Archivum Immunologiae et Therapiae Experimentalis, 2001, **49**, 101–110
PL ISSN 0004-069X

Review

Neuropeptides as Modulators of Macrophage Functions. Regulation of Cytokine Production and Antigen Presentation by VIP and PACAP

DOINA GANEA[1]* and MARIO DELGADO[1, 2]

[1] Department of Biological Sciences, Rutgers University, Newark, NJ 07102, USA, [2] Departamento Biologia Celular, Facultad de Biologia, Universidad Complutense, Madrid 28040, Spain

Abstract. Vasoactive intestinal peptide (VIP) and the structurally related neuropeptide pituitary adenylate cyclase activating polypeptide (PACAP), present in the microenvironment of lymphoid organs, modulate the function of inflammatory cells through specific receptors. VIP and PACAP inhibit the production of pro-inflammatory agents and stimulate the production of anti-inflammatory cytokines in activated macrophages. The effect is mediated through specific receptors and involves shedding of the CD14 lipopolysaccharide (LPS) receptor and the transcriptional regulation of cytokine genes through effects on *de novo* expression or nuclear translocation of NFκB, cAMP-element binding protein (CREB), c-Jun, and interferon regulatory factor 1 (IRF-1). The *in vivo* administration of VIP/PACAP results in a similar pattern of cytokine modulation which, presumably, mediates the protective effect of VIP/PACAP in a high-endotoxic murine model for septic shock. VIP/PACAP reduce the expression of the costimulatory B7.1/B7.2 molecules and the subsequent stimulatory activity for T helper (Th) cells in stimulated macrophages. In contrast, in unstimulated macrophages, VIP/PACAP induce specific B7.2 expression and promote Th2 cell differentiation. We propose that VIP/PACAP act as endogenous factors that regulate immune homeostasis and that the physiological consequences of VIP/PACAP presence in the immune microenvironment depend on the timing of the neuropeptide release and the activation stage of the neighboring immune cells.

Key words: neuropeptides; macrophages; cytokines; B7 molecules; Th1/Th2 cells; VIP/PACAP.

Introduction

The functional interactions between the nervous and immune systems depend on anatomical connections and mediators released and recognized by one or both systems. Both primary and secondary lymphoid organs and tissues possess an extensive peptidergic innervation in close proximity to the immune cells, with vasoactive intestinal peptide (VIP), neuropeptide Y (NPY), somatostatin (SOM) and galanin (Gal) as prevalent neuro-

Abbreviations used: cAMP – cyclic adenosine monophosphate, ConA – concanavalin A, CREB – cAMP-element binding protein, iNOS – inducible nitric oxide synthase, IRF-1 – interferon regulatory factor 1, LPS – lipopolysaccharide, NO – nitric oxide, PACAP – pituitary adenylate cyclase activating polypeptide, PAC1 – PACAP-preferring receptor, PKC – protein kinase C, TCR – T cell receptor, Th – T helper cells, VIP – vasoactive intestinal peptide, VPAC1 – VIP/PACAP receptor 1, VPAC2 – VIP/PACAP receptor 2, PKA – protein kinase A, TGF-β – transforming growth factor β.

* Correspondence to: Doina Ganea, Ph.D., Department of Biological Sciences, Rutgers University, 135 Smith Hall, 101 Warren Street, Newark, NJ 07102, USA, e-mail: dganea@andromeda.rutgers.edu

peptides in the noradrenergic autonomic and cholinergic nerves, and substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP) in the sensory innervation[3, 26–28, 40, 60, 71]. Some of these neuropeptides were shown to be locally released in functionally relevant amounts during an immune response, both in patients and in animal experimental models[44, 57, 59].

The biological activity of neuropeptides is mediated through specific receptors. The presence of specific receptors for SP, VIP, SOM and CGRP has been reported in immune cells through binding studies, cloning, RT--PCR, and/or characterization at the protein level[6, 14, 34, 39, 54, 55, 64, 74, 75]. In most cases, however, the nature and the physiological roles of the specific receptor subtypes expressed during the activation and differentiation of immune cells are currently unknown.

The presence and release of neuropeptides within the lymphoid microenvironment and the existence of specific neuropeptide receptors on immune cells represent the framework for neuropeptides functioning as mediators of neuroimmune interactions. Although the list of neuropeptides/neurotransmitters affecting immune functions grows at a steady pace, here we review only the effects of VIP and PACAP, two structurally related neuropeptides with major immunomodulatory functions. We focus on the most recent developments regarding the effects of VIP/PACAP on macrophage functions both in innate and acquired immunity.

VIP/PACAP Sources in the Immune Organs

Increased levels of VIP have been reported in lymphoid organs, serum and peritoneal fluid following an inflammatory response *in vivo*[4, 5, 15, 44]. However, the exact cellular source of VIP remains to be defined. Although VIP-ergic nerve fibers are found in most lymphoid organs, particularly in the respiratory and gastrointestinal tract[3, 24, 61], the nature of the signal leading to VIP release during an inflammatory reaction is not known. A reasonable assumption is that nitric oxide (NO), which functions as a particularly potent signal for the VIP release from enteric ganglia[2, 36] and is produced at high levels during an inflammatory process, represents at least one of the relevant signals. In addition, immune cells themselves express VIP at both mRNA and protein level[32, 33, 35, 72] and stimulators of the immune response, such as lipopolysaccharide (LPS), cytokines, concavalin A (ConA), or anti-T cell receptor (TCR) antibodies, were recently shown to act as inducers of VIP release[51].

Much less is known about the distribution and release of PACAP in the lymphoid organs. PACAP38 was immuno-localized in lymphocyte-like cells in the mesenteric lymph nodes and duodenal mucosa[31], and PACAP gene expression was shown to be induced in the hypothalamic paraventricular nucleus following i.p. injection of LPS or IL-1β[37]. These results suggest that, similar to VIP, PACAP might be released from both innervation and immune cells during an inflammatory response.

VIP/PACAP Receptors in Macrophages

Three types of VIP/PACAP receptors have been cloned recently: VPAC1 and VPAC2 bind both VIP and PACAP with equal affinity and activate primarily the adenylate cyclase pathway and PAC1, the PACAP--preferring receptor, binds PACAP with a 300–1000 fold higher affinity than VIP and activates both adenylate cyclase and phospholipase C[38, 62]. Recent studies indicate that unstimulated murine macrophages and macrophage cell lines express VPAC1 and PAC1 mRNA, with VPAC2 being coexpressed following LPS stimulation[16, 19]. In contrast, T lymphocytes express only VPAC1 and VPAC2, with differentiated Th1 and Th2 cell lines expressing only VPAC2[10, 43, 75]. The differential regulation of these receptors during immune cell activation and differentiation suggests different physiological roles. A similar conclusion has been reached for the VIP/PACAP receptors in the CNS, based on their complimentary distribution[67]. However, whether the three receptor subtypes indeed play different physiological roles in the CNS and/or in the periphery remains to be established.

