Therapeutic approaches of vasoactive intestinal peptide as an immunomodulatory cytokine

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ABSTRACT

Vasoactive intestinal peptide (VIP) is a neuropeptide produced by the lymphoid cells, which exerts a wide spectrum of immunological functions controlling the homeostasis of immune system through different receptors expressed in various immunocompetent cells. During the last decade, VIP has been clearly identified as a potent anti-inflammatory factor, both in innate and adaptive immunity. In innate immunity, VIP inhibits the production of pro-inflammatory cytokines and chemokines from macrophages, microglia and dendritic cells. In addition, VIP reduces the expression of costimulatory molecules on the antigen-presenting cells, and therefore reduces the activation of antigen-specific CD4+ T cells. In terms of adaptive immunity, VIP promotes Th2-type responses, and reduces the pro-inflammatory Th1-type responses. Several of the molecular mechanisms involved in the inhibition of cytokine and chemokine expression, and in the preferential development and/or survival of Th2 effectors, are perfectly known. Therefore, VIP and its analogues have been recently proposed as very promising candidates, alternative to other existing treatments, for treating acute and chronic inflammatory and autoimmune diseases, such as septic shock, rheumatoid arthritis, multiple sclerosis, Parkinson’s disease, Crohn’s disease, or autoimmune diabetes. The aim of this review is firstly to update our knowledge of the cellular and molecular events relevant to VIP function on the immune system; and secondly to gather together recent data that support its role as a type 2 cytokine. Recognition of the central functions that VIP plays in cellular processes is focusing our attention on this «very important peptide» as exciting new candidates for therapeutic intervention and drug development.

KEY WORDS: Neuroimmunology / Inflammation / Autoimmunity / Neuropeptides / T helper cells / Dendritic cells / Macrophages / Apoptosis.
INTRODUCTION

For many years immunologists have considered an immune response to be a direct consequence of, and solely determined by, antigenic stimulation of an autonomous immune system. This clearly pointed out the key molecular questions for experimental investigation. However, in the past two decades, we have come to realize that the immune system is regulated by the central nervous system either directly, or by way of either the neuroendocrine axis, (the hypothalamic-pituitary-adrenal system, HPA axis), or the autonomic nervous system. The importance of these interactions is underscored by the demonstration that immune and nervous interactions, including neuropeptides, cytokines and specific immunomodulatory enzymes, have been well conserved during the course of evolution. Neuroimmunology is now sufficiently mature in facts to begin to consider organization, workings, behaviour, and applications. A crucial factor for the functioning of this intimate bi-directional network was the demonstration that the immune and neuroendocrine systems speak a mutual biochemical language. This reduces traditional differences between neurotransmitters, hormones and immune mediators, and raises the question of what can be actually considered as immune or neuroendocrine? More important, the final outcome will most probably lead to new unifying ideas and paradigms. The present developmental stage in neuroimmunology in general, and in VIP research in particular, can be characterized as a transition from analysis (the reduction of observations to key molecules) to synthesis (the integration of the parts into a whole). The difficulty to answer this question starts when we try to exactly define the terms neuropeptide and cytokine. De Wied coined the term neuropeptide in 1974 defining it as endogenous substances synthesized by nerve cells and involved in nervous system functions. However, neuropeptide has been later defined as any peptide, independently of the cell source, that has an action on the nervous system, and also as any peptide secreted from a neurone. Although the de Wied’s definition appears to correspond to that accepted by most neuroscientists, the discrepancy is served.

Cytokines, on the other hand, are defined as secreted small regulatory proteins that control the survival, growth, differentiation and effector function of tissue cells. Originally identified as being important in inflammatory processes, in the development and maintenance of the immune response and for haematopoiesis, it is now becoming evident that cytokines are involved, at least to some extent, in most if not all physiological processes. Progress with the identification of new cytokine molecules is particularly fast moving and new molecules are discovered with alarming frequency. Although cytokines were considered to be a “family”, this is a functional rather than a structural concept, as these proteins are not all chemically related. Cytokines encompass those families of regulators variously known as growth factors, colony-stimulating factors, interleukins, lymphokines, monokines, and interferons.

The confusing nomenclature of cytokines has arisen because several different streams of investigation led to the discovery of different cytokines, and for example, molecules that initially were classically considered growth factors were later reclassified as cytokines. Conversely, interleukins were defined in 1981 as molecules made by leukocytes that acted on leukocytes. Although research afterwards has revealed that some of these molecules are also made by, and act on non-leukocytes, the nomenclature has stuck.

Despite overwhelming evidence for key immunomodulatory roles, the classical endocrine hormones have not always enjoyed a good press, and modern textbooks of immunology give scant attention to these compounds, mainly because their general properties are different from those of the cytokines (Table I). Nevertheless, there are no sharp boundaries that distinguish cytokines from other regulatory proteins. Some neuropeptides are between these boundaries.

In this sense, VIP is a paradigmatic polypeptide that has been evolved from more than a mere neuropeptide/hormone to a novel agent for modifying immune function and, possibly a cytokine-like molecule. First identified by Said and Mutt in the late 60’s, VIP was originally isolated as a vasodilator and hypotensive peptide. Subsequently, its biochemistry was elucidated and within the first decade its signature features as a neuropeptide/neurotransmitter became consolidated, acting as a neuromodulator in many organs and tissues, including heart, lung, thyroid gland, kidney, immune system, urinary tract and genital organs. The widespread distribution of VIP correlates with its involvement in a wide variety of biological processes, from systemic vasodilation (with recently reported potential use as a new drug for treatment of primary pulmonary hypertension), to core clock functions being the first neuropeptide important for maintaining stable circadian clock function. It did not take long for these insights to permeate the field of immunology, out of which surprising new attributes for VIP were found in the last years. VIP is rapidly transforming into something more than a mere hormone/neuropeptide. In evolving scientifically from a neuropeptide to a novel agent for modifying immune function and, possibly a cytokine-like molecule, VIP research has engaged many physiologists, molecular biologists, biochemists, endocrinologists and pharmacologists and it
is a paradigm to explore mutual interactions between neural and neuroendocrine links in health and disease.

During the last five years, the VIP-mediated molecular machinery accountable for its anti-inflammatory effects has been unravelled. VIP is so far the first neuropeptide able to drive Th-cell differentiation. This is the framework for integrating the effects of VIP on innate and adaptive immunity, and for developing alternative strategies against chronic inflammatory diseases and autoimmune disorders. Thus, VIP may play an important role in problems related with complexity and self-organization in a manner similar to what cytokines do. In the next paragraphs we will critically highlight the major issues that have arisen in this area and offer a final reasoned outcome: that VIP should be considered as a Th2 cytokine.

