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Vasoactive Intestinal Peptide Modulates Langerhans Cell Immune Function

Sreedevi Kodali,* Wanhong Ding,* Jing Huang,* Kristina Seiffert,* John A. Wagner,† and Richard D. Granstein2*

Epidermal nerves lie in close proximity to Langerhans cells (LC) and are capable of releasing peptides that modulate LC function, including calcitonin gene-related peptide and pituitary adenylate cyclase-activating polypeptide. The neuropeptide vasoactive intestinal peptide (VIP) has also been found in cutaneous nerves and mRNA, for the VIP receptor vasoactive intestinal peptide receptor type 1, and vasoactive intestinal peptide receptor type 2 have been found in murine LC and the LC-like cell line XSI06. We examined the effects of VIP on LC function and cutaneous immunity. VIP inhibited elicitation of a delayed-type hypersensitivity response in previously immunized mice by epidermal cells enriched for LC content pulsed with Ag in vitro. VIP also inhibited the ability of unseparated epidermal cells to present Ag to a T cell clone and hybridoma and the ability of highly enriched LCs to present to the T cell clone. Inhibition of presentation to the hybridoma was observed with an antigenic peptide that does not require processing, suggesting that VIP is active at a step independent of Ag processing. To elucidate the mechanism(s) by which VIP may mediate these effects, we determined the effects of VIP on LC cytokine production using the XSI06 cell line as a surrogate for LC. VIP augmented the production of the IL-10 in LPS-stimulated XSI06 cells while down-regulating IL-12 and IL-1β production. Thus, VIP, like pituitary adenylate cyclase-activating polypeptide and calcitonin gene-related peptide, down-regulates LC function and the associated immune response. The Journal of Immunology, 2004, 173: 6082–6088.

Departments of *Dermatology and Neurology and Neurosciences, Joan and Sanford I. Weill Medical College of Cornell University, New York, NY 10021

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†Address correspondence and reprint requests to Dr. Richard D. Granstein, Department of Dermatology, Joan and Sanford I. Weill Medical College of Cornell University, 525 East 68th Street, Room F-342, New York, NY 10021. E-mail address: rdgranstein@med.cornell.edu

Abbreviations used in this paper: LC, Langerhans cell; BMDC, bone marrow-derived dendritic cell; CM, complete medium; cOVA, chicken OVA; DTH, delayed-type hypersensitivity; EC, epidermal cell; eEC, enriched EC; KLH, keyhole limpet hemocyanin; PACAP, pituitary adenylate cyclase-activating polypeptide; pLC, purified LC; TAA, tumor-associated Ag; VIP, vasoactive intestinal peptide; VPAC1, vasoactive intestinal peptide receptor type 1; VPAC2, vasoactive intestinal peptide receptor type 2.
caused, in part, by a reduction of IL-1β and IL-12 production and an increase in IL-10 production.

Materials and Methods

Reagents

VIP and VIP,10–28 (an inactive VIP fragment) (19, 20) were purchased from Peninsula Laboratories (San Carlos, CA). Recombinant murine IL-12 and IL-1β were obtained from BD Pharimingen (San Diego, CA), as was monoclonal anti-I-A^b. The VPAC1 agonist (Lys^3,Arg^6,Leu^7) VIP (L-7)/growth hormone releasing factor (8–27), the VPAC1 antagonist Ac His1 (D-Phe2, Lys 15, Arg20), and 5-cMec-Arg (23), and 5-Azo (24) were purchased from Bachem (Bubendorf, Switzerland). Anti-I-A^b (Sigma-Aldrich) for 30 min at 4°C. The cells were then washed three times to remove the Ag and VIP. The ECs were then stimulated with 0.05 μg/ml LPS (IL-12, IL-1β) or with 0.05% trypsin for 3 min at 80°C. After an additional 3 h, the cells were washed three times with CM to remove soluble VIP and KLH and then cocultured with HDK-1 cells (5 x 10^5 per well). Supernatants were collected after 72 h and analyzed for IFN-γ production. In experiments examining the possible role of IL-12 or IL-1β in the VIP effect, either IL-12 (500 pg/ml) or IL-1β (10 ng/ml) was added to each culture well at the time of coculture of ECs and HDK-1 cells.

Cytokine analysis

IFN-γ production by HDK-1 cells was analyzed by sandwich ELISA using purified rat anti-mouse IFN-γ mAb (4 μg/ml), biotinylated rat anti-mouse IFN-γ mAb (1 μg/ml), avidin-HRP (1/1000 dilution), and ABTS substrate (405 nm) as a marker of T cell stimulation (29). In the VIP effect, either IL-12 (500 pg/ml) or IL-1β (10 ng/ml) was added to each culture well at the time of coculture of ECs and HDK-1 cells.

