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Vasoactive intestinal peptide inhibits IL-8 production in human monocytes

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Abstract

Vasoactive intestinal peptide (VIP), a neuropeptide present in the lymphoid microenvironment, acts as a potent anti-inflammatory agent that inhibits the function of activated macrophages. VIP was shown to inhibit IL-6, TNFα, IL-12, chemokine, and nitric oxide production in endotoxin-activated macrophages. The present study reports the effect of VIP on IL-8 production by stimulated human monocytes. VIP inhibits IL-8 production in a dose- and time-dependent manner at the mRNA level. The specific VPAC1 receptor mediates the inhibitory effect of VIP. Two transduction pathways appear to be involved, a major cAMP-independent pathway and a secondary cAMP-dependent pathway. Of obvious physiological significance is the fact that VIP, presumably through the inhibition of IL-8 production, dramatically reduces the monocyte-induced neutrophil chemotaxis, an important event in the pathogenesis of several inflammatory and autoimmune disorders. These findings support the proposed role of VIP as a key endogenous anti-inflammatory agent and describe a novel mechanism, i.e., the inhibition of the production of monocyte-derived IL-8.

Keywords: Neuroimmunology; Neuropeptides; Monocytes; Chemokines; Inflammation; Chemotaxis

Peripheral blood monocytes (PBM), as they migrate from the vascular compartment to a site of inflammation, undergo activation and secrete proinflammatory cytokines and oxidants such as TNFα, IL-6, IL-1β, IL-12, and nitric oxide, which contribute to pathophysiological changes associated with several acute and chronic inflammatory conditions, and inflammatory chemokines which recruit and activate blood-derived leukocytes. The chemotactic factor IL-8, the best-characterized member of the α-chemokine or CXC chemokine family, is a potent activator and chemoattractant of neutrophils, but also acts on T cells, basophils, and eosinophils [1–3]. IL-8 is produced as a 72- or 77-aminoacid protein by a wide variety of cell types, including monocytes/macrophages, neutrophils, T cells, endothelial cells, synovial cells, fibroblasts, and epithelial cells after exposure to inflammatory stimuli such as the bacterial endotoxin lipopolysaccharide (LPS), or the pro-inflammatory cytokines IL-1, IL-6, and TNFα [1–7], and it is inhibited by anti-inflammatory cytokines, such as IL-10 [8]. Many diverse forms of acute and chronic inflammatory diseases are characterized by the local accumulation of inflammatory cells, including neutrophils and lymphocytes. IL-8 participates in the pathogenesis of various diseases, including rheumatoid arthritis, asthma, endotoxic shock, psoriasis, pancreatitis, inflammatory bowel disease, and acute respiratory distress syndrome [3], and its levels are often correlated with the severity of the pathology and/or disease outcome [9,10]. Therefore, although IL-8 plays a beneficial and central role in the inflammatory response, hematopoiesis, and angiogenesis [2], excessive IL-8 production can be deleterious to the host, and its selective inhibition represents an important therapeutic goal.

Vasoactive intestinal peptide (VIP) is a neuropeptide present in the lymphoid microenvironment that elicits a broad spectrum of biological functions, including actions on natural and acquired immunity [11–15]. Although VIP affects a variety of immune functions, its primary immunomodulatory function is anti-inflammatory in nature. VIP has been shown to inhibit cytokine

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production and proliferation in T cells, and to inhibit several macrophage functions, including phagocytosis, respiratory burst, and chemotaxis [11–15], as well as LPS-induced IL-6, TNFα, IL-12, NO, and chemokine production [16–25]. In agreement with its anti-inflammatory role, VIP was reported to protect mice from lethal endotoxemia, presumably by down-regulating endogenous pro-inflammatory macrophage-derived mediators [26,27].

Since IL-8 is involved in controlling the nature and magnitude of the inflammatory response, in this study we investigate the effect of VIP on the production of IL-8 by activated human monocytes, as well as the specific receptors and the intracellular signal pathways involved. This study further clarifies the role played by VIP in the attenuation of the inflammatory response.