Effects of VIP/PACAP on Macrophage Phagocytosis, Adherence, Migration and Superoxide Ion Production

Previous studies reported contradictory results regarding the effect of VIP/PACAP on the biological functions of macrophages. For example, some authors reported an inhibitory effect on phagocytosis[41, 50], whereas others observed a stimulatory effect[8, 11]. Segura et al.[63] reported that VIP inhibits macrophage adherence, whereas other authors reported increases in macrophage adherence and migration by VIP and PACAP[1, 7, 30]. Similarly, De la Fuente et al.[8] reported

a stimulatory effect of VIP on superoxide ion production, whereas others reported inhibitory effects[42, 47, 73]. A common denominator in these studies is that the stimulatory effects of VIP (whether phagocytosis, adherence and migration, or reactive oxygen production) appear to be associated with protein kinase C (PKC) activity, whereas the inhibitory effects appear to be mediated through cyclic adenosine monophosphate (cAMP). Therefore, the differences in the reported studies could be due to the predominant expression of one or the other of the macrophage VIP/PACAP receptors, depending on the differentiation stage.

The differential use of VIP/PACAP receptors in resting versus LPS-activated macrophages is also supported by the opposite effect of VIP/PACAP on IL-6 production. The two neuropeptides enhance IL-6 release and mRNA in unstimulated macrophages in a process that is predominantly VPAC1-mediated and cAMP-dependent[52]. In contrast, VIP and PACAP inhibit IL-6 production in LPS-stimulated macrophages in a PAC1-mediated and PKC-dependent process[53].

Effects of VIP/PACAP on the Production of Macrophage-Derived Cytokines

Macrophages, major participants in innate immunity, contribute to the initiation of the inflammatory response through the secretion of inflammatory cytokines and production of reactive oxygen and nitrogen intermediates. Following stimulation with microbial products like LPS, macrophages secrete several pro-inflammatory products, such as TNF-α, IL-12, IL-1, IL-6 and NO, followed later by the secretion of the anti-inflammatory cytokines IL-10 and transforming growth factor β (TGF-β)[48]. Despite the beneficial role of pro-inflammatory cytokines and NO in host defense, their sustained production can lead to serious pathological conditions[25, 69, 70]. The control of an inflammatory state depends on the local balance between pro- and anti-inflammatory factors. In this respect, a number of regulatory molecules called "macrophage deactivating factors" have received considerable interest lately. In addition to the anti-inflammatory cytokines IL-10, IL-11, TGF-β and IL-13[29, 58, 65, 66], neuropeptides such as VIP and PACAP also function as macrophage deactivating factors. VIP/PACAP inhibit the *in vitro* and *in vivo* production of the pro-inflammatory cytokines TNF-α, IL-6, IL-12 and of NO, and stimulate the production of the anti-inflammatory cytokine IL-10 (Table 1)[16–18, 21, 23, 53, 76]. In addition, VIP/PACAP exert a protective effect *in vivo* in a high-endotoxic model for septic shock, presumably by inhibiting the production of endogenous pro-inflammatory cytokines[15].

The modulatory effects of VIP/PACAP are dose-dependent and are exerted over a large range of LPS concentrations. The use of specific VIP/PACAP receptor agonists and antagonists established that the effects on TNF-α, IL-12, IL-10 and NO were mediated primarily through VPAC1 and, to a lesser degree, also through VPAC2. The inhibition of IL-6, on the other hand, was mediated primarily through PAC1 (Table 1).

As expected, the cAMP-dependent pathway plays a major role in the inhibition of TNF-α, IL-12 and NO[16, 18, 21] and in the stimulation of IL-10[17], whereas the inhibitory effect on IL-6 is mediated by PKC[52]. However, except for IL-10, two pathways, a cAMP-dependent and a cAMP-independent pathway, appear to be involved in the inhibitory effects of VIP/PACAP[9, 18, 19] (Table 1).

Several molecular mechanisms operate in the modulatory effects of VIP/PACAP on macrophage-derived cytokines and NO. One of the mechanisms involves the down-regulation of CD14, the membrane-bound LPS receptor, by inducing its rapid shedding from macrophages[12]. In addition, VIP or PACAP induce significant reductions in the steady-state levels of TNF-α, IL-12 p40, IL-6 and inducible mitric oxide synthase (iNOS) mRNA, and a significant increase in IL-10 mRNA. The effects of VIP/PACAP on cytokine and iNOS transcription are mediated through the regulation of several transcriptional factors. The reduction in TNF-α gene transcription is due to the inhibition in NFκB binding, mediated by the cAMP-independent pathway, and

Table 1. Receptors, intracellular pathaws and transcriptional factors involved in the VIP/PACAP inactivation of LPS-stimulated macrophages

Factor	Action	Receptor	Intracellular pathways	Trancriptional factors	Reference
IL-6	inhibition	PAC1	PKC	ND	53
IL-10	stimulation	VPAC1	cAMP	CREB	17
IL-12	inhibition	VPAC1>>VPAC2	cAMP/cAMP-independent	IRF-1NFκB	9, 16
TNF-α	inhibition	VPAC1>>VPAC2	cAMP/cAMP-independent	CREB/NFκB	19, 21, 23, 76
iNOS	inhibition	VPAC1	cAMP/cAMP-independent	IRF-1/NFκB	18
B7.1/B7.2	inhibition	VPAC1	cAMP	ND	13

ND – not determined.

changes in the CRE-binding complex from ^{high}c-Jun/^{low}-CREB to ^{low}c-Jun/^{high}CREB, mediated by the cAMP-dependent pathway[19]. The increase in IL-10 gene transcription is mediated entirely through an increase in cAMP-dependent CREB binding[17]. The decrease in iNOS gene transcription is mediated through a cAMP-independent reduction in NFκB binding and the cAMP-dependent inhibition of IRF-1[18]. Finally, the decrease in p40 IL-12 gene transcription is mediated through a cAMP-independent reduction in NFκB binding and a cAMP-dependent alteration of the Ets-2-binding complex[9]. The effects of VIP/PACAP on NFκB binding are due to the inhibition of p65 and c-Rel nuclear translocation. This is, in turn, due to the inhibition of cytoplasmic IκB phosphorylation, leading to its stabilization and retention of p65/c-Rel in the cytoplasmic compartment[9]. The alterations in Ets-binding complexes are due in part to the inhibition of c-Rel nuclear translocation and also to the inhibition of IRF-1 synthesis[9]. The effects of VIP/PACAP on NFκB, CREB and IRF-1 in relationship to the expression of TNF-α, IL-10, iNOS and IL-12 p40 genes are illustrated in Fig. 1.

Protective Effect of VIP/PACAP in Lethal Endotoxemia

The severe pathological consequences of the septic shock syndrome result from a hyperactive and out-of-