Statistical analysis

The significance of differences among groups in each assay system was measured using the Student’s two-tailed t test for unequal samples (Excel software; Microsoft, Redmond, WA).

Results

VIP inhibits in vitro Ag presentation to T cell clones

To determine whether VIP affects LC Ag presentation function, murine ECs were preincubated with or without VIP and then treated with the Ag KLH. The VIP and KLH were then carefully washed out, and the ECs were cocultured with the KLH-responsive TH clone HDK-1 for 72 h. IFN-γ production by the T cells was used as a marker of T cell stimulation (29). ECs not treated with KLH induced no IFN-γ production in the HDK-1 cells. Addition of KLH stimulated a significant increase in IFN-γ production;
were cocultured with the T cell hybridoma DO11.1. The negative control COVA323–339 was added. VIP and COVA323–339 were removed, and the ECs cocultured with the fragment COVA323–339, which is presented on the LC MHC receptor without processing (26). As above, murine ECs were pretreated with VIP before addition of COVA323–339. The ECs were then washed and cocultured with DO11.1 cells, and IL-2 production by the T cells was used as a marker of T cell stimulation (31). When murine ECs were pretreated with VIP before COVA323–339 addition, there was a dose-dependent decrease in IL-2 production (Fig. 1, lower panel). Thus, VIP specifically inhibits the ability of murine ECs to present Ag to stimulate Th1 cells.

To test whether VIP inhibits LC Ag processing, we used the COVA-responsive T cell hybridoma, DO11.1. These cells respond to the fragment COVA323–339, which is presented on the LC MHC receptor independent of Ag processing.

To determine which receptor(s) was involved in VIP suppression of Ag presentation, we used specific peptide inhibitors of VPAC1 and VPAC2. Murine ECs were preincubated with each peptide for 2 h, followed by addition of VIP for an additional 3 h. Then, the cells were treated with the Ag KLH. The VIP and KLH were then carefully washed out, and the ECs were cocultured with HDK-1 cells for 72 h and measurement of IFN-γ production using ELISA. Mean difference (±SD) between the groups is shown. The data shown are representative of three separate experiments (*, p < 0.05 for N vs O, P, Q, and R; and **, p < 0.01 for B vs A, C, D, E, and F; H vs G, I, and J; and N vs M and S).

![Graph](http://www.jimmunol.org/)
pLCs, we again saw the same dose-dependent inhibition of IFN-γ production with VIP from 0.1 to 1000 nM, also with a plateau in response after 10 nM (Fig. 2). Thus, VIP directly inhibits the ability of LC to present Ag to elicit a T cell response.

To examine whether VIP inhibits the Ag-presenting capability of mature LCs, ECs were cultured for 48 h in the presence of 10 ng/ml GM-CSF, a regimen known to induce maturation of LCs (33), and then tested for the ability of VIP to inhibit their presentation of KLH to HDK-1 cells. VIP indeed inhibited Ag presentation by these cells (Fig. 3), suggesting that VIP can also modulate the function of more mature LCs.

VIP inhibits the elicitation of DTH

Because VIP inhibited the ability of LC to present Ag in vitro, we examined whether a similar inhibition occurred when eliciting an in vivo immune response. Naive mice were immunized against the S1509a tumor at weekly intervals (see Materials and Methods). eECs (~12% LC) were prepared and incubated with or without VIP, followed by treatment with TAA, soluble S1509a tumor Ag (see Materials and Methods). The eECs were then washed and re-exposed to VIP and TAA and then injected into the hind footpad of mice previously immunized to the S1509a tumor. Footpad swelling compared with baseline was used as a marker of immune response. Mice injected with eECs treated with TAA had a significant swelling compared with mice injected with eECs not treated with TAA. However, eECs preincubated with VIP before TAA addition had a significantly decreased response, ~50% less than the positive control, showing VIP’s inhibition of the elicitation of DTH (Fig. 4). To rule out an effect of VIP on the nonspecific, irritant component of the response, eECs were incubated with VIP, but not treated with TAA. As expected, this group had a baseline level of swelling similar to the negative control described above (Fig. 4). Thus, VIP reduces the elicitation of an immune response in vivo.