Materials and methods

Reagents. Synthetic VIP, secretin, glucagon, VIP_{1-12}, VIP_{10-28}, and PACAP38 were purchased from Calbiochem–Novabiochem (Laufelfingen, Switzerland). The PAC1/VPAC2-antagonist $PACAP_{6-38}$ was obtained from Peninsula Laboratories (Belmont, CA). The VPAC1-antagonist [Ac-His^1, D-Phe^2, K^{15}, R^{16}, L^{27}] VIP (3–7)-GRF (8–27), the VPAC1-agonist [K^{15}, R^{16}, L^{27}] VIP (1–7)-GRF (8–27), and the VPAC2-agonist Ro 25-1553 Ac-[Glu^8, Lys^{12}, Nle^{17}, Ala^{19}, Asp^{25}, Leu^{26}, $Lys^{27,28}$, $Gly^{29,30}$, Thr^{31}]-VIP cyclo (21–25) were donated by Dr. Patrick Robberecht (Universite Libre de Bruxelles, Belgium). The synthetic PAC1 agonist maxadilan was a gift from Dr. Ethan A. Lerner (Massachusetts General Hospital, Charlestown, MA). Human recombinant TNFα and IL-8, and capture and biotinylated antibodies against human IL-8 were purchased from Pharmingen (San Diego, CA). LPS (from *E. coli* 0111:B4) and forskolin were purchased from Sigma Chemicals (St. Louis, MO), and *N*-[2-(*p*-bromocinnamylamino)ethyl]-5-iso-quinolinesulfonamide (H89) was from ICN Pharmaceuticals (Costa Mesa, CA).

Cell isolation and stimulation. Monocytes, obtained from buffy coast of healthy volunteers, provided by the local blood bank, were isolated as described [28]. In short, heparinized venous blood was mixed 1/1 with 0.9% saline and mononuclear cells were separated by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density gradient centrifugation. Isolated mononuclear cells were washed twice in complete medium and PBM were adherence purified for 1 h (37 °C) at a concentration of 10^6 cells/ml. Cells were >95% viable as assessed by Trypan blue exclusion and consisted of >95% monocytes as determined by morphology and nonspecific esterase staining.

Polymorphonuclear cells (PMN) were prepared as previously described [29]. Briefly, 10 volumes of blood was mixed with 2 volumes of glucose dextran (3% glucose, 3% dextran T250; Pharmacia), and the leukocytes were recovered following a 40-min sedimentation at room temperature. The leukocytes were then diluted 1:2 in complete medium and isolated by Ficoll-Hypaque centrifugation. Contaminating erythrocytes were lysed and the viability of PMN was assessed by counting the cells in 0.1% eosin (viability >96%). PMN were used in the chemotaxis assay.

Monocytes (10^6 cells/ml, 200 μl/well) were cultured in 96-well flat bottomed tissue culture plate (Costar, Cambridge, MA) in RPMI 1640 medium supplemented with 10% human serum (HS; Gibco-BRL), containing 10 mM Hepes buffer, 1 mM pyruvate, 0.1 M nonessential aminoacids, 2 mM glutamine, 50 mM of 2-mercaptoethanol, 100 U/ml penicillin, and 10 μg/ml streptomycin (complete medium). Cells were stimulated with different concentrations of LPS (from 100 pg/ml to 10 μg/ml) or TNFα (10 ng/ml) in the presence or absence of VIP (10^{-8} M) and/or other agents as indicated, for various times at 37 °C in a humidified incubator with 5% CO_2. Culture supernatants were harvested and stored at −20 °C until IL-8 determination by ELISA.

RNA extraction and Northern blot analysis. Northern blot analysis was performed according to standard methods. Monocytes were prepared and stimulated as described above. At the various time points, 1×10^7 cells were harvested and total RNA was extracted by the acid guanidinium–phenol–chloroform method, electrophoresed on 1.2% agarose–formaldehyde gels, transferred to S & S Nytran membranes (Schleicher and Schuell, Keene, NJ), and cross-linked to the nylon membrane using UV light.