The key characteristics for a molecule to be considered as a Th2 cytokine are: 1) to be produced by Th2 cells in response to a specific antigen stimulation; 2) to stimulate and to inhibit Th2 and Th1 function/generation, respectively; 3) to mediate autocrine/paracrine actions, involving the expression of specific receptors in neighbouring cells, and 4) to act in a pleiotropic and redundant way inducing a Th2 biological action (reducing the cellular-mediated inflammatory response, favouring the humoral response). VIP combines all of them.

VIP IS PRODUCED BY TH2 CELLS

VIP belongs to the secretin/glucagon family of peptides and its aminoacid sequence has been well conserved during evolution, suggesting an important biological role. VIP is produced by neurons in different areas of the central and peripheral nervous system and by endocrine cells, such as the pituitary lactotrophes and cells of the endocrine pancreas. Moreover, VIP is also present in inflammatory and immune cells. VIP is produced by lymphocytes in central (thymus) and peripheral (spleen and lymph nodes) organs. Additionally, immune cells themselves express and secrete VIP upon stimulation with LPS, cytokines, ConA, or anti-TCR antibodies. More recently, we have demonstrated that VIP is preferentially produced by type 2 CD4+ and CD8+ cells but not by type 1 T effectors upon specific antigen stimulation, both in vivo and in vitro.

VIP RECEPTORS ARE EXPRESSED BY DIFFERENT IMMUNOCOMPETENT CELLS

VIP exerts its biological actions through three different receptors of the family 2 of G protein-coupled receptors (GPCR) that are shared with the structurally related pituitary adenylate cyclase-activating polypeptide (PACAP). To date, three types of VIP/PACAP receptors have been cloned, which according to the International Union of Pharmacology (IUPHAR) nomenclature (15) have been classified as follows: the VPAC1 receptor (also known as VIP1, VIP/PACAP type II, or PVR2), and the VPAC2 receptor (also termed VIP2, VIP/PACAP type III, or PVR3) bind both VIP and PACAP with equal affinity, and activate primarily the adenylate cyclase pathway; and the PAC1 receptor (also known as VIP/PACAP type I receptor, or PVR1), the PACAP preferring receptor, binds PACAP with 300-1000 fold higher affinity than VIP, and activates both adenylate cyclase and phospholipase C.

Our group has recently studied the expression and distribution of the different VIP/PACAP receptors in various immune cell populations. VPAC1 is expressed in rat thymocytes, peripheral T and B lymphocytes, murine thymocytes, and peripheral CD4+ and CD8+ T lymphocytes, murine macrophages, and human monocytes. In contrast to VPAC1, which seems to be constitutively expressed in

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| TABLE I. General differences between cytokines and endocrine hormones |
|--------------------------------|-----------------|-----------------|
| Property                   | Cytokines       | Hormones       |
| Sites of production        | Many            | Few            |
| Cell targets               | Few             | Many           |
| Biological role            | Immunity        | Homeostasis    |
|                           | Inflammation    |                |
|                           | Fighting infection |             |
|                           | Tissue repair   |                |
| Redundancy                 | High            | Low            |
| Pleiotropic Action         | High            | Low            |
| Circulatory levels         | Very low        | Moderate/High  |
| Influence range            | Local: Autocrine/Paracrine | Systemic: widespread |
| Inducers                   | External insults | Physiologic variation |
both unstimulated and stimulated lymphocytes and macrophages, VPAC2 is expressed only following stimulation through the TCR-associated CD3 molecule in lymphocytes or endotoxin in macrophages, and represents the only VIP receptor expressed in T cell lines such as EL4.IL-2, D10-G4.1, 2B4.11, and A1.1(16). The physiological significance of the distinct anatomical distribution and regulation of VPAC1 and VPAC2 is unresolved at the present time, but it seems to be a general characteristic because in most organs VPAC1 and VPAC2 do not overlap anatomically, and in some cases, particularly in brain, the distribution is complementary(17). Also, the expression of VPAC2 appears to be developmentally regulated in several systems(17). The expression of VPAC1 and VPAC2 in T lymphocytes and macrophages seems to follow a similar pattern, with VPAC2 mRNA being induced during lymphocyte and macrophage activation. Involvement of different transduction signals could explain the differential distribution and regulation of both receptors. The best characterized effect of VIP in various tissues, including immune cells, is the accumulation of intracellular cAMP and the subsequent activation of protein kinase A(15). Although both VPAC1 and VPAC2 induce intracellular cAMP accumulation, more subtle differences may exist between transduction pathways affected specifically by any of these receptors.

Regarding PAC1, macrophages and monocytes are the only immunocompetent cells that have been shown to constitutively express PAC1(18). Although a highly preference of this receptor for PACAP versus VIP has been extensively reported, mainly in the central nervous system, we have shown that PAC1 expressed in peritoneal macrophages binds PACAP and VIP with a similar affinity(19); moreover, both neuropeptides activate in a similar way the protein kinase C/ phospholipase C system coupled to PAC1(18), suggesting that PAC1 expressed in macrophages is essentially different to that expressed in the nervous system, since at least five different subtypes of this receptor have been described(19).

VIP IS AN ANTI-INFLAMMATORY AGENT IN INNATE IMMUNITY

The immune system responds to pathogen invasion with two temporarly separate but physically linked responses, mediated by different types of cells. The first response, termed innate immunity involves neutrophils, NK cells, monocytes/macrophages and dendritic cells in the periphery and microglia in the CNS. The innate response is rapid and based on the recognition of conserved pathogen-associated molecular patterns. In contrast, the adaptive response occurs later, following activation of T and B lymphocytes through specific receptors. In contrast to the innate response, adaptive immunity leads to the development of memory for a specific antigen.

Successful elimination of pathogens requires the initiation and participation of both innate and adaptive immunity. However, once the pathogen is eliminated, cells participating in innate and adaptive immunity have to be deactivated or eliminated, to re-establish immune homeostasis. Uncontrolled activation of the immune system leads to serious consequences for the host, such as tissue destruction, organ failure, or even death. In addition to apoptosis of activated immune cells, several endogenous agents, such as anti-inflammatory cytokines, lipid mediators, and glucocorticoids downregulate inflammation. Recently, several neuropeptides, including VIP and PACAP, have been added to the list of endogenous anti-inflammatory agents.