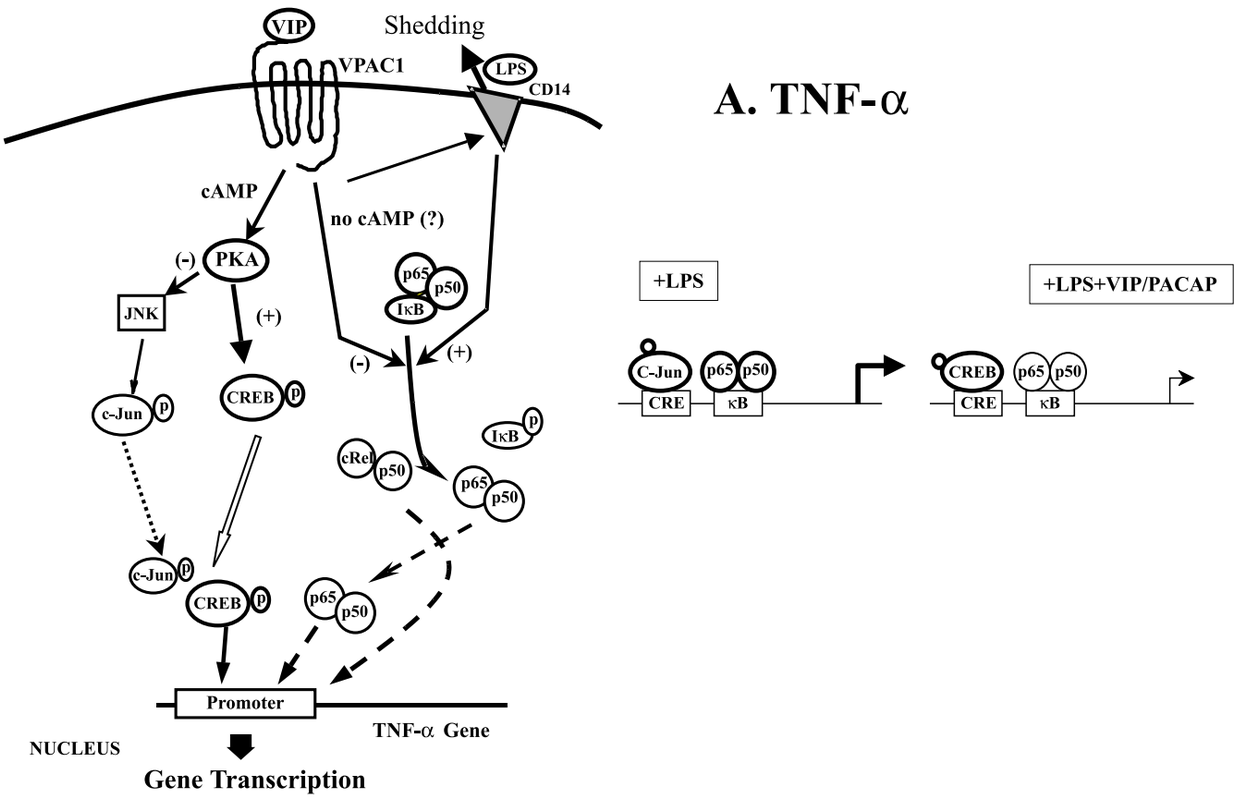
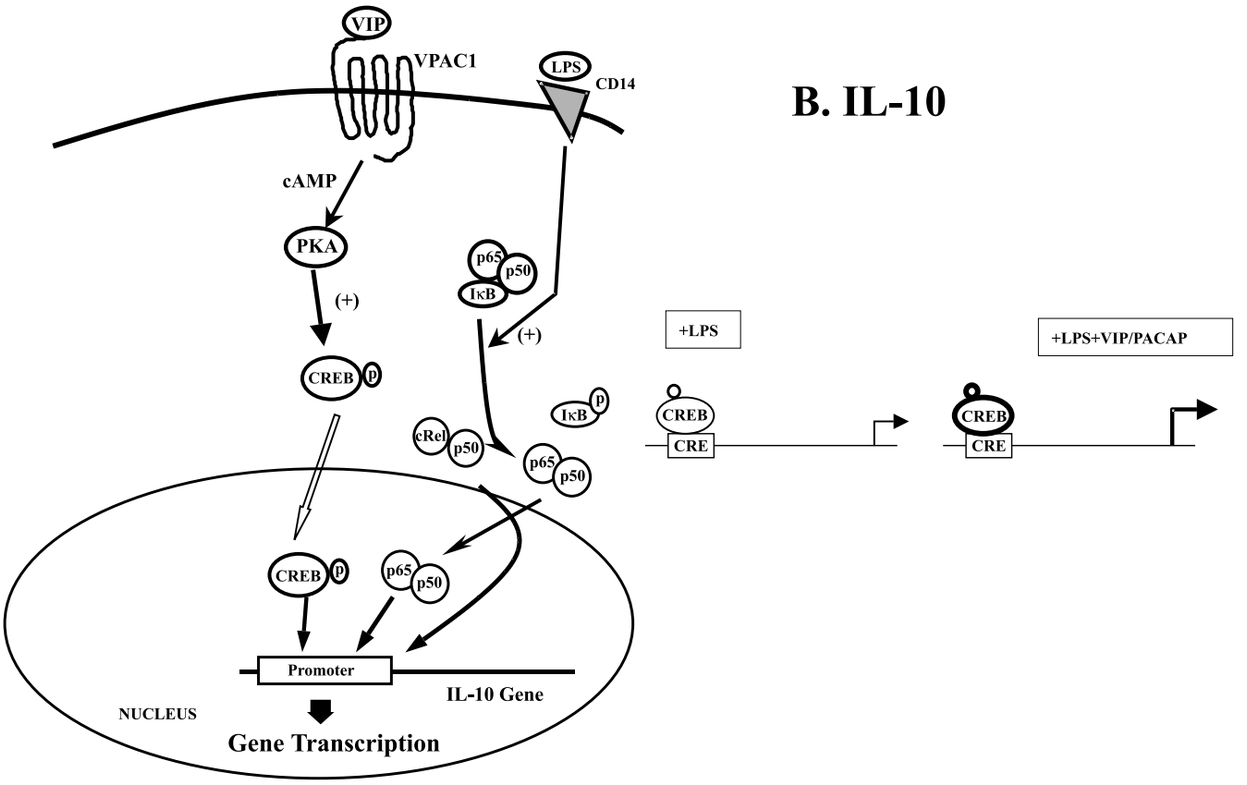
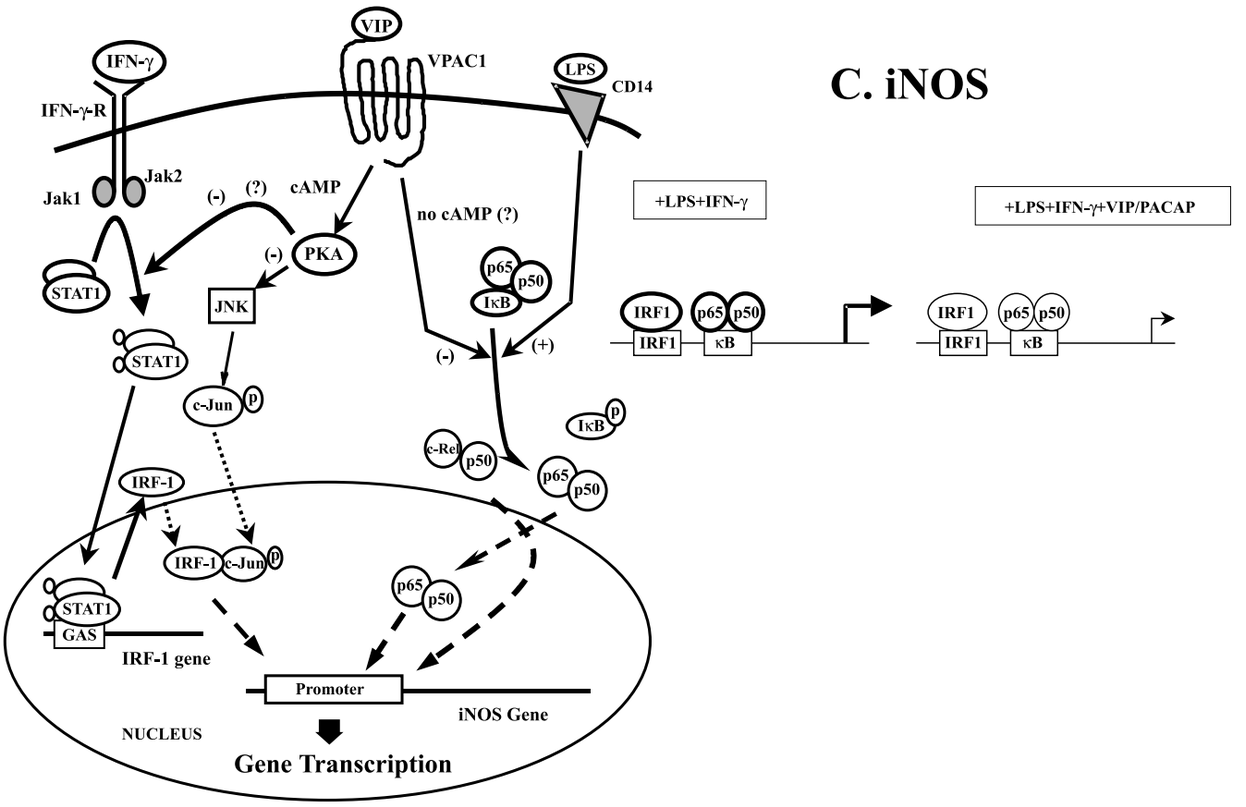
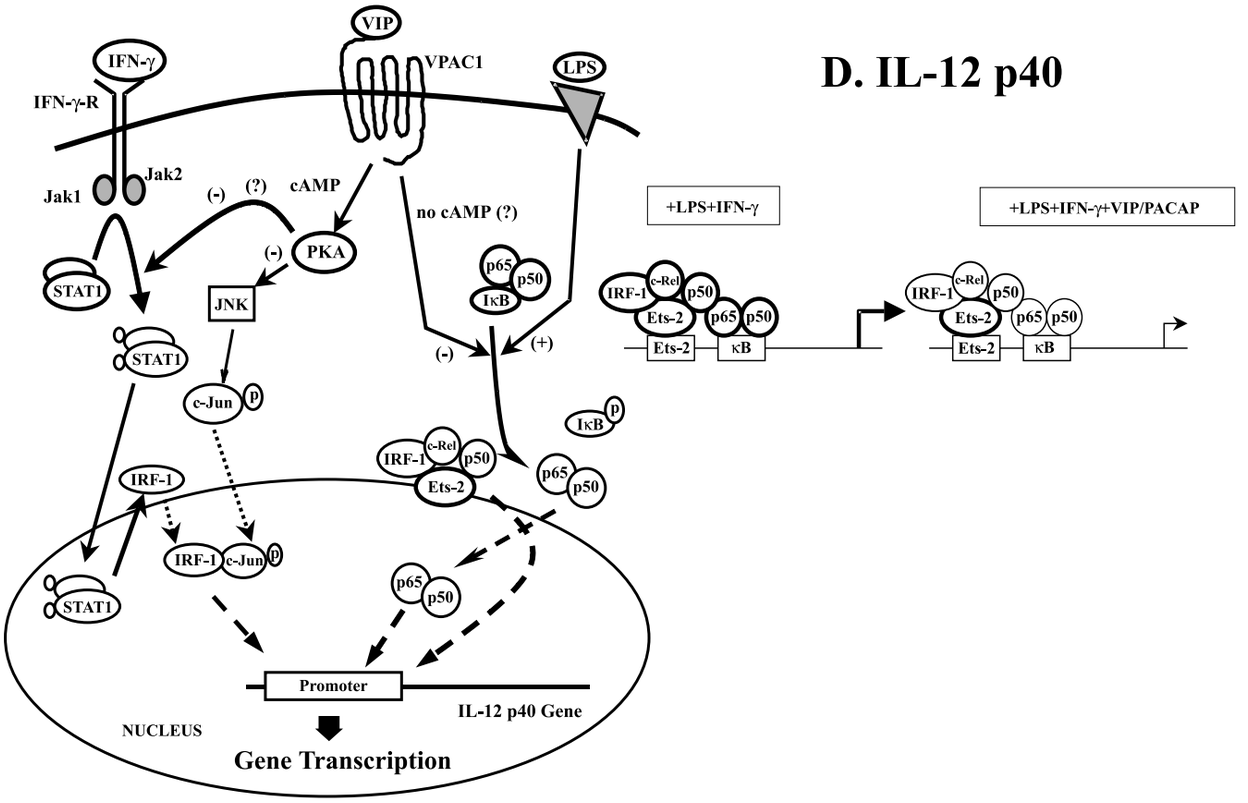