The probe for human IL-8 (Oncogene, Cambridge, MA) was end-labeled with [γ-^{32}P]ATP (3000 Ci/mmol, Amersham, Arlington, IL) by using T4 polynucleotide kinase. The RNA-containing membranes were prehybridized for 16 h at 42 °C and then hybridized at 60 °C for 16 h with the appropriate probes. The membranes were then washed twice in 2× SSC containing 0.1% SDS at room temperature (20 min each time), once at 37 °C for 20 min, and once in 0.1× SSC containing 0.1% SDS at 50 °C (20 min). The prehybridization and hybridization buffers were purchased from 5′-Prime–3′ Prime (Boulder, CO). The membranes were exposed to X-ray films (Kodak, Rochester, NY). Signal quantitation was performed in a PhosphorImager SI (Molecular Dynamics, Sunnyvale, CA). To assure equal loading, the membrane was stripped and rehybridized with [^{32}P]ATP-labeled β-actin probe (Stratagene, La Jolla, CA).

IL-8 ELISA. IL-8 levels in culture supernatants were determined using a human IL-8-specific sandwich ELISA (Pharmingen) following the manufacturer's instructions.

In vitro migration assay. PMN migration was evaluated using a 96-well chemotaxis microchamber. Briefly, different dilutions of the supernatants from monocyte cultures (24 h) previously treated with LPS (100 ng/ml) in the presence or absence of VIP (10^{-8} M) were added to the lower wells of the chemotaxis chamber (Neuroprobe, Pleasanton, CA). Complete medium was used as basal control. In some experiments, rhIL-8 (100 ng/ml) was added in the lower chemotaxis chambers, and in others, culture supernatants were neutralized with anti-IL-8 mAb (20 μg/ml). A polycarbonate filter (5-μM pore size, Neuroprobe) was layered onto the wells and covered with a silicon gasket and the top plate. Freshly isolated PMN (1.5×10^6 cells/ml, 50 μl) were seeded in the upper chamber. After 90 min incubation at 37 °C with 5% CO_2, cells transmigrated into the lower chamber were recovered and counted with a FACScalibur for 60 s at a flow rate of 60 μl/min. Results are shown as migration index, which represents the ratio between PMN that migrated to the lower chamber in the presence of IL-8 or monocyte culture supernatants and cells that migrated in response to medium alone.

Results and discussion

The majority of human septic shocks, which are systemic responses to severe bacterial infections resulting in high mortality, are caused by Gram-negative bacterial endotoxins [30]. Indeed, systemic administration of LPS, an integral outer membrane component of Gram-negative bacteria, in experimental animals leads to pathophysiological changes similar to the human septic shock syndrome. The toxic effects of endotoxin are exerted through the generation of endogenous pro-inflammatory cytokines. Systemic exposure to bacterial endotoxins initiates a rapid, coordinated recruitment of neutrophils, monocytes/macrophages, and T cells into specific host tissues [1–3]. The infiltration and activation

of inflammatory leukocytes, together with overproduction of proinflammatory mediators, initiate the tissue damage that precedes multiple organ failure. IL-8 is a human chemokine that plays a crucial role in the pathogenesis of various inflammatory diseases [3], its levels being correlated with the severity of the pathology and/or disease outcome [9,10]. Similar to other anti-inflammatory cytokines such as IL-10 and IL-13 [31,32], exogenous administration of VIP protects mice from the lethal effect of high endotoxemia, presumably by down-regulating the production of proinflammatory mediators such as IL-6, TNFα, IL-12, nitric oxide, and IFNγ [26,27]. The present study investigates a novel property of VIP that might contribute to its anti-inflammatory effects, namely the inhibition of IL-8 production in activated human monocytes.

