experiment of seven are shown. CD4⁺ T cells are gated. Numbers in quadrants indicate percentages of positive cells. B, The average IFN-γ/IL-4 MFI ratios from seven independent experiments are shown. Error bars indicate SEM. C and D, IL-10 (C) and TGF-β (D) contents in CD4⁺ T cell/PDC coculture supernatants were determined by ELISA. Error bars indicate SEM of seven independent experiments.
by itself is able to induce activation of PDC, as reflected by the up-regulation of MHC class I and of the chemokine receptor CCR7. VIP-induced modulation of CCR7 is of particular interest because CCR7 interacts with CCL19/CCL21 and may be relevant for mature PDC to enter afferent lymph vessels to reach lymph nodes (34). Therefore, one effect of VIP on PDC in tissues could be to facilitate maturation and transport to regional lymph nodes. The fact that VIP alone has an effect on this molecule suggests that VIP is not just an inhibitor of PDC function. It rather appears to be an immune modulator that changes PDC phenotype at distant sites in a way that supports migration to secondary lymphoid organs, where they can encounter and interact with specific T cells, even in the absence of more potent stimuli like viral DNA.

In contrast, our results indicate that VIP inhibits parts of the immunological function of PDC. First, the neuronal receptor Neuropilin-1 (identical with BDCA-4 (35)) is down-modulated by VIP. Neuropilin-1 has been demonstrated to be essential for establishing the initial contact between DCs and resting T cells and thus the initiation of the primary immune response (21). Second, in MLRs performed with differentially treated PDC and allogeneic CD4+ T cells, we found that VIP-treated PDC are less active in inducing T cell proliferation as compared with PDC matured in the absence of VIP. Comparable results were found by Delgado et al. (6, 33), who demonstrated that VIP-primed bone marrow-derived DC or resting macrophages had a reduced capacity to stimulate T cell proliferation.

Another critical point where VIP has been proposed to interfere with immune regulation is in the induction of Treg by DC. Delgado et al. (32) have proposed that monocyte-derived DCs treated with VIP become tolerogenic and give rise to an increased frequency of Treg (17). We therefore evaluated the typical cytokines Treg produce, either predominantly IL-10 (Treg1) or TGF-β (Treg2/Th3) (36). Surprisingly, we found no significant impact of VIP-primed PDC on IL-10 secretion by T cells. However, unexpectedly, TGF-β levels were significantly lower in the presence of VIP-primed PDC than in the presence of untreated PDC. CpG ODN had a similar effect that was not further affected by VIP. CTLA-4 surface expression on cocultured CD4+ T cells was not altered by VIP pretreated PDC (data not shown). It may be relevant that VIP did not prevent DC maturation, unlike in studies with other DC types describing that VIP induced a stable immature phenotype (17, 32). Our findings suggest that the effects of VIP on DCs strongly depend on the DC type and the maturation stimuli present at the time of priming. Our data do not support the hypothesis that VIP-treated PDC may acquire the capacity to induce Treg in vitro. It remains to be further elucidated whether or not VIP-treated PDC can shape the character of encountering T cells in a different way in the presence of other maturation stimuli.

The DC type we have characterized in this study is substantially different from the ones used by Delgado et al. (15, 17, 33). Human mDCs as opposed to PDCs might be closer to the monocyte-derived or bone-marrow-derived DC model systems used by Delgado et al. (15, 17, 33) and should be studied in more detail in the future.

In conclusion, our study demonstrates for the first time that human PDCs express receptors for and show biological responses to VIP. VIP significantly inhibits CpG ODN-induced secretion of IFN-α by PDC and changes their immune phenotype in such a way that their interaction with CD4+ T cells is facilitated and a Th2 response is promoted, whereas T cell proliferation is inhibited. VIP may therefore be involved in the modulation of immune responses to viral infections, thereby helping to prevent immunological overdrive. Although independent of IL-10 or TGF-β, VIP might contribute to the development and the maintenance of immune tolerance, which could have substantial implications for the treatment of Th1-dominated autoimmune diseases such as lupus erythematosus, rheumatoid arthritis, and multiple sclerosis.

Acknowledgments

We thank Jean Ross for excellent assistance with immunofluorescence staining and confocal microscopy and Justin Fishbaugh and Gene Hess for excellent technical assistance with flow cytometry.

Disclosures

The authors have no financial conflict of interest.

References


