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REVIEW

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# Vasoactive intestinal peptide in the immune system: potential therapeutic role in inflammatory and autoimmune diseases

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**Abstract** Vasoactive intestinal peptide (VIP), a neuropeptide that is produced by lymphoid as well as neural cells, exerts a wide spectrum of immunological functions, controlling the homeostasis of the immune system through different receptors expressed in various immunocompetent cells. In the last decade, VIP has been clearly identified as a potent anti-inflammatory factor, which acts by regulating the production of both anti- and pro-inflammatory mediators. In this sense, VIP has been described to prevent death by septic shock, an acute inflammatory disease with a high mortality. In addition, VIP regulates the expression of co-stimulatory molecules, this being an action that may be related to modulating the shift toward Th1 and Th2 differentiation. We have recently reported that VIP prevents the deleterious effects of an experimental model of rheumatoid arthritis, by downregulating both inflammatory and autoimmune components of the disease. Therefore, VIP has been proposed as a promising candidate alternative treatment for acute and chronic inflammatory and autoimmune diseases such as septic shock, arthritis, multiple sclerosis, Crohn disease, or autoimmune diabetes.

**Keywords** Vasoactive intestinal peptide · Inflammation · Autoimmunity · Arthritis · Endotoxic shock · Neuroimmunology

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## Introduction

The inflammatory process is vital to the survival of all complex organisms, and it plays a profound role in health and disease. Although as a localized protective response it serves to destroy the injurious agent, the sustained production of inflammatory mediators can lead to serious pathological conditions. The accumulation and subsequent activation of leukocytes are central events in the pathogenesis of virtually all forms of inflammation. The elimination of foreign material proceeds by a series of integrated steps, the first being the recognition of the agent.

Inflammatory responses are self-controlled by anti-inflammatory mediators secreted during the ongoing process. The ability to control an inflammatory state depends on the local balance between pro- and anti-inflammatory factors. An insufficient response could compromise the viability of the organism, while an excessive re-

sponse could lead to pathological conditions such as systemic inflammatory response syndrome or inflammatory bowel disease [1, 2, 3]. Inflammation is also a key aspect of autoimmunity. It is believed that diseases such as multiple sclerosis, rheumatoid arthritis, and insulin-dependent diabetes mellitus are initiated by a small number of antigen-specific T cells that recognize a tissue antigen. Additional cells are recruited to the site, namely T cells, B cells, macrophages, and other antigen-presenting cells, that can also activate resident cells [4, 5, 6]. The role of inflammation in autoimmune diseases has been generally thought of as a tissue-destructive one.

Current strategies for the treatment of inflammatory and autoimmune diseases have focused on blocking individually the main players of the inflammatory processes. Anti-inflammatory cytokines, such as interleukin-10 (IL-10), antibodies that neutralize pro-inflammatory cytokines, such as anti-tumor necrosis factor (TNF), and agents targeted to prevent gene activation of inflammatory mediators, such as corticosteroids and nonsteroidal anti-inflammatory drugs, have been used to block the actions of cytokines and chemokines, the expression of cell adhesion molecules, and the recruitment of inflammatory cells [7, 8, 9, 10]. However, the tissue specificity of each inflammatory process, its multistep nature, and the cellular and molecular heterogeneity of the different pathological conditions have yielded controversial results. In the last decade, neuropeptides, hormones, and other components of the neuroendocrine system, which are also produced by cells of the immune system, have been included in the list of potential candidates to treat inflammatory and autoimmune disorders [11, 12, 13].

The circuit between the three main systems involved in homeostasis, nervous, endocrine, and immune systems, is well established. An important feature of this circuit is that the involved cells that synthesize and secrete common substances and share the same receptors reduce traditional differences between neurotransmitters, hormones, and immune mediators. To date, we know of at least 27 different neuroendocrine mediators that could be produced by cells of central and peripheral lymphoid organs, with the most-recent members being the glucocorticoids [14], calcitonin gene-related peptide (CGRP) [15, 16], and pituitary adenylate cyclase-activating polypeptide (PACAP) ([17]; Abad et al., submitted for publication).