VIP regulates cytokine production by activated macrophages and microglia

Activated macrophages initiate local inflammation through the secretion of pro-inflammatory cytokines, chemokines, and production of reactive oxygen and nitrogen intermediates. Microglia play a similar role in the CNS. Microbial products such as LPS induce macrophages and microglia to secrete pro-inflammatory products such as TNFα, IL-12, IL-1, IL-6, and nitric oxide (NO). However, with the exception of IL-10, a second, cAMP-independent pathway is also involved.

The inhibitory effects of VIP on the expression of TNFα (both in macrophages and microglia), IL-12, and iNOS, and the stimulation of IL-10 expression are mediated through VPAC1(21-27). As expected, a cAMP-dependent transduction pathway mediates the effects on TNFα, IL-12, iNOS, and IL-10. However, with the exception of IL-10, a second, cAMP-independent pathway is also involved. All four cytokines and iNOS are regulated at the transcriptional level. In macrophages, VIP affects TNFα expression through reduction in NFkB and changes in the composition of the CRE-binding complex(28). The stimulatory effect on IL-10 expression is mediated through the increase
in phosphorylated CREB\textsuperscript{26}. For both iNOS and IL-12p40 expression, VIP reduces NFkB and IRF-1/ets-2\textsuperscript{26, 28}. The following common conclusions were reached from these studies. The inhibition of TNF\alpha, IL-12p40 and iNOS expression is mediated through two secondary messengers, i.e. cAMP, the expected mediator, which affects CREB and IRF-1, and a second, still unidentified mediator, which reduces NFkB binding. We have recently reported on the detailed transduction pathways leading to the VIP inhibition of IRF-1 synthesis, changes in AP-1, and the inhibition of NFkB transactivating activity in macrophages. VIP inhibits IRF-1 gene transcription by affecting Jak1/2-STAT1 phosphorylation\textsuperscript{27, 29}. VIP inhibits \textit{c}-Jun phosphorylation by affecting the MEKK1/MEK4/JNK pathway, and induces \textit{Jun}B phosphorylation through the CAMP pathway, both in macrophages and microglia\textsuperscript{30, 31}. In macrophages, VIP inhibits NFkB transactivating activity at three levels\textsuperscript{32}. First, it prevents IkB phosphorylation by inhibiting IKK activity, in a CAMP-independent manner. This results in reduced p65 nuclear translocation, due to the stabilization of the p65-p50/IkB cytoplasmic complex. Second, VIP induces CREB phosphorylation, leading to the sequestration of nuclear CBP (CREB-binding protein, the transcriptional coactivator required for NFkB transactivating activity). Third, VIP reduces TBP (TATA-box binding protein) phosphorylation by affecting the MEKK1/MEK3-6/p38 pathway, and further reduces NFkB transactivation. The effects on CBP and TBP are CAMP-dependent. Recently, we have demonstrated that VIP has similar effects in microglia (33). Treatment of LPS-stimulated microglia with VIP results in inhibition of NFkB p65 nuclear translocation, and reduction in CBP/p65 complexes due to the preferential binding of CBP by phosphorylated CREB.

**VIP inhibits the production of macrophage- and microglia-derived pro-inflammatory chemokines (CK)**

Accumulation of immune cells at the site of pathogen invasion is mediated through inflammatory CK and immune cells expressing the appropriate CK receptors (CK-R). The inflammatory CK are released primarily by cells involved in innate immunity, i.e. macrophages, dendritic cells (DC), and microglia.

At present there are few reports regarding the effects of VIP on inflammatory CK production, and no information as to its effects on CK-R. We reported that VIP inhibits the expression of two CXC chemokines (CXCL2/MIP2 and CXCL1/KC), and of four CC chemokines (CCL3/MIP\textit{\alpha}, CCL4/MIP\textit{\beta}, CCL2/MCP1, CCL5/RANTES), in LPS-stimulated macrophages and microglia\textsuperscript{25, 34}. The inhibition is mediated by VPAC1 and correlates with a reduction in NFkB binding and transactivating activity. The two CXC chemokines CXCL2 and CXCL1 function as chemoattractants for neutrophils, whereas the CC chemokines attract monocytes/macrophages and T cells. Accordingly, VIP treatment of microglia resulted in a reduced \textit{in vitro} chemotactic activity for peripheral leukocytes, and the \textit{in vivo} administration of VIP in a model of acute peritonitis led to a significant reduction in the recruitment of neutrophils, macrophages, and lymphocytes in the peritoneal cavity\textsuperscript{21, 34}.

The possibility that VIP might modulate the expression not only of CK but also of CK-R, opens new territories in neuroimmunomodulation, since differences in chemokine receptors impose the different migratory patterns of immature versus mature DC, and of Th1 versus Th2 cells\textsuperscript{35, 36}. Therefore, neuropeptides might be active participants in the maturation and function of DC, and in the differential development and function of Th1/Th2 effector cells.

**VIP inhibits the expression of costimulatory molecules on activated macrophages, dendritic cells, and microglia, and reduces their capacity to stimulate antigen-specific T cells**

In addition to being active participants in innate immunity, macrophages, DC and microglia serve as a link to adaptive immunity, by presenting antigen and stimulating antigen-specific CD4\textsuperscript{+} T cells. Several signals are required for the activation of naïve T cells. The stimulatory signal is provided by the interaction between the MHC class II/antigenic peptide on the antigen-presenting cell (APC) and the TCR on T cells. The costimulatory signals are provided by interactions between the B7 family and CD40 on APCs, and CD28 and CD40L on T cells, respectively. Although all APCs express MHC class II, DC are the only ones expressing B7.1 and B7.2 constitutively, which makes them the most efficient APCs for activation of naïve T cells. However, all three types of APCs, i.e. DC, macrophages and microglia, express Toll-like receptors (TLR) involved in the recognition of pathogen-associated molecular patterns, and upregulate both stimulatory and costimulatory molecules upon encountering pathogens.