Fig. 1. Transduction pathways and transcriptional factors affected by VIP/PACAP in LPS or LPS/IFN-γ-stimulated macrophages. **A. TNF-α** – the cAMP-dependent pathway leads to the inhibition of JNK and therefore reduction in c-Jun, and an increase in CREB phosphorylation. As a result, c-Jun is replaced by CREB at the CRE site. The cAMP-independent pathway inhibits IκB phosphorylation and prevents the nuclear translocation of c-Rel and p65. Decreases in nuclear NFκB and changes in the CRE-binding complexes lead to reduced TNF-α transcription. **B. IL-10** – the cAMP, dependent pathway induces CREB phosphorylation and the increase in transcriptionally active CREB increases IL-10 gene transcription. **C. iNOS** – the cAMP-dependent pathway inhibits JNK activity and, possibly, STAT1 phosphorylation. As a result, IRF-1 transcription is inhibited. The cAMP-independent pathway inhibits IκB phosphorylation and prevents the nuclear translocation of c-Rel and p65. Decreases in nuclear NFκB and IRF-1 reduce iNOS gene transcription. **D. IL-12 p40** – the cAMP-dependent pathway inhibits JNK activity and, possibly, STAT1 phosphorylation. As a result, IRF-1 transcription is inhibited. IRF-1 participates as a member of the Ets-2 complex to the transcriptional activation of the IL-12 p40 gene. The reduction in IRF-1 and the decrease in nuclear NFκB through the cAMP-independent pathway lead to a reduced IL-12 p40 gene transcription. Explanations: (–) indicates an inhibitory effect; (+) a stimulatory effect. On the right side of each of the figures, bold circling means increased, and thin circling reduced levels of the transactivating factors.


B. IL-10
VIP
VPAC1
LPS
CD14
cAMP
PKA
(+)
p65
p50
IκB
(+)
CREB
P
IκB
P
cRel
p50
p65
p50
+LPS
CREB
CRE
+LPS+VIP/PACAP
CREB
CRE
CREB
P
p65
p50
Promoter
IL-10 Gene
NUCLEUS
Gene Transcription


C. iNOS
VIP
VPAC1
LPS
CD14
IFN-γ
IFN-γ-R
Jak1
Jak2
(-)
(?)
cAMP
no cAMP (?)
(-)
PKA
p65
p50
IκB
STAT1
JNK
(-)
(+)
STAT1
c-Jun
P
IκB
P
c-Rel
p50
p65
p50
IRF-1
IRF-1
c-Jun
P
p65
p50
STAT1
GAS
IRF-1 gene
Promoter
iNOS Gene
NUCLEUS
Gene Transcription
+LPS+IFN-γ
IRF1
p65
p50
IRF1
κB
+LPS+IFN-γ+VIP/PACAP
IRF1
p65
p50
IRF1
κB



-control network of endogenous pro-inflammatory cytokines[68]. Since VIP/PACAP inhibit the production of pro-inflammatory macrophage-derived cytokines *in vivo* and *in vitro*, it was expected that the two neuropeptides might protect against endotoxemia. Indeed, VIP/PACAP had a protective effect against lethal high endotoxemia in a murine model for septic shock[15]. VIP/PACAP acted in a dose-dependent and time-dependent manner, and the surviving VIP/PACAP-treated individuals did not present any of the histopathological alterations associated with septic shock. Initial experiments indicated a reduction of both TNF-α and IL-6 at the protein and mRNA levels. Subsequent experiments showed similar reductions in IL-12, IFN-γ and NO in serum and peritoneal fluid, associated with decreases in the corresponding mRNAs and an increase in IL-10[20]. These results suggest that VIP/PACAP exert their protective effect through a generalized inhibition of the pro-inflammatory cytokine network and through the up-regulation of other anti-inflammatory agents such as IL-10. Since VIP/PACAP appear to affect multiple cytokines and inflammatory factors, they might provide a more efficient therapeutical alternative to the use of specific cytokine antibodies or antagonists.