## VIP is secreted by lymphocytes

Vasoactive intestinal peptide (VIP) was isolated for the first time from the porcine duodenum in 1970 [18], and in 1974, Mutt and Said [19] established its amino acid sequence. VIP contains 28 amino acid residues, with a highly conserved sequence in vertebrates, a fact that is consistent with its important biological role [20]. Today, it is known that VIP is a pleiotropic peptide produced by neurons in different areas of the central and peripheral nervous system [21, 22] and by endocrine cells such as the pituitary lactotrophes and cells of the endocrine pancreas [23]. Moreover VIP is also present in inflammatory and immune cells [24, 25, 26]. Thus, VIP is one of the signal molecules of the neuroendocrine-immune network. In the immune system our first evidence of the role of VIP in immunity was the demonstration of VIP immunoreactivity in the cytoplasm of lymphocytes [25]. These results were later confirmed by the biochemical characterization of VIP by reverse-phase high-performance liquid chromatography and radioimmunoassay in lymphoid cell suspensions [27]. VIP mRNA expression was also demonstrated by in situ hybridization and reverse transcriptase polymerase chain reaction in T and B lymphocytes, and in double- and single-positive thymocytes [28, 29, 30]. Finally, we developed a specific enzyme-linked immunosorbent assay in order to demonstrate that VIP produced by lymphocytes is secreted in central and peripheral lymphoid organs. Our results showed for the first time that agents that mediate important immune functions, such as proliferation and antigen stimulation [concanavalin A, lipopolysaccharide (LPS), and anti-T cell receptor (TCR) antibody], inflammation (LPS, TNF$\alpha$, IL-6, and IL-1$\beta$),or apoptosis (dexamethasone) induced the production and release of VIP to the lymphoid microenvironment [31]. More recently, it has been demonstrated that VIP is preferentially produced by type 2 T cells [32].

## VIP receptors in immune cells

VIP, together with PACAP, secretin, and GRF receptors, constitute a subfamily based on the homology of both ligands and receptors. To date, three VIP/PACAP receptors have been identified that are membrane-bound receptors belonging to the family 2 of G protein-coupled receptors (GPCR) [33]. The six families of GPCR have a common central domain constituted of seven transmembrane helices. The family 2 is characterized by a large *N*-terminal domain, which plays an important role in the binding of the ligand [34]. For VIP receptors, both extracellular and transmembrane domains are also involved [35, 36]. The three VIP/PACAP receptors cloned are the VPAC1 and VPAC2 receptors that bind VIP and PACAP with equal affinity [37, 38], and the PAC1 receptor that is PACAP selective. In micromolar concentration VIP is a heterologous ligand [39], with eight variants to date produced from alternative splicing of the transcript [40, 41, 42]. VPAC1, VPAC2, and PAC1 receptors stimulate primarily the adenylate cyclase (AC) pathway. In addition, it has been demonstrated that only in transfected cell systems, VPAC1, at high expression levels, may stimulate the inositol triphosphate (IP)/PLC system [43], and can increase intracellular calcium levels [44]. There is also some evidence, in a transfected cell system, that VPAC2 stimulates IP synthesis [45], but a clear link to the PLC/IP system remains to be found. Seven splice variants of the PAC1 receptor are involved in the activation of both AC and IP/PLC systems, and the eighth

PAC1 receptor variant, which is also a CGRP receptor, is not linked to AC or IP/PLC systems, but activates an L-type calcium channel [46]. VPAC1, VPAC2, and PAC1 receptors are expressed in different cell populations in both central nervous system and peripheral tissues [47, 48].

### VPAC1 receptor

In the immune system, the first VIP receptors were identified using binding techniques in human peripheral blood lymphocytes [49], human monocytes [50], murine lymphocytes [51, 52], rat alveolar macrophages [53], and murine peritoneal macrophages [54, 55]. We recently studied the expression and distribution of VIP/PACAP receptors in different immune cell sub-populations, describing, for the first time, the gene expression of VPAC1 in murine isolated thymocytes and T and B lymphocytes from spleen and lymph nodes [56]. We also demonstrated VPAC1 gene expression in freshly double-positive (CD4+ CD8+) and single-positive thymocyte subsets, whereas double-negative subsets lack VPAC1 receptor expression [57]. Moreover, we have described VPAC1 gene expression in lymphocytes and macrophages from peritoneal suspensions [58].