One of the components of the general anti-inflammatory action of VIP could be the reduction in stimulatory/costimulatory activity of APCs for antigen-specific T cells. We reported earlier that in LPS/IFN\gamma-activated macrophages, VIP downregulates B7.1/B7.2 expression, both at mRNA and protein level, without affecting MHC class II or CD40\textsuperscript{37}. The inhibitory effects of VIP on B7 expression correlate with the reduction in the stimulation of antigen-specific T cell proliferation. In microglia, VIP affects B7.2 and CD40 expression in LPS-stimulated primary microglia and the
VIP: A FACTOR PROMOTING TH2-TYPE IMMUNE RESPONSES

VIP affects CD4+ T cell differentiation in favour of Th2 effectors

Following stimulation, CD4+ T cells proliferate and differentiate into Th1 and Th2 effectors, characterized by specific cytokine profiles and functions. The cytokine microenvironment represents the major deciding factor for the differentiation into Th1 or Th2 effectors, with IL-12 being the major Th1, and IL-4 the major Th2 promoting cytokine. Recently, other endogenous factors such as progesterone, glucocorticosteroids, and norepinephrine have been reported to favour Th2 differentiation.

The question whether neuropeptides affect Th1/Th2 differentiation is just starting to be addressed. Several lines of investigation indicate that VIP/PACAP promote Th2-type immune responses. We have previously reported that macrophages treated in vitro with VIP gain the ability to induce Th2-type cytokines (IL-4 and IL-5) and inhibit Th1-type cytokines (IFNγ, IL-2) in antigen-primed CD4+ T cells. In agreement with the in vitro results, in vivo administration of VIP in immunized mice results in a decreased number of IFNγ-secreting cells (Th1) and an increased number of IL-4 secreting cells (Th2). Similar results have been recently obtained with DC generated from bone marrow. Two recent studies confirmed the prevalence of Th2-type responses in mice overexpressing the human VPAC2 receptor in CD4+ T cells, and of Th1-type responses in mice deficient in VPAC2. The role of endogenous, Th2-derived VIP, in maintaining the Th2 bias has been recently confirmed in studies in which elimination of VIP from TCR-stimulated T cells with Vipase IgG resulted in the readjustment of the Th1/Th2 balance. These studies confirm and extend the concept that VIP affects the Th1/Th2 balance in vivo, and indicate the prevalent role of VPAC2 in this process.

Mechanisms by which VIP promotes Th2-type immune responses

Several non-excluding mechanisms could contribute to the Th2 bias by VIP. The neuropeptide could act at the level of Th1/Th2 generation, either directly or through its effects on APCs, and/or at the level of the already generated effectors, by preferentially promoting Th2 proliferation, survival, or accumulation.

There is evidence that VIP affects APCs in at least two ways that are relevant to the generation of Th1/Th2 effectors. First, VIP inhibits IL-12 production from activated macrophages. Since differentiation into Th1 cells is controlled primarily by the availability of IL-12, the Th1/Th2 balance will be altered in favour of Th2 in the presence of suboptimal doses of IL-12. Second, the presence of costimulatory molecules on APCs seems to be significantly more important for the development of Th2, compared to Th1 cells. We demonstrated previously that VIP induces B7.2 expression in resting macrophages and immature DC. Moreover, the induction of Th2-type cytokines in antigen-primed T cells by resting macrophages and DC treated with VIP is abolished in the presence of neutralizing anti-B7.2 Abs. This supports the role of VIP-induced B7.2 in promoting Th2-type responses.

VIP could also affect Th1/Th2 differentiation by acting directly on T cells. Indeed, addition of VIP to TCR-transgenic T cells cultured with mitomycin C-treated APCs and antigenic peptide, in the presence or absence of polarizing cytokines (IL-12 for Th1 and IL-4 for Th2 differentiation) leads to increased levels of IL-4 and decreased levels of IFNγ, suggesting that VIP promotes the development of Th2 cells directly by acting on the differentiating CD4+ T cells. Whether VIP affects the expression of the master transcriptional factors T-bet and GATA-3, required for Th1 and Th2 differentiation, respectively, remains to be established. Previous studies indicated that VIP induces expression of JunB, one of the transcriptional factors required for Th2 differentiation, in both macrophages and newly stimulated T cells.

Finally, VIP could act on the already generated Th1/Th2 effectors. The majority of Th1/Th2 effectors are eliminated through apoptosis following a relatively short period of intense activity. The relatively few surviving T cells become antigen-specific memory cells, which differ from naive T cells in terms of homing patterns and activation requirements. The elimination of effector T cells occurs through either active or passive apoptosis, depending on whether the antigen persists or is eliminated. Based on its anti-inflammatory role, the expectation was that VIP would promote T cell apoptosis. However, contrary to the expected outcome, VIP...
proteins activated CD4+ T cells against active (antigen-induced) cell death (AICD) both in vitro and in vivo, through the inhibition of Fas L ligand (FasL) expression\(^{(35, 31)}\).

How can the VIP anti-inflammatory effects be reconciled with the protective effect against AICD? At first glance, a higher number of surviving effector CD4+ T cells should result in higher levels of activity, hence a more intense inflammatory response. However, following the proliferative stage, CD4+ T cells differentiate into Th1 effectors primarily involved in cell–mediated immunity, and Th2 effectors involved in humoral immunity. From a functional point of view, Th1, and not Th2 cells, mediate acute inflammation, through the mobilization and activation of potent inflammatory cells such as neutrophils and macrophages within the inflammatory site. In addition to their known functional differences, Th1 and Th2 effectors also differ in terms of inflammatory site. In agreement with the observed in vivo Th2-type immune responses upon administration of exogenous VIP\(^{(40, 44)}\), or in mice overexpressing the VPAC2 receptor in CD4+ T cells\(^{(45)}\), the preferential effect of VIP on the survival of Th2 effectors in vivo and in vitro was indeed confirmed in a recent study\(^{(53)}\). The selective recruitment of effector T cells to an inflammatory site is mediated through CK and CK-Rs. Th1 and Th2 cells have been shown recently to express different CK-R, and therefore, home differently\(^{(35)}\). Th1 cells express CXCR3 and CCR5, whereas Th2 cells preferentially express CCR3, CCR4 and CCR8. Among CKs, IP-10 (CXCL10), MIG (CXCL9), and I-TAC (CXCL11) bind only to CXCR3, expressed on activated Th1 cells, NK cells and thymocytes, whereas MDC (CCL22), TARC (CCL17), and TCA-3 (CCL1) bind to CCR4 and/or CCR8, expressed on activated Th2 cells, NK cells, and thymocytes. Activated Th1 and Th2 cells were reported to migrate in response to IP-10 (CXCL10) and MDC (CCL22), respectively\(^{(50, 51)}\). Since VIP induces Th2-type responses, we investigated the possibility that, in addition to the effects on the generation and survival of Th2 cells, VIP might also promote specific Th2 recruitment. We focused on the effects of VIP/PACAP on two of the Th1/Th2 recruiting CK, i.e. IP-10/CXCL10 and MDC/CCL22. In a recent study we reported that VIP downregulates CXCL10, and up-regulates CCL22 in vivo and in vitro in spleen cells\(^{(52)}\) and in DC generated from BM\(^{(53)}\). The VIP effects on CCL22 and CXCL10 translated into differences in chemotaxis for Th1 and Th2 cells.