Effects of VIP/PACAP on Macrophage B7 Expression and the Subsequent Th1/Th2 Differentiation

In addition to their function in innate immunity, macrophages participate in the initiation of acquired or specific immunity through their capacity to present the antigen to T cells expressing the appropriate receptor. To activate T helper (Th) cells, macrophages have to provide a second, costimulatory signal in addition to the MHC class II/antigen complex recognized by the specific T cell receptor. Among the costimulatory molecules, B7.1 and B7.2 play major roles. In macrophages, B7.1 and B7.2 are expressed only following activation, with B7.2 being induced earlier and at higher levels than B7.1[49].

VIP and PACAP belong to the family of endogenous factors that regulate B7 expression in macrophages. Interestingly, the two neuropeptides affect B7 expression in resting and activated macrophages in an opposite manner. In resting macrophages, VIP/PACAP up-regulate B7.2, but not B7.1 expression at mRNA and protein level both *in vivo* and *in vitro*. In contrast, in LPS/IFN-γ-activated macrophages, VIP/PACAP down-

-regulate both B7.1 and B7.2 expression[22]. The effect of VIP/PACAP on B7 expression correlates with effects on the costimulatory activity for T cells[22].

The different subsets of effector Th cells play different roles in the immune response, with Th1 being primarily involved in cell-mediated immunity and Th2 in antibody production. Although the biological significance of the differential expression of B7.1 and B7.2 is not clear, several *in vivo* models suggest the involvement of B7.2 in Th2 differentiation[45, 46]. In agreement with the capacity to induce B7.2, but not B7.1 in unstimulated macrophages, VIP/PACAP favor the development of Th2 cells both *in vivo* and *in vitro*[13]. As a consequence, the *in vivo* administration of VIP/PACAP also induces a shift in antibody isotype, increasing IgG1 and decreasing IgG2a levels[13].

If the reduction in B7.1/B7.2 expression in activated macrophages is easy to incorporate into the general physiological role proposed for VIP/PACAP as endogenous anti-inflammatory agents, the induction of B7.2 in unstimulated macrophages, and the subsequent induction of costimulatory activity for Th cells, appears to contradict the proposed model. However, since the final outcome is the predominant development of a Th2 response, with subsequent inhibition of Th1 differentiation, and since the Th1 response is associated with inflammatory reactions typical for cell-mediated immunity, the VIP/PACAP inhibition of Th1 development may represent an additional mechanism for their general anti-inflammatory activity.

Conclusions

VIP, and the structurally related neuropeptide PACAP, present in the microenvironment of lymphoid organs, modulate the function of inflammatory cells through specific receptors. Production and release of both pro-inflammatory and anti-inflammatory cytokines by activated phagocytes is a crucial event in the initiation and subsequent down-regulation of the immune response. VIP and PACAP inhibit the production

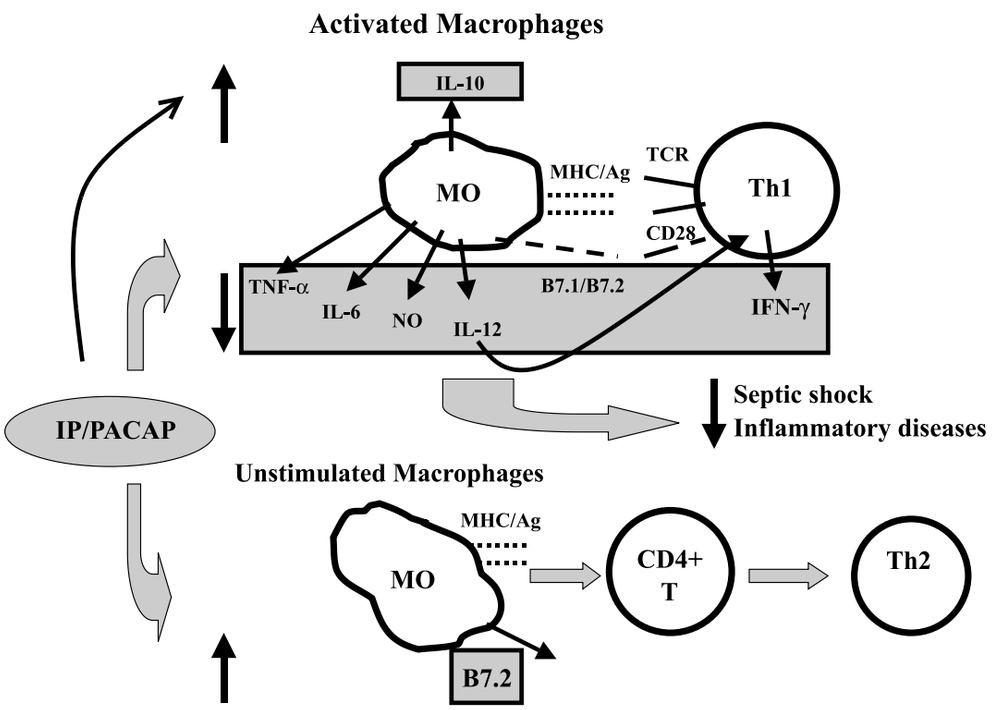


Fig. 2. Model for the *in vivo* immunomodulatory functions of VIP/PACAP on macrophages. **Activated macrophages** – VIP/PACAP inhibit the release of NO and of the pro-inflammatory macrophage-derived cytokines IL-6, TNF-α and IL-12, and of the IL-12, induced release of IFN-γ from Th1 cells. They also inhibit the expression of the costimulatory molecules B7.1/B7.2 and the subsequent stimulation of Th cells. In contrast, VIP/PACAP augment the production of the anti-inflammatory cytokine IL-10. Through their effects on both the pro- and anti-inflammatory cytokine network, VIP/PACAP might protect from the deleterious consequences of a hyperactive pro-inflammatory cytokine network in septic shock and inflammatory diseases. **Unstimulated macrophages** – VIP/PACAP induce the expression of B7.2 and subsequent activation of Th cells, leading to the preferential development of Th2 effector cells

of the pro-inflammatory cytokines TNF-α, IL-6, IL-12 and nitric oxide and stimulate the production of the anti-inflammatory cytokine IL-10, in activated macrophages *in vitro* and *in vivo*. The protective effect of VIP/PACAP in a high-endotoxic murine model for septic shock is, presumably, mediated through the effects on the cytokine network.