### VPAC2 receptor

This receptor was described for the first time as a VIP/helodermin-preferring receptor in the human lymphoma cell line SUP T1 [59]. Data from our laboratory have demonstrated that in contrast to VPAC1, which is expressed constitutively in lymphocytes and macrophages, VPAC2 expression is inducible in these cells. Thus, VPAC2 is detected only following stimulation through the TCR-associated CD3 molecule in lymphocytes [60] or LPS in macrophages [61]. Moreover, VPAC2 receptor is detected in mononuclear cells by immunohistochemical techniques 2 days after the detection of VPAC1 at sites of inflammation and antigen recognition [62]. However, VPAC2 receptor is the only receptor of the VIP/PACAP receptor family expressed in some murine T cell lines [63], and its constitutive expression has been reported in human lymphoid cell lines [64]. The best-characterized VIP effects exerted through interaction with VPAC1 and VPAC2 in the immune system are mediated by the AC pathway.

### PAC1 receptor

This receptor is expressed only in macrophages, as lymphocytes lack its expression [61, 65]. Although PAC1 receptor is the PACAP-selective receptor that binds PACAP with higher affinity (100–1,000×) than VIP [39], especially in the central nervous system, the PAC1 expressed in freshly isolated macrophages possesses similar affinity for both VIP and PACAP, and is coupled to the IP/PLC system [65]. The splice variant of PAC1 receptor present in these important cells of the immune system must be elucidated. Moreover, different lines of evidence support the notion that new members of the VIP/PACAP receptor family are waiting to be discovered.

## VIP is a natural anti-inflammatory factor

Inflammatory stimuli such as bacterial products activate macrophages and other cells to produce pro-inflammatory cytokines, chemokines, adhesion molecules, and enzymes [66]. Critical in gene expression during the response are nuclear transcription and coactivator factors such as NF-κB, AP-1, CREB-binding protein (CBP), and others [67, 68]. The functional status of each factor, leading to gene expression, is regulated by complex transduction systems controlled by extracellular stimuli. Anti-inflammatory cytokines, such as IL-10, transforming growth factor-β, or IL-1Ra, eicosanoids, and neuropeptides counteract the inflammatory stimuli, inhibiting the production of pro-inflammatory factors and overcoming the inflammatory response [69, 70, 71, 72, 73].

Although VIP has been extensively reported to regulate a wide variety of functions, participating in the control of the homeostasis of the immune system [13], its major role is probably as a potent anti-inflammatory factor. The anti-inflammatory properties of VIP are exemplified by its ability to inhibit several macrophage functions, including phagocytosis, respiratory burst, and chemotaxis [74], and also by inhibiting T cell proliferation and decreasing lymphocyte migration [75]. VIP has also been reported to inhibit the production of pro-inflammatory cytokines such as TNFα, interferon-γ (IFNγ), IL-6, and IL-12 [61, 76, 77, 78], to reduce the activity of inducible nitric oxide synthase (NOS) [78], and to enhance the production of the anti-inflammatory cytokines IL-10 and IL-1Ra [79, 80].

The anti-inflammatory properties of VIP represent the coordinated action of the three types of receptors present on both macrophages and lymphocytes, each associated with specific transduction systems and differential gene expression. Figure 1 represents the different receptors, transduction pathways, and cytokines implicated. VIP inhibition of TNFα, IL-12, and inducible NOS (iNOS) is mediated through VPAC1 receptors and, to a lesser degree, also through VPAC2 receptors [81]. cAMP-activated protein kinase A (PKA) plays a major role in this inhibition, but a cAMP-independent pathway is also involved. However, the inhibition of IL-6 is mediated primarily through PAC1 receptors present in macrophages, and is mediated by protein kinase C (PKC) [77]. On the other hand, VIP stimulates the production of the anti-inflammatory cytokine IL-10 by macrophages through its action on VPAC1 receptors in a cAMP-dependent pathway [79].