To determine the effect of VIP on IL-8 production, human monocytes were activated with LPS or TNFα, two potent activators of cells of the monocyte/macrophage cell lineage, in the absence or presence of various doses of VIP, and the amounts of IL-8 released in the culture supernatants were assayed at different time periods. Unstimulated monocytes produce very low amounts of IL-8 (Fig. 1A). However, LPS and TNFα stimulation of monocyte cultures resulted in a time-dependent increase in the production of IL-8, reaching peak levels between 24 and 48 h (Fig. 1A). VIP inhibited in a dose- and time-dependent manner the IL-8 production by activated monocytes (Fig. 1). IL-8 production was significantly inhibited as early as 6 h, with maximum inhibitory effect after 24–48 h of culture (Fig. 1A). The dose–response curve shows a maximal effect around 10^{-8} M (Fig. 1C). This is the dose range in which VIP modulates several other immunological functions [11–25]. The inhibitory effect of this neuropeptide was observed over a wide range of LPS concentrations, showing maximal effect at 10–500 ng/ml LPS (Fig. 1B). Moreover, the reduction of IL-8 production was maintained throughout the 72-h incubation period (data not shown), indicating that VIP does not delay, but rather reduces, IL-8 release.

The inhibitory effects were not the result of a decreased number of monocytes, as VIP did not affect cell numbers or viability of stimulated monocytes after 36 h of culture (viabilities determined by Trypan-blue exclusion were in the range of 89–95% with or without neuropeptide).

In experiments described so far, VIP was added to cells at the same time as LPS. We also investigated the effect of exposing monocytes to VIP after LPS stimulation by adding VIP at different times after (from 0 to 3 h) LPS stimulation. Supernatants were collected 24 h after the initiation of the cultures (LPS stimulation) and were assayed for IL-8 production. The addition of VIP up to 2 h after LPS stimulation resulted in significant levels of inhibition (50%) (Fig. 1D).

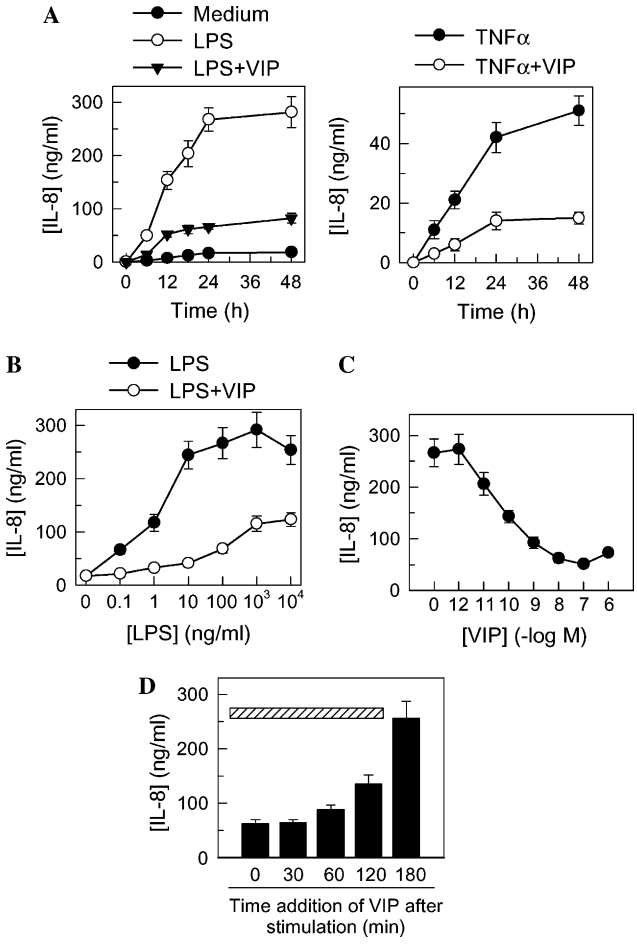

Fig. 1. VIP inhibits LPS- and TNFα-induced IL-8 production in human monocytes. (A) Time-dependent inhibition. Monocytes (10^6 cells/ml) were incubated with medium alone or stimulated with LPS (100 ng/ml) or with TNFα (10 ng/ml), in the absence or presence of 10^{-8} M VIP, and supernatants harvested at different time points were assayed for IL-8 content by ELISA. (B) LPS-dose response. Monocytes were stimulated with different doses of LPS (from 100 pg/ml to 10 μg/ml), in the absence or presence of VIP (10^{-8} M), and 24 h later, IL-8 content was determined in culture supernatants. (C) Dose-dependent inhibition. Monocytes were stimulated with LPS (100 ng/ml) and treated with various concentrations of VIP for 24 h. IL-8 contents in the culture supernatants were determined by ELISA. (D) Effects of delayed addition of VIP on IL-8 production. Monocytes were stimulated with LPS (100 ng/ml) at time 0. VIP (10^{-8} M) was added at different times after the initiation of the cultures. Supernatants were collected 24 h after the initiation of the cultures and assayed for IL-8 production. Dashed horizontal bar represents control values from cultures incubated with LPS alone (266 ± 32 ng/ml). Each result is the mean ± SD of four separate experiments performed in duplicate.