Macrophages function as antigen-presenting cells, initiating the activation of Th cells. VIP/PACAP reduce the expression of the costimulatory B7.1/B7.2 molecules and the subsequent stimulatory activity for Th cells in stimulated macrophages. In contrast, in unstimulated macrophages, VIP/PACAP induce specific B7.2 expression and, subsequently, promote T cell differentiation into Th2 effector cells.

We propose the following model for the immunomodulatory role of VIP/PACAP *in vivo* (Fig. 2). Antigenic stimulation initiates the early production of pro-inflammatory cytokines by activated macrophages, followed by the later secretion of anti-inflammatory factors. Among these, the neuropeptides VIP and PACAP are released from the peptidergic innervation or the immune cells, bind to macrophages expressing the VIP/PACAP receptors and modulate cytokine and iNOS expression. The production of pro-inflammatory agents such as TNF-α, IL-6, IL-12 and NO is inhibited and the production of anti-inflammatory cytokines such as IL-10 is stimulated. In septic shock the physiological "macrophage deactivating agents", including VIP/ /PACAP, are overwhelmed by the excessive pro-inflammatory cytokine network. However, exogenous administration of VIP/PACAP might have a significant therapeutical potential, since, in contrast to neutralizing antibodies and receptor antagonists directed against a single cytokine, VIP and PACAP directly interact with macrophages to suppress the LPS-induced production of TNF-α, IL-6, IL-12 and NO. In addition, VIP/PACAP inhibit B7.1/B7.2 expression in activated macrophages and reduce their subsequent stimulatory capacity for T cells.

Although most of the immunomodulatory functions of VIP/PACAP are in agreement with an *in vivo* anti--inflammatory role, recent observations suggest a more complex pattern of immune regulation, depending on the activation and developmental stage of the cellular targets. For example, VIP/PACAP induce B7.2 expression and IL-6 production in unstimulated macrophages, in contrast to their inhibitory effect on activated macrophages. Therefore, the physiological consequences of VIP/PACAP presence in the immune microenvironment may depend on the timing of their release and the activation stage of the neighboring immune cells.

References

1. AHMED A. A., WAHBI A., NORDLIND K., KHARAZMI A., SUNDQVIST K. G., MUTT V. and LIDEN S. (1998): *In vitro Leishmania major* promastigote-induced macrophage migration is modulated by sensory and autonomic neuropeptides. Scand. J. Immunol., **48**, 79–85.
2. ALLESCHER H. D., KURJAK M., HUBER A., TRUDRUNG P. and SCHUSDZIARRA V. (1996): Regulation of VIP release from rat enteric nerve terminals: evidence for a stimulatory effect of NO. Am. J. Physiol., **271**, G568–G574.
3. BELLINGER D. L., LORTON D., ROMANO T. D., OLSCHOWKA J. A., FELTEN S. Y. and FELTEN D. L. (1990): Neuropeptide innervation of lymphoid organs. Ann. NY Acad. Sci., **594**, 17–33.
4. BRANDTZAEG P., OKTEDALEN O., KIERULF P. and OPSTAD P. K. (1989): Elevated VIP and endotoxin plasma levels in human Gram-negative septic shock. Regul. Pept., **24**, 37–44.
5. BRONER C. W., O'DORISIO M. S., ROSENBERG R. B. and O'DORISIO T. M. (1995): Cyclic nucleotides and VIP production in a rabbit model of *E. coli* septicemia. Am. J. Med. Sci., **309**, 267–277.
6. COOK G. A., ELLIOT D., METWALI A., BLUM A. M., SANDOR M., LYNCH R. and WEINSTOCK J. V. (1994): Molecular evidence that granuloma T lymphocytes in murine schistosomiasis mansoni express an authentic substance P (NK-1): receptor. J. Immunol., **152**, 1830–1835.
7. DE LA FUENTE M., DELGADO M., DEL RIO M., GARRIDO E., LECETA J., HERNANZ A. and GOMARIZ R. P. (1994): Vasoactive intestinal peptide modulation of adherence and mobility in rat peritoneal lymphocytes and macrophages. Peptides, **15**, 1157–1163.
8. DE LA FUENTE M., DELGADO M., DEL RIO M., MARTINEZ C., HERNANZ A. and GOMARIZ R. P. (1993): Stimulation by VIP of phagocytic function in rat macrophages. Protein kinase C involvement. Regul. Pept., **48**, 345–353.
9. DELGADO M. and GANEA D. (1999): Vasoactive intestinal peptide and pituitary adenylate cyclase activating polypeptide inhibit IL-12 transcription by regulating NFκB and Ets activation. J. Biol. Chem., **274**, 31930–31940.
10. DELGADO M. and GANEA D. (2000): VIP and PACAP inhibit antigen-induced apoptosis of mature T lymphocytes by inhibiting FasL expression. J. Immunol., **164**, 1200–1210.
11. DELGADO M., GARRIDO E., DE LA FUENTE M. and GOMARIZ R. P. (1996): PACAP-38 stimulates rat peritoneal macrophage functions. Peptides, **17**, 1097–1105.
12. DELGADO M., LECETA J., ABAD C., MARTINEZ C., GANEA D. and GOMARIZ R. P. (1999): Shedding of membrane-bound CD14 from LPS-stimulated macrophages by VIP and PACAP. J. Neuroimmunol., **99**, 61–71.
13. DELGADO M., LECETA J., GOMARIZ R. P. and GANEA D. (1999): VIP and PACAP stimulate the induction of Th2 responses by upregulating B7.2 expression. J. Immunol., **163**, 3629–3635.
14. DELGADO M., MARTINEZ C., JOHNSON M. C., GOMARIZ R. P. and GANEA D. (1996): Differential expression of vasoactive intestinal peptide receptors 1 and 2 (VIP-R1 and VIP-R2) mRNA in murine lymphocytes. J. Neuroimmunol., **68**, 27–38.
15. DELGADO M., MARTINEZ C., POZO D., CALVO J. R., LECETA J., GANEA D. and GOMARIZ R. P. (1999): Vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating polypeptide (PACAP) protect mice from lethal endotoxemia