The cAMP-independent pathway associated with the VPAC1 receptors leads to the inhibition of NF-κB nu-

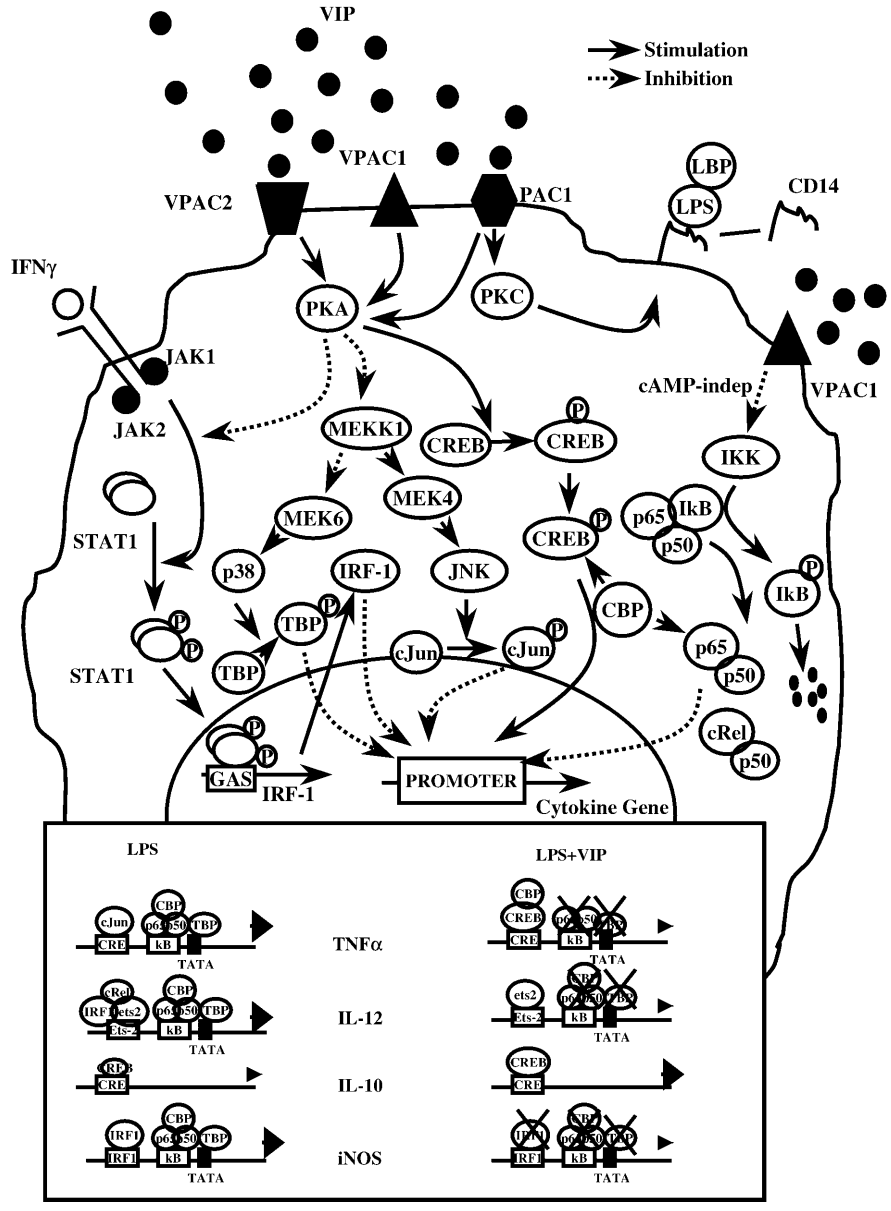


**Fig. 1** Vasoactive intestinal peptide (*VIP*) exerts its anti-inflammatory action through various molecular mechanisms. VIP binds to the VPAC1 receptor on macrophages and activates both the cAMP/PKA pathway, and a cAMP/PKA-independent pathway. Interferon-γ (*IFNγ*) initiates the Jak1/Jak2-STAT1 pathway resulting in the generation of phosphorylated STAT1 dimers, their translocation to the nucleus, and subsequent binding to the GAS site in the IRF-1 promoter. VIP, through cAMP/PKA activation, prevents IRF-1 transcription by inhibiting the phosphorylation of Jak1/2 and STAT1. The intermediary between PKA and Jak1/2 phosphorylation is not known. On the other hand, lipopolysaccharide (*LPS*) activates the MEKK1/MK4/JNK mitogen-activated kinase pathway leading to the phosphorylation of c-Jun, and subsequent activation as a transcriptional factor. VIP inhibits, in a cAMP-dependent manner, the MEKK1/MEK4/JNK pathway and c-Jun phosphorylation. In addition, VIP activates JunB through the cAMP/PKA pathway. Replacement of c-Jun with JunB leads to an inactive transcriptional complex for many of the macrophage-derived cytokines. The cAMP-independent pathway stabilizes IkB by inhibiting the kinase activity of IKKa. The stabilized IkB sequesters the p65/p50 complexes in the cytoplasm. This results in a decreased NF-kB binding to promoters. The cAMP-dependent pathway phosphorylates CREB, leading to its nuclear translocation and subsequent binding to CBP (CREB-binding protein). In the absence of the coactivator CBP, the transcriptional complexes are not fully active. In addition, the binding of activated CREB to IL-10 promoter activates its transcription. Moreover, by inhibiting the MEKK1/MEK3/6/p38 pathway, VIP reduces the phosphorylation of TBP (TATA-box binding protein), resulting in a reduced recruitment of RNA polymerase II

clear translocation in stimulated macrophages, and the subsequent inhibition of the transcription of TNFα, IL-12, and iNOS [73, 82]. The cAMP-dependent pathway is associated with the absence of interferon regulatory factor (IRF-1) in the Ets-2 site of the promoter of the IL-12 p40 gene [83]. This is correlated with a decreased transcription of the inducible component of IL-12 p40. Since IL-12 enhances IFNγ secretion by T lymphocytes, VIP indirectly reduces the secretion of this macrophage-activating cytokine [61]. IRF-1 synthesis