Next we investigated whether the inhibitory effect of VIP is mediated through specific receptors. First, we compared the effect of VIP to that of related peptides, such as pituitary adenylate cyclase-activating polypeptide (PACAP), secretin, glucagon, and the VIP fragments VIP_{1-12} and VIP_{10-28}. Whereas PACAP showed a similar dose-dependent inhibitory effect to that of VIP, IL-8 production was not affected by secretin and glucagon

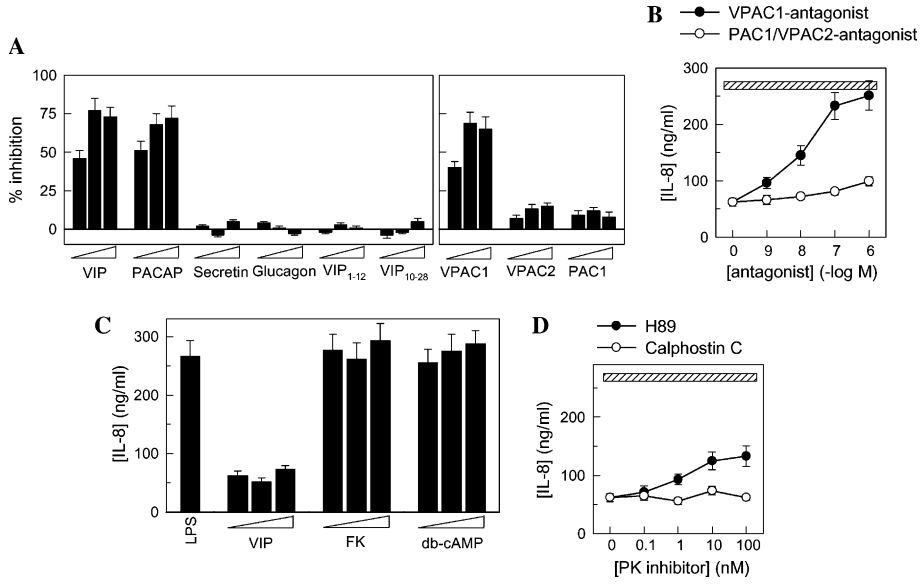

Fig. 2. Receptor and intracellular pathway involved in the VIP inhibition of IL-8 production. (A) Comparative effects of VIP, VIP-related peptides, VIP-fragments, and VIP receptor agonists. Monocytes were stimulated with LPS (100 ng/ml) in the presence or absence of different concentrations (10^{-10}, 10^{-8}, and 10^{-6} M) of VIP, PACAP38, secretin, glucagon, VIP_{1-12}, VIP_{10-28}, maxadilan (a PAC1-agonist), Ro 25–1553 (a VPAC2-agonist), and [K^{15}, R^{16}, L^{27}]VIP(1–7)-GRF(8–27) (a VPAC1-agonist). Supernatants collected 24 h later were assayed for IL-8 production by ELISA. Percentage inhibition was calculated by comparison with controls containing LPS alone. Each result is the mean ± SD of four experiments performed in duplicate. (B) Effect of PAC1- and VPAC-antagonists. Monocytes were stimulated with LPS (100 ng/ml) and treated simultaneously with VIP (10^{-8} M), and different concentrations of the VPAC1-antagonist, [Ac-His^1, D-Phe^2, K^{15}, R^{16}, L^{27}]VIP(3–7)-GRF(8–27), or the PAC1/VPAC2-antagonist ($PACAP_{6-38}$). Supernatants collected 24 h later were assayed for IL-8 production. The VPAC1 antagonist (10^{-6} M) and $PACAP_{6-38}$ (10^{-6} M) did not affect cytokine production in LPS-treated monocytes. Dashed horizontal bar represents control values from cultures incubated with LPS alone. Each result is the mean ± SD of four experiments performed in duplicate. (C) Effect of cAMP-inducing agents. Monocytes were stimulated with LPS (100 ng/ml) in the presence or absence of different concentrations (10^{-8}, 10^{-7} and 10^{-6} M) of VIP, forskolin or db-cAMP. Supernatants collected 24 h later were assayed for IL-8 production. Each result is the mean ± SD of five experiments performed in duplicate. (D) Comparative effects of calphostin C (a PKC-inhibitor) and H89 (a PKA-inhibitor). Monocytes were stimulated with LPS (100 ng/ml), incubated with or without VIP (10^{-8} M), in the absence or presence of different concentrations of calphostin C or H89. Supernatants collected 24 h later were assayed for IL-8 production. The dashed horizontal bar represents control values from cultures incubated with LPS alone. Each result is the mean ± SD of four experiments performed in duplicate.