### ROLE OF VIP IN INFLAMMATORY AND AUTOIMMUNE DISORDERS.

#### CLINICAL IMPLICATIONS

Due to its pleiotropic and multifunctional characteristics, VIP has been used in the development of therapies for several disorders, including impotence, skin disorders, asthma, alterations of circadian rhythms, tumours (glioblastoma, prostate, lung, pancreatic and breast cancer), heart and lung ischemia/reperfusion injuries, type 2 diabetes, and neuronal defects, learning and memory defects\(^{(56)}\). However, we will only review the potential therapeutic applications of VIP in immunological disorders. Due to its inhibition of exacerbated inflammatory responses, and its ability to shift the immune response toward a Th2-type response, VIP emerges as an attractive therapeutic factor for inflammatory disorders and/or Th1-type autoimmune diseases.

#### Septic Shock

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. Indeed, the administration of the endotoxin LPS to experimental animals leads to pathological changes similar to the human septic shock syndrome. Our understanding of the pathophysiology of septic shock has increased markedly over the past few years. Septic shock begins with bacteria overwhelming the host defenses, and the release of toxic products that activate plasma factors (complement and clotting molecules) and cells of the immune system (polymorphonuclear cells, monocytes/macrophages,
lymphocytes). Invading bacteria and bacterial products trigger the release of a complex array of host mediators, including cytokines. Normally cytokines are autocrine and paracrine molecules that act locally to control the host response to invading organisms. In fact, by affecting coagulation and leukocyte mobilization and by activating professional phagocytes, cytokines contribute to the control of a local infection. It is generally accepted that the severe pathological consequences of the septic shock syndrome result from a hyperactive and out-of-balance network of endogenous pro-inflammatory cytokines, including TNFα, IL-12, IL-6, and IFNγ. The overproduction of inflammatory cytokines generates systemic activation, which affects vascular permeability, cardiac function, and induces metabolic changes that can lead to tissue necrosis, and eventually to multiple-organ failure and death.

Since VIP inhibits the production of pro-inflammatory macrophage-derived cytokines in vivo and in vitro, it was expected to protect against endotoxemia. Indeed, VIP administration protects against high lethal endotoxemia in a mouse model for septic shock syndrome. Endotoxic animals suffer from generalized intravascular coagulation with multiple organ failure as evidenced by severe congestion, haemorrhage, hyperemia, fibrin deposits, oedema, thrombosis, and massive accumulation of leukocytes in lungs and the intestinal tract, as well as severe congestion of the medullar sinusoids in the spleen, and segmental ischemia of the bowel with regions of haemorrhage or necrosis, and an infarcted caecum. In contrast, VIP-treated animals did not present any of the histopathological alterations associated with septic shock, such as disseminated intravascular coagulation, leukocyte infiltration and inflammation in various organs, mesenteric ischemia, and acute tubular necrosis in the kidneys. VIP acts presumably by down-regulating the pro-inflammatory mediators such as TNFα, IFNγ, IL-6, IL-12, and NO and up-regulating production of IL-10, PGE2, and COX-2. In addition, the inhibitory effect of VIP on the production of proinflammatory chemokines is also crucial in its preventive action on endotoxemia, because transmigration of inflammatory cells to target inflamed organs is one of the initial events.

Studies with specific VPAC agonists and the use of a PAC1−/− mouse indicated that VPAC1 is the main mediator involved in the preventive effect of VIP on endotoxemia, although VPAC2 and PAC1 also play a role. VPAC1, and probably VPAC2, are involved in the inhibitory action of VIP in the TNFα-induced cascade, affecting the infiltration of activated inflammatory cells in certain organs, production of cytotoxic inflammatory factors and the subsequent tissue necrosis. PAC1 is mainly related to the inhibition of IL-6 production and the subsequent activation of acute phase proteins and disseminated coagulation.

The current strategies of adjunctive therapy for human septic shock are mainly derived from observations made in animal models. Promising experimental results prompted large-scale randomized clinical trials with a variety of agents such as anti-endotoxin monoclonal antibodies, glucocorticoids or ibuprofen for nonspecific downregulation of inflammation, antagonists of platelet activating factor, antagonists of bradykinin, IL-1 receptor antagonists and monoclonal anti-TNF antibodies or soluble dimeric TNF receptor fusion proteins (reviewed in NLM Identifier NCT00004494: ClinicalTrials.gov). Unfortunately, despite some promising results during preliminary trials, all the major clinical studies of immunomodulators in sepsis have yielded disappointing results. Several causes may explain this relative disappointment. In animal models, the cascade of events starting from the initial stimulus and usually ending with the death, and the resulting cytokine cascade production generally follows a predictable time course. Thus, experimental protocols designed to block one cytokine cascade or another are relatively straightforward. In contrast, in human septic shock syndrome, the sequence of events is more complex, and the course of the disease generally lasts days rather than hours, as seen in most animal models. The design of a clinical trial is based essentially on experimental models using parenteral injections of LPS/bacteria, in which cytokine blockade is beneficial, not on animal models with focal infections, where such therapy is usually not effective. The organisms and site of infections are diverse, and importantly the patients have a large variety of underlying diseases, which is not the case in animal studies. Interventions in animal models have been successful only when applied early, before the disease is well established, which is the usual in patients.

In view of these findings, which are the possibilities to translate the promising results of VIP observed in animal models to an effective treatment of the human septic shock syndrome? A clinical study is on the way [phase I] in patients with acute respiratory distress syndrome and sepsis with very promising and hopefully results (NLM Identifier NCT00004494: ClinicalTrials.gov). Probally, due to its pleiotropic effects inhibiting a wide spectrum of pro-inflammatory mediators, including mediators which appear later during the inflammatory response, VIP protects from endotoxemia if given two hours after endotoxin injection. Therefore, due to a wider therapeutic window, VIP may represent a better biological therapeutic alternative than anti-inflammatory cytokines or anti-TNFα antibodies, which are only effective in the very early states. Furthermore, additional mechanisms could be participating...
in the therapeutic effect of VIP on endotoxemia. Indeed, other studies\(^{62}\) suggested that the protective effect of VIP on septic shock syndrome is mediated through the regulation of serum levels of hormones, such as adrenaline and cortisol, which control haemodynamic constants, important events in the pathology of sepsis.