is also inhibited by VIP through the cAMP-dependent pathway that interferes with the Jack/STAT1 pathway [84]. The cAMP-dependent pathway is also implicated in a change in the transactivator complex associated with the cAMP response element (CRE) located at the TNFα promoter. This element is necessary for maximal induction of the TNFα gene. VIP reduces cJun binding at this level through the inhibition of the MEKK1/MEK4/JNK signaling pathway by PKA activation [85, 86].

Since other molecules implicated in inflammatory responses, such as chemokines and adhesion molecules, are controlled by NF-κB, these molecules can also be targets for VIP regulation. Chemokines are essential for leukocyte trafficking and inflammatory processes. Their involvement in such processes further suggests that the chemokines are important players in inflammatory disorders. The selective chemoattractant activities for macrophage and lymphocyte subsets make them ideal candidates to play a key role in leukocyte trafficking, getting the correct sub-population of cells to migrate into the tissues [87]. Recently VIP has been shown to reduce chemokine production by activated macrophages [88]. The CXC chemokine MIP-2, which induces the migration of polymorphonuclear leukocytes, and the CC chemokines MIP-1α, MIP-1β, MCP-1, and RANTES, which are chemotactic for monocyte/macrophages and T cells, were inhibited in a long-lasting way. Inhibition has also been shown in vivo [80, 88]. In a murine model of abdominal acute inflammation, VIP reduced the migration of polymorphonuclear leukocytes, macrophages, and lymphocytes to the peritoneal cavity, presumably through the inhibition of chemokine production [88]. The inhibition of cellular recruitment to sites of inflammation may also hamper the possible in situ antigen presentation that may contribute to exacerbation of clinical signs in chronic inflammation and reactivity to these antigens in autoimmune disorders.