VIP modulates cytokine production in XS106 cells

Clearly, VIP inhibits the ability of LC to effectively elicit a T cell-mediated immune response. To elucidate a mechanism contributing to this phenomenon on a molecular level, we determined the effects of VIP on cytokine production in the LC-like cell line XS106. We chose to focus on IL-1β, IL-12, and IL-10 because all three have been shown to play important roles in LC function and cutaneous immunity. IL-1β and IL-12 are proinflammatory, up-regulating T cell-mediated immune responses, while IL-10 has been shown to be anti-inflammatory in its effects (34–37).

When XS106 cells were treated with LPS, there was a significant increase in IL-1β, IL-12, and IL-10 compared with cells treated with medium alone; however, pretreatment with VIP before and during the period of LPS stimulation inhibited the induction of IL-1β and IL-12 in a dose-dependent manner (Fig. 5). In contrast, VIP augmented the LPS-induced stimulation of IL-10 production (Fig. 5). These results cannot be accounted for by VIP-induced cell death, as trypan blue staining indicated no increase in cell death with VIP. In addition, cell death would be expected to either increase or decrease all levels of cytokines, not specifically increase one while decreasing others. Thus, VIP modulates cytokine production in XS106 cells.

These findings suggest that changes in cytokine expression induced by VIP in dendritic cells might be responsible, at least in part, for the VIP effect on Ag presentation. To examine this possibility, IL-12 or IL-1β was added to tissue culture wells of VIP-treated ECs used to present KLH to HDK-1 cells. Addition of IL-12 (500 pg/ml) fully restored the response of HDK-1 cells to VIP-treated ECs (Fig. 6). Addition of IL-1β (10 ng/ml) had no effect at the dose used (data not shown).

Discussion

We have described a novel role for VIP as an inhibitor of LC immune function. We demonstrated that in vitro, VIP inhibits the ability of murine ECs and pLCs to present Ag to elicit a Th1-mediated immune response. A similar effect was also shown in vivo, in which VIP inhibited the ability of murine eECs to elicit a...
DTH response in previously immunized mice. We believe that VIP may mediate its effects, at least in part, through its modulation of cytokine production. In the XS106 LC-like line, VIP suppressed the LPS-stimulated induction of IL-12 and IL-1β while augmenting IL-10 production. Stimulation of XS106 cells by LPS was used as a surrogate for activation of LCs in vivo, and, of course, correlation of our results with cellular and molecular events in vivo must be inferred with caution.

A recent study reported that VIP enhances the Ag-presenting capacity of immature bone marrow-derived dendritic cells, while inhibiting the Ag-presenting capability of mature bone marrow-derived dendritic cells (32). Our findings demonstrate that VIP suppresses the Ag-presenting capacity of freshly obtained LCs, usually thought of as immature. These discordant findings may relate to intrinsic differences between LCs and bone marrow-derived dendritic cells. Alternatively, freshly obtained LCs may be more mature than the cells used in that study.

LC are the only known source of cutaneous IL-1β (38). IL-1β has been shown to be necessary for LC migration to regional lymph nodes to present Ag to T cells (39). Furthermore, IL-1β increases the expression of I-A, B7-2, CD40, and ICAM-1 in LC, all of which are crucial in stimulating T cells (40). At the T cell level, IL-1β promotes T cell proliferation by up-regulating T cell production of IL-2 and IL-2R (41). It is unclear whether LC production of IL-1β acts in an autocrine fashion on the LC, on the T cells, or both. By down-regulating IL-1β production in LC, VIP may contribute to an anti-inflammatory milieu within the skin. However, at the concentrations examined, addition of IL-1β failed to restore the ability of VIP-treated ECs to present KLH to HDK-1 cells.

The cytokine IL-12 is an important positive regulator of Th1 immune responses and augments IFN-γ production by T cells and induces proliferation of activated T cells (42). IL-12 is composed of two subunits, p35 and p40, which together make the biologi-
Perfusates were analyzed for 72-h IFN-\(\gamma\)-treated with neuropeptide or KLH before coculture with HDK-1 cells. Supernatants were analyzed for 72-h IFN-\(\gamma\) production using ELISA. Mean difference (\(\pm\)SD) between the groups is shown. The data shown are representative of three separate experiments (*, \(p < 0.01\) for C vs A, B, and D; and not significant for C vs E and F).

**FIGURE 6.** IL-12 restores the Ag-presenting capability of VIP-treated EC. Murine ECs were incubated in CM (A, B, C, and F) or CM containing VIP (D and E) and then exposed to the Ag KLH. Neuropeptide and KLH were then removed, and the ECs were cocultured with the Th1, KLH-specific clone HDK-1. At the time of coculture, IL-12 (500 pg/ml) was added to wells of B, E, and F. The negative controls (A and B) were not treated with neuropeptide or KLH before coculture with HDK-1 cells. Supernatants were analyzed for 72-h IFN-\(\gamma\) production using ELISA. Mean difference (\(\pm\)SD) between the groups is shown. The data shown are representative of three separate experiments (*, \(p < 0.01\) for C vs A, B, and D; and not significant for C vs E and F).