(Fig. 2A). Similar to the effect on other cytokines such as IL-2, IL-6, TNFα, IL-10, and IL-12 [11–25], the inhibition of IL-8 requires an intact VIP molecule (Fig. 2A). This is in agreement with previous reports showing that either C- or N-terminal truncations of VIP lead to significant losses in biological activity [33,34].

The immunological actions of VIP are exerted through a family of receptors consisting of VPAC1, VPAC2, and PAC1 [11–25]. It has been demonstrated that human monocytes express VIP binding sites [35,36] and RT-PCR analysis indicates that the human monocytic cell line THP1 constitutively expresses both PAC1 and VPAC1 mRNA and VPAC2 mRNA following LPS-stimulation [37]. In order to determine which of the VIP receptors is involved in the inhibition of IL-8 production, we used specific receptor agonists and antagonists. The effects of a VPAC1-agonist [38], a VPAC2 agonist (Ro 25–1553) [39], and of maxadilan, a PAC1 agonist [40], were tested. The VPAC1 agonist, but not the VPAC2 or the PAC1 agonist, inhibited IL-8 release, with a potency similar to that of VIP (Fig. 2A). In addition, we investigated the ability of $PACAP_{6-38}$, an antagonist of PAC1 and, to a lesser degree of VPAC2 [41], and of a specific VPAC1-antagonist [42], to reverse the inhibitory effect of VIP. Increasing concentrations of the antagonists (10^{-9}–10^{-6} M) were added simultaneously with 10^{-8} M VIP. The VPAC1-antagonist reversed the effects of VIP in a dose-dependent manner (Fig. 2B). In contrast, $PACAP_{6-38}$ did not reverse the inhibitory effect (Fig. 2B). Together these results confirm the specificity of the VIP and PACAP inhibitory activity, and indicate that VIP exerts its action primarily through VPAC1. However, the apparent major role of VPAC1 could reflect the balance between VPAC1 and VPAC2 expression. Because VPAC2 is expressed relatively late during monocyte activation (12 h), VPAC1 is probably the major receptor type present during early culture period.

The VPAC1 is coupled primarily to the adenylate cyclase system and VIP increases intracellular cAMP levels in human monocytes [43]. To fully understand the mechanism of the action of VIP, it is important to clarify which transduction pathways are involved in the inhibition of IL-8 in monocytes. We investigated the