Because of its anti-inflammatory properties, VIP has been reported to protect against several inflammatory pathological conditions, such as toxic shock syndrome and rheumatoid arthritis (RA). TNFα and IL-6 are overproduced in these states and VIP has been shown to reduce the circulating levels of these cytokines in animal models of such pathologies [89]. Exogenous administration of VIP protects mice from the lethal effects of high endotoxemia, presumably by downregulating the pro-inflammatory mediators such as TNFα, IFNγ, IL-6, IL-12, and NO [81]. Probably due to its pleiotropic effects of inhibiting pro-inflammatory mediators that appear later during the inflammatory response, VIP protects against endotoxemia if given 2 h after endotoxin injection, and also ameliorates rheumatoid symptoms in animals with an advanced state of pathology. So, VIP may represent a better biological therapeutic alternative than other agents such as anti-inflammatory cytokines or anti-TNFα antibodies, which are only effective in the early states of these pathologies or have no effect on other inflammatory mediators [13].

## VIP is a natural anti-arthritic factor

RA is a chronic and debilitating autoimmune disease of unknown etiology that leads to chronic, progressive, and symmetrical inflammation in the joints and subsequent erosive destruction of the cartilage and bone. The drugs and agents currently used to treat RA have multiple effects, some of which are undesirable, and in the long term, some of them do not prevent joint damage. In order to find therapeutic alternatives, several strategies have been designed based on the two deleterious aspects of RA, i.e., inflammation and autoimmunity.

The majority of treatments of RA have been developed to try to decrease chronic joint inflammation, which is a multifactorial response dependent upon both regulatory cytokines and proinflammatory chemokines. Therefore, agents that inhibit secretion of inflammatory mediators, especially TNFα, or that can block their binding to cell surface receptors, are increasingly viewed as potential therapeutic agents that might provide increased specificity compared with traditional drugs [90].

An alternative therapeutic strategy that has been explored in RA is the alteration of the T cell response, and CD4 T cells in particular have been targeted for immunotherapy. The balance of Th1-/Th2-type cytokines may play a significant role in the regulation of autoimmune diseases. Although the contribution of Th1 and Th2 responses in RA is not completely understood, several studies in animal models revealed that the Th1 cytokine profile predominates at the induction and acute phases of the disease, whereas Th2-mediated responses are associated with the remission phase of the disease [91, 92, 93, 94, 95]. This suggests a pathogenic role of Th1-derived cytokines.

Since a specific causative agent or antigen has not yet been identified, bypassing the potential antigen and targeting the cytokine imbalance might represent a solid way to control RA.

Because VIP has been clearly identified as a potent anti-inflammatory factor, and some evidence indicates that VIP preferentially induces differentiation toward a Th2 response following antigen stimulation [96], VIP has emerged as a very attractive candidate for the treatment of arthritis. In fact, in a recent report [80], by using collagen-induced arthritis (CIA), a murine experimental model induced by immunization with type II collagen (CII), which shares a number of common clinical, histological, and immunological features with human RA, we have demonstrated that treatment of arthritic mice with VIP decreases the frequency of arthritis, delaying the onset, reducing the severity of arthritic symptoms, and preventing joint damage (Fig. 2). The therapeutic effect of VIP on arthritis is associated with a striking reduction of the two deleterious components of the disease, i.e., autoimmune and inflammatory responses.

The effect of VIP on the autoimmune component of arthritis consists of the reduction in the titers of autoreactive antibodies against CII (particularly IgG2a antibodies), a major factor in determining susceptibility to CIA.