through the inhibition of TNF-α and IL-6. J. Immunol., **162**, 1200–1205.
16. DELGADO M., MUNOZ-ELIAS E. J., GOMARIZ R. P. and GANEA D. (1999): VIP and PACAP inhibit IL-12 production in LPS--stimulated macrophages. Subsequent effect on IFN-γ synthesis by T cells. J. Neuroimmunol., **96**, 167–181
17. DELGADO M., MUNOZ-ELIAS E. J., GOMARIZ R. P. and GANEA D. (1999): Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide enhance IL-10 production by murine macrophages: *in vitro* and *in vivo* studies. J. Immunol., **162**, 1707–1716.
18. DELGADO M., MUNOZ-ELIAS E. J., GOMARIZ R. P. and GANEA D. (1999): VIP and PACAP prevent inducible nitric oxide synthase transcription in macrophages by inhibiting NF-κB and interferon regulatory factor 1 activation. J. Immunol., **162**, 4685–4696.
19. DELGADO M., MUNOZ-ELIAS E. J., KAN Y., GOZES I., FRIDKIN M., BRENNEMAN D. E., GOMARIZ R. P. and GANEA D. (1998): Vasoactive intestinal peptide and pituitary adenylate cyclase--activating polypeptide inhibit TNF-α transcriptional activation by regulating NF-κB and CREB/c-Jun. J. Biol. Chem., **273**, 31427–31436.
20. DELGADO M., MUNOZ-ELIAS E. J., MARTINEZ C., GOMARIZ R. P. and GANEA D. (1999): VIP and PACAP38 modulate cytokine and nitric oxide production in peritoneal macrophages and macrophage cell lines. Ann. NY Acad. Sci., **897**, 401–414.
21. DELGADO M., POZO D., MARTINEZ C., LECETA J., CALVO J. R., GANEA D. and GOMARIZ R. P. (1999): Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit endotoxin-induced TNF-α production by macrophages: *in vitro* and *in vivo* studies. J. Immunol., **162**, 2358–2367.
22. DELGADO M., SUN W., LECETA J. and GANEA D. (1999): VIP and PACAP differentially regulate the costimulatory activity of resting and activated macrophages through the modulation of B7.1 and B7.2 expression. J. Immunol., **163**, 4213–4223.
23. DEWIT D., GOURLET P., AMRAOUI Z., VERTONGEN P., WILLEMS F., ROBBERECHT P. and GOLDMAN M. (1998): The vasoactive intestinal peptide analogue RO25-1553 inhibits the production of TNF and IL-12 by LPS-activated monocytes. Immunol. Lett., **60**, 57–60.
24. DEY R. D., SHANNON W. A. and SAID S. I. (1981): Localization of VIP-immunoreactive nerves in airways and pulmonary vessels of dogs, cats and human subjects. Cell Tissue Res., **220**, 231–238.
25. EVANS C. H. (1995): Nitric oxide: what role does it play in inflammation and tissue destruction? Agents Actions, **47**, 107–116.
26. FELTEN D. L., FELTEN S. Y., BELLINGER D. L., CARLSON S. L., ACKERMAN K. D., MADDEN K. S., OLSCHOWKA J. A. and LIVNAT S. (1987): Noradrenergic sympathetic neural interactions with the immune system: Structure and function. Immunol. Rev., **100**, 225–260.
27. FELTEN D. L., FELTEN S. Y., BELLINGER D. L. and LORTON D. (1992): Noradrenergic and peptidergic innervation of secondary lymphoid organs: role in experimental rheumatoid arthritis. Eur. J. Clin. Invest., **22** (suppl. 1), 37–41.
28. FINK T. and WEIHE E. (1988): Multiple neuropeptides in nerves supplying mammalian lymph nodes: Messenger candidates for sensory and autonomic neuroimmunomodulation. Neurosci. Lett., **90**, 39–54.
29. FIORENTINO D. F., ZLOTNIK A., MOSSMAN T. R., HOWARD M. and O'GARRA A. (1991): Interleukin-10 inhibits cytokine production by activated macrophages. J. Immunol., **147,** 3815–3822.
30. GARRIDO E., DELGADO M., MARTINEZ C., GOMARIZ R. P. and DE LA FUENTE M. (1996): PACAP38 modulates lymphocyte and macrophage functions: stimulation of adherence and opposite effect on mobility. Neuropeptides, **30**, 583–595.
31. GAYTAN F., MARTINEZ-FUENTES A. J., GARCIA-NAVARRO F., VAUDRY H. and AGUILAR E. (1994): PACAP immunolocalization in lymphoid tissues of the rat. Cell Tissue Res., **276**, 223–227.
32. GOETZL E. J., KODAMA K. T., TURCK D. W., SCHIOGOLEV A. S. and SREEDHARAN S. P. (1989): Unique pattern of cleavage of VIP by human lymphocytes. Immunology, **66**, 554–558.
33. GOMARIZ R. P., DE lA FUENTE M., HERNANZ A. and LECETA J. (1992): Occurrence of VIP in lymphoid organs from rat and mouse. Ann. NY Acad. Sci., **650**, 13–18.
34. GOMARIZ R. P., GARRIDO E., LECETA J., MARTINEZ C., ABALO R. and DELGADO M. (1994): Gene expression of VIP receptor in rat lymphocytes. Biochim. Biophys. Res. Commun., **203**, 1599–1604.
35. GOMARIZ R. P., LECETA J., GARRIDO E., GARRIDO T. and DELGADO M. (1994): VIP mRNA expression in rat T and B lymphocytes. Regul. Pept., **50**, 177–184.
36. GRIDER J. R. and JIN J.-G. (1993): Vasoactive intestinal peptide release and L-citrulline production from isolated ganglia of the myenteric plexus: evidence for regulation of vasoactive intestinal peptide release by nitric oxide. Neuroscience, **54**, 521–526.
37. HANNIBAL J., JESSOP D. S., FAHRENKRUG J., HARBUZ M. S. and LARSEN P. J. (1999): PACAP gene expression in neurons of the rat hypothalamo-pituitary-adrenocortical axis is induced by endotoxin and interleukin-1beta. Neuroendocrinology, **70**, 73–82.
38. HARMAR A. J., ARIMURA A., GOZES I., JOURNOT L., LABURTHE M., PISEGNA J. R., RAWLINGS S. R., ROBBERECHT P., SAID S. I., SREEDHARAN S. P., WANK S. A. and WASHECK J. A. (1998): Nomenclature of receptors for vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase activating polypeptide (PACAP). Pharmacol. Rev., **50**, 625–627.
39. HIRUMA K., KOIKE T., NAKAMURA H., SUMIDA T., MAEDA T., TOMIOKA H., YOSHIDA S. and FUJITA T. (1990): Somatostatin receptors on human lymphocytes and leukemia cells. Immunology, **71**, 480–485.
40. ICHIKAWA S., SREEDHARAN S. P., GOETZL E. J. and OWEN R. L. (1994): Immunohistochemical localization of peptidergic nerve fibers and neuropeptide receptors in Peyer's patches of the cat ileum. Regul. Pept., **54**, 385–395.
41. ICHINOSE M., SAWADA M. and MAENO T. (1994): Inhibitory effect of VIP on phagocytosis in mouse peritoneal macrophages. Regul. Pept., **54**, 457–466.
42. ISHIZUKA T., KUROSAWA M., HANAWA K., AOKI S., KOBAYASHI H. and MORI M. (1992): Inhibitory effects of vasoactive intestinal peptide on superoxide anion generation from stimulated human inflammatory cells. Arerugi, **41**, 504–511.
43. JIANG X., WANG H.-Y., YU J. and GANEA D. (1998): VIP1 and VIP2 receptors but not PVR1 mediate the effect of VIP/PACAP on cytokine production in T lymhpocytes. Ann. NY Acad. Sci., **865**, 397–407.
44. KALTREIDER H. B., ICHIKAWA S., BYRD P. K., INGRAM D. A., KISHIYAMA J. I., SREEDHARAN S. P., WARNOCK M. L., BECK J. M. and GOETZL E. J. (1997): Upregulation of neuropeptides and neuropeptide receptors in a murine model of immune in-