effects of calphostin C (a PKC inhibitor), H89 (a PKA inhibitor), forskolin (a strict cAMP inducing agent), and db-cAMP (a cAMP analog). As previously described [44–47], forskolin and db-cAMP did not inhibit IL-8 production in LPS-activated monocytes (Fig. 2C). However, a partial, although minimal, involvement of cAMP in the VIP effect is suggested by the results obtained with the two protein kinase inhibitors. Whereas calphostin C did not affect the inhibitory effect of VIP, H89 induced a slight reversal (Fig. 2D). Similar to VIP, β-adrenergic agonists inhibit IL-8 production by LPS-stimulated THP1 cells, involving increases in cAMP levels [48]. In addition, adenosine that increases intracellular cAMP through stimulation of adenylate cyclase inhibits IL-8 production in monocytes [49], although the exact molecular mechanisms remain to be elucidated. Our results suggest that VIP inhibits IL-8 production in activated monocytes mainly through a cAMP-independent mechanism, and only partially through a cAMP-dependent pathway. We have recently shown that the major cAMP-independent pathway preferentially blocks nuclear translocation of NF-κB and its binding to the kB site of the IL-8 promoter, and that the minor cAMP-dependent pathway inhibits the activation and binding to the IL-8 promoter of both CREB-binding protein (CBP) and TATA box-binding protein (TBP), two transcriptional cofactors strictly required for transactivating activity of NF-κB (Delgado and Ganea, submitted). The involvement of both cAMP-dependent and cAMP-independent pathways was shown previously for the inhibitory effect of VIP on TNFα, IL-12, chemokine, and nitric oxide production in macrophages [16,17,19,21–25], on TNFα production in monocytes [37], and on IL-2 and IL-10 production in lymphocytes [50]. However, the relative involvement of these pathways in the later cases and in the IL-8 production is different. Whereas the effect of VIP on TNFα, IL-12, chemokine, and nitric oxide production is mediated primarily through the cAMP-dependent pathway, the major mediator in the inhibition of IL-8 production is cAMP-independent. The nature of the cAMP-independent transduction pathway remains to be determined.

Having demonstrated that VIP inhibits IL-8 production, we sought to determine whether this action occurs at a transcriptional level. We stimulated human monocytes with LPS in the presence or absence of 10^{-8} M VIP for 2, 4, 8, and 12 h, and total RNA was prepared and subjected to Northern blot analysis. Although very little IL-8 mRNA is detectable in unstimulated cells, progressively increased levels of IL-8 mRNA are present in LPS-stimulated cells (Fig. 3). At all time points, VIP inhibited the levels of IL-8 mRNA (Fig. 3), with a maximum effect at 4 and 8 h. These results indicate that VIP inhibits IL-8 steady-state mRNA levels. The precise molecular mechanisms that account for the VIP inhibition of IL-8 expression are largely unknown, and it

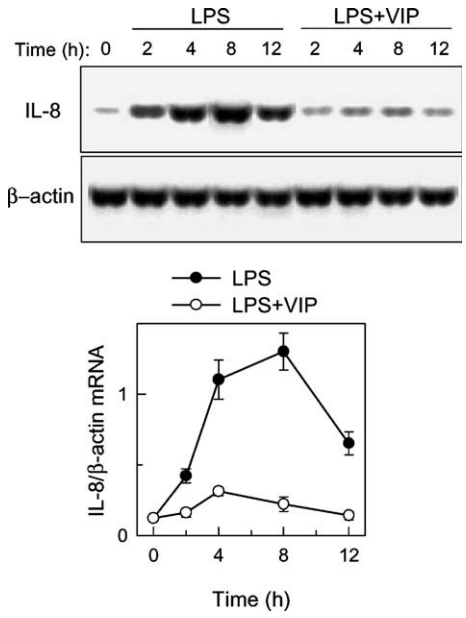

Fig. 3. VIP regulates IL-8 mRNA expression by LPS-stimulated monocytes. Monocytes were stimulated with LPS (100 ng/ml) in the presence or absence 10^{-8} M VIP. At different time points, the cells were lysed and subjected IL-8 to Northern blot analysis. Results (means ± SD of two samples performed in duplicate) are expressed in arbitrary densitometric units normalized for the expression of β-actin in each sample.

remains to be established whether the reduction in steady-state IL-8 mRNA levels results from a decrease in de novo transcriptional rate, message stabilization, or both. However, some evidence points to a direct effect of VIP on the novo transcription as the most likely possibility. First, delayed treatment with VIP did not affect IL-8 production, suggesting that VIP inhibits an early event in IL-8 expression. Second, VIP inhibits LPS- and TNFα-induced IL-8 promoter gene activation (Delgado and Ganea, submitted).