**Rheumatoid Arthritis**

Rheumatoid arthritis (RA) is a common, chronic and debilitating autoimmune inflammatory disease of unknown aetiology that leads to chronic, progressive and symmetrical inflammation of the joints and subsequent erosive destruction of the cartilage and bone. RA affects approximately 1% of the population, it has a disproportionately high incidence in women compared with men (3:1 ratio) and in older individuals, and it is a significant cause of disability, chronic ill health and premature mortality. In order to find therapeutic alternatives, several strategies have been designed based in the two deleterious aspects of RA, i.e., inflammation and autoimmunity.

The majority of RA treatments were developed with the purpose of decreasing chronic joint inflammation, a multifactorial response dependent upon both regulatory cytokines and proinflammatory chemokines. Therefore, agents that inhibit secretion of inflammatory mediators, especially TNF\(\alpha\), or that can block receptor binding, are increasingly being viewed as potential therapeutic agents with increased specificity as compared to traditional drugs\(^{63}\).

An alternative therapeutic strategy explored in RA is the alteration of the T cell response, with CD4\(^+\) T cells as targets 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 remission\(^{63}\).

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

VIP has been clearly identified as a potent anti-inflammatory factor, and some evidence indicates that VIP preferentially induces differentiation toward a Th2 response. Therefore, VIP has emerged as an attractive candidate for the treatment of arthritis. In fact, two recent reports\(^{64, 65}\) using the animal model of collagen-induced arthritis (CIA), which shares a number of common clinical, histological, and immunological features with human RA, have demonstrated that treatment of arthritic mice with VIP decreases the frequency, delays the onset, and reduces the severity of the disease. The therapeutic effect of VIP on arthritis is associated with a striking reduction of the two deleterious components of the disease, i.e. the autoimmune and inflammatory response.

VIP reduces the titre of autoreactive anti-collagen antibodies (particularly IgG2a antibodies), in response to a reduction in the collagen-specific T cell response. This is accompanied by a shift from Th1 to Th2 type responses, characterized by a reduction in IFN\(\gamma\) production, and an increase in IL-4.

Regarding its effect in joint inflammation, VIP strongly reduces the inflammatory response during arthritis development by down-regulating the production of several pro-inflammatory agents in the inflamed joints and synovial cells, including TNF\(\alpha\), IL-6, IL-1\(\beta\), iNOS, IL-12 and IL-18, as well as various chemokines (RANTES, MCP-1, MIP-1\(\alpha\), MIP-1\(\beta\), MIP-2) reported to play a role in inflammation and in the development of the arthritic responses\(^{63}\). In addition, VIP increases the production of the anti-inflammatory cytokines IL-10 and IL-1Ra, which have been reported to ameliorate arthritis symptoms\(^{63}\). Chemokines are responsible for the infiltration and activation of various leukocyte populations, which are contributing to pannus development and the subsequent pathology of RA. A similar inhibitory action of VIP on the production of inflammatory mediators (TNF\(\alpha\) and IL-8) has been described in synovial cells from arthritic patients\(^{66}\), suggesting a potential application of this neuropeptide in human RA. By inhibiting chemokine production, VIP prevents leukocyte infiltration of the synovium. The capacity of VIP to regulate a wide spectrum of inflammatory mediators may offer an advantage over neutralizing antibodies and receptor antagonists directed against a single cytokine.

VIP prevention of cartilage destruction and bone erosion has to be attributed, at least partially, to its inhibitory effect on the expression and activity of some matrix metalloproteases. The latter have been assigned pivotal roles in the depletion of proteoglycan and collagen observed in the joints, in addition to proinflammatory cytokines such as TNF\(\alpha\) and IL-1\(\beta\), leading to cartilage and bone erosion in patients with RA\(^{67}\).

The rheumatoid synovium shows hyperplasia of fibroblast-like synovial lining cells and is infiltrated with various mononuclear cells, among which macrophages and T lymphocytes predominate. Although the involvement of other cells cannot be ruled out, macrophages and synoviocytes appear to be the target cells of VIP.

Of obvious biological significance is the fact that VIP levels, similar to those of other recent described anti-arthritic
neuropeptides and hormones, such as CGRP and αMSH (66, 68), are specifically increased in serum and joints of arthritic mice during the development of the disease (69). This demonstrates that endogenous neuroimmune factors act as natural anti-arthritic agents activated in response to autoimmune/inflammatory conditions, to counterbalance the effects of inflammatory mediators. Nevertheless, arthritic-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 down-regulate 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, the exogenous administration of these anti-inflammatory mediators could offer an alternative to existing treatments for arthritis and other inflammatory/Th1-autoimmune diseases, such as multiple sclerosis, inflammatory bowel diseases, or autoimmune diabetes.

However mice do not naturally develop RA, and although the results with VIP are encouraging, it requires a leap of faith to extrapolate our findings to the clinic. Also, there are possible side effects to the chronic administration of VIP, including gastrointestinal effects and generalized immunosupression. No such adverse effects were observed short-term in the experimental CIA model. Extending the use of VIP or VIP analogs to humans will depend on the dosage and way of administration. In addition, VIP gene transfer could be attractive as potential means to deliver consistent, prolonged therapeutic titres of this anti-inflammatory factor with fewer side effects and without the need for repeated administrations.

**Crohn’s Disease**

Human inflammatory bowel disease (IBD) is a worldwide, chronic, idiopathic disease of the distal small intestine and the colon mucosa, clinically characterized by two overlapping phenotypes, i.e. ulcerative colitis and Crohn’s disease. Crohn’s disease is an incurable autoimmune disease with a prevalence of 0.01-0.08% that leads to chronic transmural inflammation resulting in a range of gastrointestinal and extraintestinal symptoms, including abdominal pain, rectal bleeding, diarrhoea, weight loss, skin and eye disorders, as well as delayed growth and sexual maturation in children (70).

These symptoms can greatly impact the patients’ well-being, quality of life, and capacity to function. Crohn’s disease is chronic and typically has an onset before 30 years of age, thus generally requiring lifelong treatment. Its aetiology is unknown, but Crohn’s disease is marked by an exaggerated gut-associated lymphoid tissue-developed immune response. This gives rise to a prolonged severe inflammation of the intestinal mucosa, characterized by uncontrolled production of proinflammatory cytokines, and oligoclonal expansion and activation of CD4+ T cells, specifically associated with a Th1 response (71).