Globally active protein IL-12 p70. LC have been shown to secrete both p40 and biologically active p70 (43). Furthermore, IL-12 produced by LC has been shown to be important for stimulating immune responses. One group demonstrated that LC taken from normal mice and incubated with Ag were subsequently able to immunize naive mice to that Ag; however, LC from IL-12 knockout mice were completely unable to immunize naive mice (44). Similarly, murine ECs treated with IL-12 significantly augmented IFN-\(\gamma\) production in an allogeneic mixed epidermal lymphocyte reaction as compared with untreated EC; however, this increase was not due to any change in CD86, CD80, or MHC II expression in IL-12-treated ECs (37). VIP’s suppression of IL-12 may be a key method by which it suppresses Th1-mediated immune responses. Our finding that addition of IL-12 restores the ability of VIP-treated ECs to present Ag supports this concept. Of course, it is not definitive proof, as addition of IL-12 could be compensating for another change induced by VIP.

In contrast to IL-12, IL-10 attenuates Th1-mediated immune responses. IL-10 inhibits IL-2 production and blocks T cell stimulation via the CD28 costimulation pathway. Following Ag stimulation, IL-10 inhibits LC migration. In IL-10 knockout mice, LC showed an enhanced capacity to migrate to regional lymph nodes upon Ag exposure (45). Furthermore, IL-10 down-regulates LC ICAM1, B7-1, and B7-2 expression and inhibits the continuous proinflammatory cytokine production (46, 47). Previous work by our group has shown that murine LC can be stimulated to produce at least some IL-10 (48); however, human LC have been shown to produce very little IL-10, with only 5% of human LC expressing enough IL-10 for detection by an Ab-based technique (49). Another group reports that human LC have enhanced IL-10 production after cutaneous occlusion (50). As a whole, our findings support the anti-inflammatory nature of VIP within the skin environment.

The endogenous mediators of VIP release in the skin are not well understood. VIP can be released from cutaneous nerves, and one study demonstrated that in the enteric ganglia, NO was a potent stimulator of VIP release (51). NO is produced in high levels during an inflammatory response, and this may serve as a mechanism for VIP release from cutaneous nerves (52). Another source of VIP may be immune cells. Recently, murine lymphocytes were shown to release VIP upon treatment with LPS, cytokines, or anti-TCR Abs (53). Thus, release of VIP from either nerves or lymphoid cells during inflammatory processes may be an endogenous mechanism for suppressing existing inflammation or preventing excessive inflammation and initiation of autoimmune diseases.

Receptor-specific knockout mice demonstrate that VIP plays an important role in the Th1/Th2 balance. Studies of mice that are deficient in the VPAC2 receptor support the hypothesis that VIP inhibits Th1 responses while augmenting Th2 responses. As expected, these mice have an enhanced hapten-evoked DTH response, but diminished cutaneous anaphylaxis and anti-hapten IgE Abs (54). Stimulated splenic CD4+ T cells in these mice produced higher levels of IL-2 and IFN-\(\gamma\), but lower levels of the Th2 cytokine IL-4 (55). In contrast, transgenic mice overexpressing the VPAC2 receptor demonstrate the opposite phenotype favoring Th2 responses (54). These reports support our preliminary finding that VIP signaling through the VPAC2 receptor may be responsible for decreased Ag presentation in the systems we have used. These findings also support a previous in vitro study demonstrating that VIP-treated macrophages induce Th2 cytokine production and inhibit Th1 cytokine production in Ag-primed CD4+ T cells (55). VIP was also found to support the proliferation and survival of Th2, but not Th1 effectors (56). Our findings are consistent with the hypothesis that VIP inhibits Th1 responses. We saw an inhibition in vivo in the DTH response, and VIP inhibited LC Ag presentation to a Th1 clone. Furthermore, our finding that VIP augments IL-10 production, but suppresses IL-12 production by XS106 cells supports the concept of a shift from Th1 to Th2 processes.

The immunomodulatory capacity of VIP provides an important therapeutic tool in Th1-mediated diseases. In murine models of rheumatoid arthritis and Crohn’s disease, administration of VIP had significant anti-inflammatory effects (57, 58). This raises the possibility that VIP may be an effective treatment in Th1-mediated skin diseases such as psoriasis.

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