In view of the chemotactic action of IL-8 toward leukocytes, especially neutrophils, we investigated whether monocyte cell-free conditioned medium from LPS-stimulated cultures in the presence or absence of VIP contained such chemotactic activities. As shown in Fig. 4, supernatants from monocytes cultured for 24 h in the presence of 100 ng/ml LPS were highly chemotactic for human neutrophils. The chemotactic activity decreased upon dilution of the supernatants (not shown). The chemotactic activity is mainly due to the presence of IL-8, because neutralizing anti-IL-8 Abs almost completely abrogated the chemotactic activity, and addition of rhIL-8 did not significantly increase the chemotactic activity (Fig. 4). In contrast, conditioned medium from LPS-stimulated monocytes cultured in the presence of VIP exhibited a much lower chemotactic activity (Fig. 4). These results show a strong correlation between the VIP-mediated inhibition of IL-8 production and the chemotactic activity for neutrophils. Although

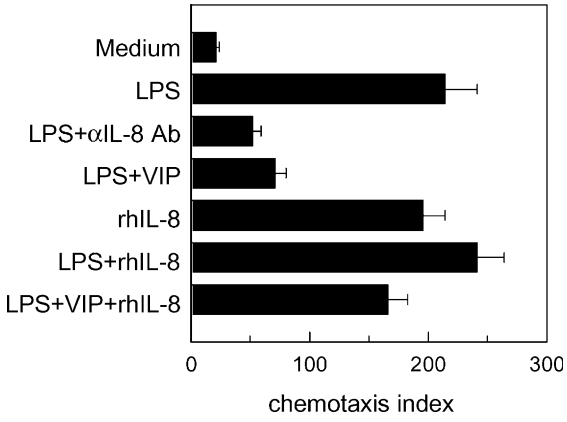

Fig. 4. VIP inhibits monocyte-induced chemotactic activity for human neutrophils. Monocytes were stimulated with LPS (100 ng/ml) in the presence or absence of VIP (10^{-8} M) or neutralizing anti-IL-8 Ab (20 ng/ml). Supernatants collected after 24 h were seeded in lower wells of 96-well chemotaxis microchamber, and chemotaxis of freshly isolated human PMN cells was assayed at 37 °C for 90 min as described in Materials and methods. Complete medium instead of culture supernatants was used as basal control (medium). Some culture supernatants were supplemented with rhIL-8 (50 ng/ml). Results are expressed as means ± SD of triplicate determinations of one representative experiment of four.

the involvement of other monocyte-derived chemokines beside IL-8 cannot be excluded, since VIP inhibits the production of several chemokines (MIP-1α, MIP-1β, RANTES, KC, MIP-2, and MCP-1) by activated macrophages [25], IL-8 appears to play a major role. Indeed, addition of rhIL-8 to conditioned medium from LPS-stimulated monocytes cultured in the presence of VIP restores almost completely the chemotactic activity (Fig. 4). Inflammatory agents such as nitric oxide induce VIP release from the innervation; in addition, immune cells, particularly antigen-stimulated Th2 cells, secrete functional VIP [51]. In this context, we propose that VIP derived from local sources, including newly recruited $CD4^+$ T cells, inhibits in vivo IL-8 production from activated monocytes. This results in a reduced influx of neutrophils. In addition, since low concentrations of IL-8 have been shown to be more potent lymphocyte than neutrophil chemoattractants [1–3], VIP could switch the cellular nature of the ongoing inflammatory response from a predominant acute neutrophilic influx to a more chronic accumulation of mononuclear cells.

Collectively, the present findings support a novel role for VIP as a key anti-inflammatory agent in vivo: as a result of the VIP-mediated inhibition of LPS-induced IL-8 production, the neutrophil infiltration, and subsequent release of proinflammatory mediators, into the inflammatory site would to be attenuated. In addition, the inhibition of IL-8 production by VIP may have therapeutic potential, because excessive IL-8 production has been implicated in the tissue damages characteristic for several inflammatory and autoimmune diseases, such as sepsis, arthritis, and neuroimmunologic disorders.

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