Current therapeutic agents used for Crohn’s disease include aminosalicylates, corticosteroids, azathioprine, 6-mercaptopurine, antibiotics and methotrexate, but they are not entirely effective, nonspecific and with multiple adverse side effects. In most cases surgical resection is the ultimate alternative (70). Therefore, the present therapeutic strategy is aimed to find drugs or agents that specifically modulate both components of the disease, i.e. the inflammatory and Th1-driven responses.

In a recent report, we have used the established murine model of Crohn’s disease induced by intrarectal administration of trinitrobenzene sulphonic acid (TNBS) to demonstrate that treatment with VIP reduced the clinical and histopathological severity of TNBS-induced colitis, abrogating body weight loss, diarrhoea and intestinal inflammation (72). The therapeutic effects of VIP occurred in all phases of the disease (early, acute and chronic), and were associated with the down-regulation of both inflammatory and Th1-driven autoimmune responses. In the early acute phase of bowel inflammation, there is an overlapping of innate and adaptive immune responses, with multiple mediators involved, such as chemokines and cytokines. VIP significantly reduced the inflammatory response by down-regulating the production of different proinflammatory mediators involved in the local and systemic damage, such as TNFα, IL-1β, IL-6, IL-12, MIP-1α, MCP-1, MIP-2 and KC. This broad spectrum of action is related to a decrease in colitis-associated inflammation and in the infiltration of neutrophils, macrophages and CD4+ T cells in the lamina propria. The VIP treatment partially prevented TNBS-induced generation of CD4+ T cells and of Th1 responses. Moreover, spleen and lamina propria CD4+ T cells from mice treated with VIP preferentially showed a Th2 pattern, with increased production of IL-4 and IL-10 after antigenic stimulation. Novel therapeutic strategies for Crohn’s disease include the blockade of some of the proinflammatory and Th1 cytokines (such as TNFα, IL-6 and IL-12) (73). Therefore, the capacity of VIP to regulate a wide spectrum of inflammatory and Th1 mediators in TNBS-induced colitis represents a
therapeutic advantage over current treatments directed against a single mediator. From a therapeutical point of view, it is important to point out that VIP reduced disease severity even when given after the onset of the disease, and that VIP dramatically reduced disease recurrence upon administration of a second dose of TNBS. Therefore, VIP could represent a possible multistep therapeutic agent for Crohn’s disease.

Parkinson’s Disease

Parkinson’s disease (PD) is a neurodegenerative disorder involving a progressive degeneration of the dopaminergic neurons of the substantia nigra pars compacta (SNpc), and the subsequent loss of their projecting fibres in the striatum. Although current treatments alleviate some of the symptoms, chronic use of these drugs is effectiveness in dampening the progression of PD, and has been associated with debilitating side effects. In addition, the aetiology of PD remains unknown, and this has impeded the development of effective therapies. However, insights into the pathogenesis of PD have been achieved experimentally by using the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP produces irreversible clinical, biochemical, and neuropathological effects that mimic those observed in idiopathic PD, including the dramatic neurodegeneration of the nigrostriatal dopaminergic pathway. This meperidine analogue is metabolised to 1-methyl-4-phenylpyridinium (MPP+), the enzyme monoamine oxidase B. MPP+ is subsequently transported selectively into neurons by the dopamine reuptake system on the dopaminergic terminals, and concentrated in neural mitochondria in the SN, where it binds to and inhibits complex I of the electron transport chain, thereby producing the same biochemical defects as those detected in SNpc of PD patients. Several mechanisms, including mitochondrial dysfunction, oxidative damage, excitotoxicity, and α-synuclein deposition, have been proposed to initiate neuronal damage and subsequent cell death characteristic of this disorder. In addition to these specific mechanisms, in several neurodegenerative disorders, including PD, Alzheimer’s disease, multiple sclerosis, and AIDS dementia, neuronal cell death occurs subsequent to inflammatory responses, mediated at least partially by activated microglia. Proinflammatory cytokines are known to participate in mitochondrial impairment and oxidative stress, and therefore, an inflammatory response may serve as an integral feature of the mechanistic underpinning related to PD pathogenesis. Because VIP has been shown to act as a «microglia-deactivating factor» under inflammatory conditions, and to prevent inflammation-induced neurodegeneration, this neuropeptide emerges as a plausible candidate for neuroprotection under inflammatory conditions, including PD.

We have recently demonstrated that the stereotaxic administration of VIP into the SNpc in the MPTP mouse model of PD is beneficial, reducing SNpc dopaminergic neuronal degeneration and nigrostriatal nerve fibre loss of VIP prevents MPTP-induced activation of microglia in the SNpc and striatum and the expression of the cytotoxic mediators, iNOS, IL-1β and TNFα and NADPH-oxidase. This effect seems to be mediated through VPAC1 and a PKA-dependent pathway. Based on the correlation between the neuroprotective effect of VIP and its ability to inhibit MPTP-induced microglial activation, it has been proposed that VIP exerts its neuroprotective activity through its effect as a «microglial deactivating factor». The involvement of astrocytes in the mediation of VIP, although minimal, cannot be discarded, because VIP slightly reduced MPTP-induced astroglia. It is unlikely that the reduction of MPTP-induced microglia activation by VIP is secondary to the attenuation of neuronal loss rather than the reverse, but a direct action of VIP on neurons cannot be ruled out. Interestingly, systemic administration of VIP (i.p. injection) significantly inhibits MPTP-induced loss of TH immunoreactivity; it is, however, much less effective, since 15-fold higher doses are required to obtain a significant effect, which is 50% less efficient than the cerebral VIP administration. In addition to the dilution factor in a systemic administration, the difference in effectiveness could be due to a diminished entry of VIP into the brain parenchyma through the blood brain barrier, even in inflammatory conditions, where the blood brain barrier is slightly compromised.