flammation in lung parenchyma. Am. J. Respir. Cell. Mol. Biol., **16**, 133–144.
45. KEANY-MYERS A. M., GAUSE W. C., FINKELMAN F. D., XHOU X.-D. and WILLS-KARP M. (1998): Development of murine allergic asthma is dependent upon B7-2 costimulation. J. Immunol., **160**, 1036–1043.
46. KUCHROO V. K., DAS M. P., BROWN J. A., RANGER A. M., ZAMVIL S. S., SOBEL R. A., WEINER H. L., NABAVI N. and GLIMCHER L. H. (1995): B7.1 and B7.2 costimulatory molecules activate differentially the Th1/Th2 developmental pathways: application to autoimmune disease therapy. Cell, **80**, 707–718.
47. KUROSAWA M. and ISHIZUKA T. (1993): Inhibitory effects of vasoactive intestinal peptide on superoxide anion formation by N-formyl-methionyl-leucyl-phanylananine-activated inflammatory cells *in vitro*. Int. Arch. Allergy Immunol., **100**, 28–34.
48. LASKIN D. L. and PENDINO K. J. (1995): Macrophages and inflammatory mediators in tissue injury. Ann. Rev. Pharmacol. Toxicol., **35**, 655–677.
49. LENSCHOW D. J., WALUNAS T. L. and BLUESTONE J. A. (1996): CD28/B7 system of T cell costimulation. Annu. Rev. Immunol., **14**, 233–258.
50. LITWIN D. K., WILSON A. K. and SAID S. I. (1992): VIP inhibits rat alveolar macrophage phagocytosis and chemotaxis *in vitro*. Regul. Pept., **40**, 63–74.
51. MARTINEZ C., DELGADO M., ABAD C., GOMARIZ R. P., GANEA D. and LECETA J. (1999): Regulation of VIP production and secretion by murine lymphocytes. J. Neuroimmunol., **93**, 126–138.
52. MARTINEZ C., DELGADO M., POZO D., LECETA J., CALVO J. R., GANEA D. and GOMARIZ R. P. (1998): VIP and PACAP enhance IL-6 release and mRNA levels in resting peritoneal macrophages: *in vitro* and *in vivo* studies. J. Neuroimmunol., **85**, 155–167.
53. MARTINEZ C., DELGADO M., POZO D., LECETA J., CALVO J. R., GANEA D. and GOMARIZ R. P. (1998): Vasoactive intestinal peptide and pituitary adenylate cyclase activating polypeptide modulate endotoxin-induced IL-6 production by murine peritoneal macrophages. J. Leukoc. Biol., **63**, 591–601.
54. MCCORMACK R. J., HART R. P. and GANEA D. (1996): Expression of NK-1 receptor mRNA in murine T lymphocytes. NeuroImmunoModulation, **3**, 35–46.
55. MCGILLIS J. P., HUMPHRIES S. and REID S. (1991): Characterization of functional calcitonin gene-related peptide receptors on rat lymphocytes. J. Immunol., **147**, 3482–3489.
56. METWALI A., ELLIOTT D., BLUM A. M., LI J., SANDOR M. and WEINSTOCK J. V. (1996): T cell vasoactive intestinal peptide receptor subtype expression differs between granulomas and spleen of schistosome-injected mice. J. Immunol., **157**, 265–270.
57. MOSMANN B. L., WHITE M. V., HOHMAN R. J., GOLDRICH M. S., KAULBACH H. C. and KALINER M. A. (1993): Substance P, calcitonin gene-related peptide, and vasoactive intestinal peptide increase in nasal secretions after allergen challenge in atopic patients. J. Allergy Clin. Immunol., **92**, 95–104.
58. MUCHAMUEL T., MENON S., PISACANE P., HOWARD M. C. and COCKAYNE D. A. (1997): IL-13 protects mice from LPS-induced lethal endotoxemia. J. Immunol., **158,** 2898–2903.
59. NIEBER K., BAUMGARTEN C. R., RATHSACK R., FURKERT J., OEHME P. and KUNKEL G. (1992): Substance P and beta-endorphin-like immunoreactivity in lavage fluid of subjects with and without allergic asthma. J. Allergy Clin. Immunol., **90**, 646–654.
60. NOHR D. and WEIHE E. (1991): The neuroimmune link in the bronchus-associated lymphoid tissue (BALT): of cat and rat: peptides and neural markers. Brain Behav. Immun., **5**, 84–101.
61. PEARSE A. G. E., POLAK J. M. and BLOOM S. R. (1977): The newer gut hormone. Cellular sources, physiology, pathology and clinical aspects. Gastroenterology, **72**, 746–761.
62. RAWLINGS S. R. and HEZAREH M. (1996): Pituitary adenylate cyclase activating polypeptide and PACAP/vasoactive intestinal polypeptide receptors: actions on the anterior pituitary gland. Endocrine Rev., **17**, 4–29.
63. SEGURA J. J., GUERRERO J. M., LOPEZ-GONZALEZ M. A. and CALVO J. R. (1993): VIP inhibits substrate adherence capacity of rat peritoneal macrophages by a mechanism that involves cAMP. Cell. Adhes. Commun., **1**, 213–221.
64. SREEDHARAN S. P., KODAMA K. T., PETERSON K. E. and GOETZL E. J. (1989): Distinct subsets of somatostatin receptors on cultured human lymphocytes. J. Biol. Chem., **264**, 949–952.
65. TREPICCHIO W., BOZZA M., PEDNEAULT G. and DORNER A. (1996): Recombinant human IL-11 attenuates the inflammatory response through down-regulation of proinflammatory cytokine release and nitric oxide production. J. Immunol., **157**, 3627–3634.
66. TSUNAWAKI S., SPORN M., DING A. and NATHAN C. F. (1988): Deactivation of macrophages by transforming growth factor β. Nature, **334,** 260–262.
67. USDIN T. R., BONNER T. I. and MRZEY E. (1994): Two receptors for vasoactive intestinal peptide with similar specificity and complementary distributions. Endocrinology, **135**, 2662–2680.
68. VAN DEUREN M., DOFFERHOFF A. S. and VAN DER MEER J. W. (1992): Cytokines and the response to infections. J. Pathol., **168**, 349–356.
69. VAN SNICK J. (1990): Interleukin 6: an overview. Annu. Rev. Immunol., **8**, 253–278.
70. VASSALLI P. (1992): The pathophysiology of the tumour necrosis factor. Annu. Rev. Immunol., **10**, 411–452.
71. WEIHE E., NOHR D., MICHEL S., MULLER S., ZENTEL H.-J., FINK T. and KREKEL J. (1991): Molecular anatomy of the neuro-immune connection. Int. J. Neurosci., **59**, 1–23.
72. WEINSTOCK J. V. and BLUM A. M. (1990): Detection of VIP and localization of its mRNA within granulomas of murine schistosomiasis. Cell. Immunol., **125**, 291–300.
73. WIIK P. (1989): Vasoactive intestinal peptide inhibits the respiratory burst in human monocytes by a cyclic AMP-mediated mechanism. Regul. Pept., **25**, 187–197.
74. XIA M., SREEDHARAN S. P. and GOETZL E. J. (1996): Predominant expression of type II vasoactive intestinal peptide receptors by human T lymphoblastoma cells. J. Clin. Immunol., **16**, 21–30.
75. XIN Z., JIANG X., WANG H.-Y., DENNY T. N., DITTEL B. N. and GANEA D. (1997): Effect of vasoactive intestinal peptide (VIP) on cytokine production and expression of VIP receptors in thymocyte subsets. Regul. Pept., **72**, 41–54.
76. XIN Z. and SRIRAM S. (1998): Vasoactive intestinal peptide inhibits IL-12 and nitric oxide production in murine macrophages. J. Neuroimmunol., **89**, 206–212.

Received in December 1999
Accepted in January 2000