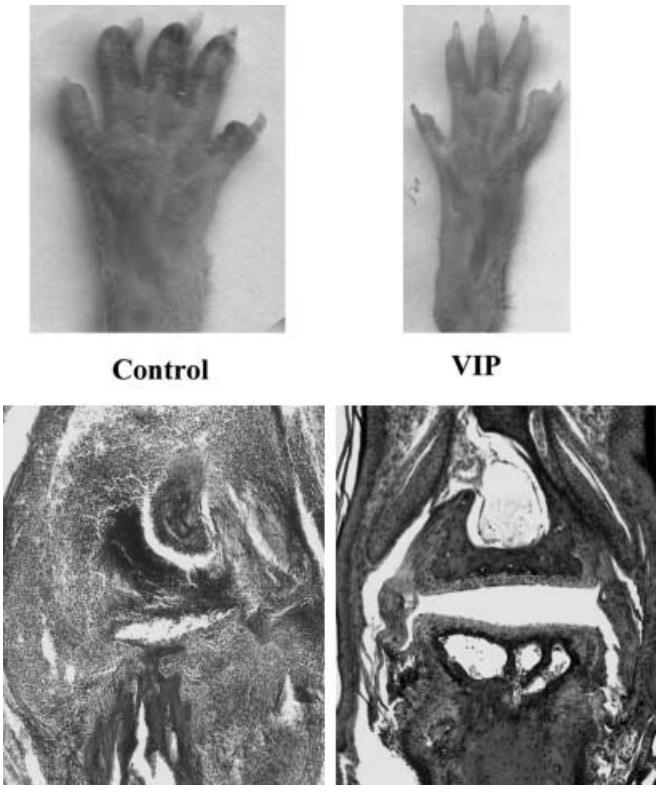


**Fig. 2** VIP is an anti-arthritic factor. The swelling and edema of paws of arthritic mice (controls) in comparison with arthritic mice treated with VIP that do not show any symptoms of the disease. Histological sections (*lower panels*) show that arthritic mice have a massive inflammatory infiltration in the joint, with an almost complete destruction of bone and cartilage, in comparison with VIP-treated mice that show a normal morphology of the joint

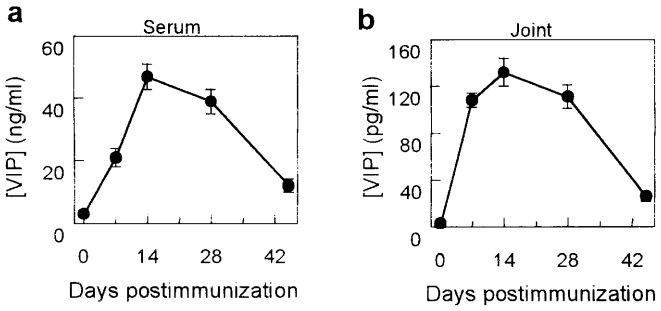


**Fig. 3a, b** VIP is an anti-arthritic factor endogenously produced in response to disease development

This is the result of a decreased CII-specific T cell response, accompanied by a specific shift in the Th phenotype from Th1- toward Th2-type response, with the subsequent inhibition of IFNγ and stimulation of IL-4 production.

VIP strongly reduces the inflammatory response during the development of arthritis by downregulating the production of several pro-inflammatory agents in inflamed joints and synovial cells, including TNFα, IL-6, IL-1β, iNOS, IL-12, and IL-18, as well as various chemokines (RANTES, MCP-1, MIP-1α, MIP-1β, MIP-2) that have been reported to participate in inflammation and in the development of arthritic responses [90]. In addition, VIP increases production of the anti-inflammatory cytokines IL-10 and IL-1Ra, which have been reported to ameliorate arthritis symptoms [90]. Chemokines are responsible for the infiltration and activation of various leukocyte populations in joint tissue, which are contributing factors to pannus development and the subsequent pathology of RA. By inhibiting chemokine production, VIP could prevent leukocyte infiltration in the synovium of arthritic mice. The capacity of VIP to regulate a wide spectrum of inflammatory mediators may offer an advantage against neutralizing antibodies and receptor antagonists directed against a single cytokine.

The ameliorative effect of VIP treatment on cartilage destruction and bone erosion has to be attributed, at least partially, to its inhibitory effect on expression and activity of some matrix metalloproteinases (MMP), which in addition to proinflammatory cytokines, such as TNFα and IL-1β, have pivotal roles in the depletion of proteoglycan and collagen observed in the joints, which leads to the cartilage and bone erosion in patients with RA [97].