These findings invite to important future directions, including the possible therapeutic use of VIP in brain disorders, such as PD itself, multiple sclerosis, Alzheimer’s disease, and AIDS dementia, where the inflammatory response plays a major role. Since an inflammatory response is involved in PD, antioxidants and newly developed non-steroidal anti-inflammatory drugs, such as iNOS inhibitors, cyclo-oxygenase inhibitors or minocycline have been proposed for treatment. However, although several drugs offered neuroprotection in animal models, there has been little or no success in the clinical treatment of PD. This may indicate that either the animal models do not reflect the events in PD, or that neuronal cell death involves a cascade of events, which cannot be prevented by a single neuroprotective drug. Therefore, consideration should be given to multi-drug therapy, similar to the approach taken in AIDS and cancer therapy. It is also possible that agents such as VIP, which affect a large spectrum of inflammatory mediators, might be at an advantage compared to other anti-inflammatory agents with a more restricted effect.
Brain Trauma

A recent study has demonstrated the potential therapeutic effect of VIP in brain trauma, a pathological condition associated with inflammation(76). The increase in the levels of proinflammatory cytokines is a normal and early feature of the CNS response to trauma(68). However, it remains controversial as to whether inflammation in the injured CNS serves a beneficial or detrimental purpose(81). Several reports demonstrated that the administration of inflammatory cytokines to injured areas was neuroprotective and/or promoted axonal regeneration(81). It has been reported that blood-derived macrophages modify the properties of CNS white matter near mechanical lesions, converting a nonpermissive state to one promoting axonal growth. In contrast, a number of studies have shown the involvement of proinflammatory mediators such as TNF-α, IL-1β and NO in neuronal and oligodendroglial death(78). Treatment until about 6 h after lesion, whereas T lymphocytes and other tissues. Thus, neutrophils do not infiltrate appreciably into other tissues. Hence, the influx of leukocytes in the CNS is delayed in response to an acute insult in comparison to other tissues. Thus, neutrophils do not infiltrate appreciably until about 6 h after lesion, whereas T lymphocytes and monocytes appear 12 to 24 h later(69), suggesting that the CNS mounts an early and intrinsic inflammatory response upon trauma. Microglia has been proposed as the origin of the early increase in CNS inflammatory cytokines following injury(80). It has been proposed that VIP reduces neuronal cell loss in the vicinity of the lesion site through the inhibition of microgial-derived proinflammatory mediators such as TNF-α and IL-1β and of chemokines responsible for the influx of blood-derived leukocytes into the parenchyma surrounding the injured region. It is unlikely that the reduction of activation of microglia by VIP is secondary to the attenuation of neuronal loss rather than the reverse, but a direct action of VIP on neurons cannot be ruled out(77). Similarly, neurotrophic effects mediated through astrocytes cannot be excluded. A neuroprotective action of VIP in response to different neuronal insults, through the production of neuroprotective factors (i.e., ADNF) by astrocytes, has been previously described(85).

CONCLUDING REMARKS AND PERSPECTIVES

VIP, released in the lymphoid organs by activated immune cells, modulates the function of inflammatory cells through specific receptors, affecting both innate and adaptive immunity. Macrophages and dendritic cells in the periphery and microglia in the CNS are major players in innate immunity, and serve as a link to adaptive immunity by functioning as APCs. Activated macrophages, dendritic cells, and microglia decrease the pathogen load through the release of cytotoxic cytokines, oxygen radicals, and nitric oxide, and through the mobilization of additional immune cells in response to inflammatory chemokines. Responding to stimulatory and costimulatory signals delivered by APCs, CD4+ T cells proliferate and differentiate into effector Th1 and Th2 cells. At the conclusion of an immune response, both activated APCs and T cells have to be deactivated and/or eliminated, to avoid excessive tissue and organ damage. A number of endogenous factors, particularly anti-inflammatory cytokines, contribute to the downregulation of the innate response. Neuropeptides, such as VIP and related peptides, have been recently added to the list of endogenous anti-inflammatory molecules (Fig. 1). VIP exerts its anti-inflammatory function in several ways: 1) direct inhibition of pro-inflammatory cytokine production (TNF-α, IL-6, IL-12) by activated macrophages and microglia; 2) upregulation of IL-10 production (a potent anti-inflammatory cytokine); 3) inhibition of the expression and release of pro-inflammatory chemokines from activated macrophages and microglia; 4) inhibition of B7.1/B7.2 expression in activated macrophages and dendritic cells, and subsequent inhibition of their stimulatory activity for antigen-specific T cells; 5) inhibition of Th1 responses (reduction in both the amount of Th1 cytokines and the number of cytokine-producing Th1 cells). Along with these well-defined anti-inflammatory functions, VIP supports the generation and long-term survival of Th2 cells, representing the first described neuropeptide with a possible role in the generation of memory Th2 cells. VIP, produced and secreted by Th2 cells following antigen stimulation, participates in a Th2 auto-regulatory loop, where it promotes Th2-type responses, through multiple non-excluding and probably interrelated mechanisms, including direct effects on differentiating CD4+ T cells, indirect effects on APCs, and modulation of proliferation, survival and recruitment of already generated T effectors. From a physiological point of view, the fact that VIP promotes the generation and long-
term survival of Th2 cells is particularly relevant to the concept of immune privilege. In privileged organs such as brain and eye, there likely is an active process of immune deviation mediated by regulatory T cells generated in the presence of Th2-derived cytokines. From a pathological point of view, these findings open the possibility of using VIP and its analogues in the treatment of inflammatory and autoimmune diseases with a prevalent Th1 background, and partially explain the beneficial effect showed by VIP in models of endotoxic shock, Parkinson’s disease, brain trauma, rheumatoid arthritis and Crohn’s disease.

Could VIP be considered a type 2 cytokine? The findings reviewed here clearly indicate that VIP shares most of the Th2-like cytokine characteristics. Moreover, it is clear that VIP is pleiotropic to an extreme. Further research in our functional genomics era will address how many genes are regulated by VIP at a molecular level. Most probably, these genes will be more or less pleiotropic themselves. Therefore, a single molecule like VIP, in a well-known cytokine manner is pleiotropic at the most basic causal level. The functional overlap between VIP and different immune-related molecules raises another well-known cytokine characteristic, that of redundancy. VIP and other Th2 cytokines will only partially overlap in their effects; each molecule produces effects that the others do not. This degenerate redundancy (partially overlapping pleiotropisms) is one of the hallmarks of cytokines.

Therefore, if Said and Mutt had discovered VIP in the immune system, instead of the small intestine, VIP would have been considered a type 2 cytokine instead of a neuropeptide. While this current «assessment of its potential» is necessarily broad and multi-disciplinary, one can anticipate an even greater interface with other disciplines in the future as the full spectrum of VIP’s biological action become apparent. Only time will tell whether these advances will translate into new therapeutic approaches, but for sure the detailed characterization of VIP effects on the immune system has opened up entirely new areas for basic and clinical investigation.

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