The rheumatoid synovium shows hyperplasia of fibroblast-like synovial lining cells and is infiltrated with various mononuclear cells, including macrophages and predominately T lymphocytes. Although the involvement of other cells can not be ruled out, macrophages and synoviocyte cells seem to be the target cells through which VIP exerts its effects on arthritis.

Of obvious biological significance is the fact that VIP levels, like those of other recently described anti-arthritic neuropeptides and hormones, such as CGRP and melanocyte-stimulating hormone (α-MSH) [98, 99], are specifically increased in the serum and joints of arthritic mice during the development of the disease (Fig. 3), suggesting that endogenous neuroimmune factors form a clear natural anti-arthritic machinery that is activated in response to autoimmune/inflammatory conditions, such as arthritis, in an attempt to counterbalance the effects of inflammatory mediators. Nevertheless, arthritis-induced endogenous VIP levels are two to three orders of magnitude lower than the concentrations of protective exogenous VIP. We propose that during a normal immune response, the timely production and release of VIP and other neuroimmune factors within the lymphoid microenvironment following antigenic/inflammatory stimulation serves to downregulate the ongoing immune/inflammatory response, mostly through modulation of cytokine production. During arthritis, however, due to severe inflammation and overstimulation of the immune system, the effect of checkpoint factors such as VIP, PACAP, CGRP, αMSH, and other anti-inflammatory mediators, including IL-10, IL-1Ra, and IL-13, is overwhelmed by the inflammatory cytokine network. However, based on the protective effect of these factors in different arthritic disorders, the exogenous administration of these anti-inflammatory mediators could offer an alternative to existing treatments for arthritis and other inflmmatory/Th1

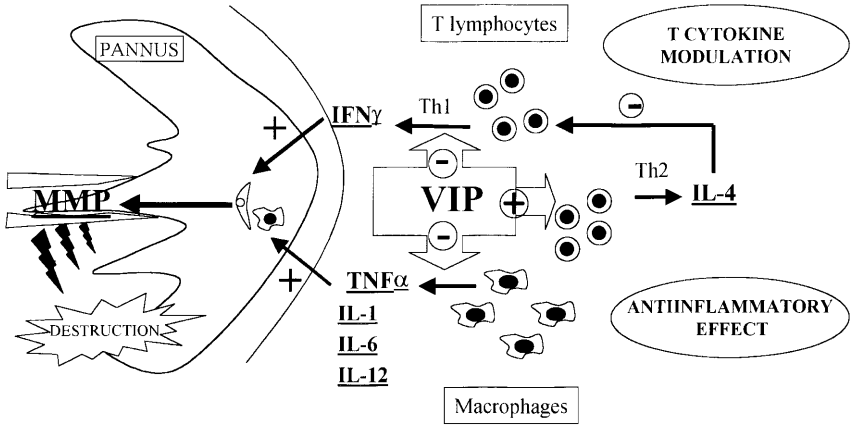


**Fig. 4** VIP prevents arthritis by downregulating both components of the disease, i.e., inflammation and autoimmunity, affecting both macrophage and T cell function

autoimmune diseases, such as multiple sclerosis, inflammatory bowel diseases, or autoimmune diabetes.

Unfortunately, animals do not develop RA, and in a comment on our recent work [80], Firestein states [100] that "although these results are encouraging, it requires a leap of faith to extrapolate our findings to clinic, and there could be some pitfalls in this approach, such as side effects to chronic administration of VIP, including detrimental gastrointestinal effects and a general immunosuppression". However, in our experimental system, we did not observe any adverse effects of the neuropeptide. The fact that VIP reshapes both the autoimmune and inflammatory responses (Fig. 4) presents an advantage over existing treatments for RA. Extending the use of VIP to humans will depend on the dosage and means of administration. In addition, VIP gene transfer could be an attractive means of delivering consistent, prolonged therapeutic titers of this anti-inflammatory factor with fewer side effects and without the need for repeated administrations.

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