Translational Therapies for Immune-based Disorders Review Series

J. Cell. Mol. Med. Vol 12, No 5B, 2008 pp. 1830-1847

Guest Editor: D. Pozo

Endogenous anti-inflammatory neuropeptides and pro-resolving lipid mediators: a new therapeutic approach for immune disorders

Per Anderson, Mario Delgado*

Instituto de Parasitologia y Biomedicina, Consejo Superior de Investigaciones Cientificas, Granada 18100, Spain

Received: April 21, 2008; Accepted: June 5, 2008

Abstract

Identification of the factors that regulate the immune tolerance and control the appearance of exacerbated inflammatory conditions is crucial for the development of new therapies of inflammatory and autoimmune diseases. Although much is known about the molecular basis of initiating signals and pro-inflammatory chemical mediators in inflammation, it has only recently become apparent that endogenous stop signals are critical at early checkpoints within the temporal events of inflammation. Some neuropeptides and lipid mediators that are produced during the ongoing inflammatory response have emerged as endogenous anti-inflammatory agents that participate in the regulation of the processes that ensure self-tolerance and/or inflammation resolution. Here we examine the latest research findings, which indicate that neuropeptides participate in maintaining immune tolerance in two distinct ways: by regulating the balance between pro-inflammatory and anti-inflammatory factors, and by inducing the emergence of regulatory T cells with suppressive activity against autoreactive T-cell effectors. On the other hand, we also focus on lipid mediators biosynthesized from ω-3 and ω-6 polyunsaturated fatty-acids in inflammatory exudates that promote the resolution phase of acute inflammation by regulating leucocyte influx to and efflux from local inflamed sites. Both anti-inflammatory neuropeptides and pro-resolving lipid mediators have shown therapeutic potential for a variety of inflammatory and autoimmune disorders and could be used as biotemplates for the development of novel pharmacologic agents.

Keywords: inflammation • autoimmunity • regulatory T cells • tolerance • lipid mediators • lipoxins • neuropeptide • resolvins

Introduction

Immune tolerance: a matter of life or death

The immune system responds to pathogen invasion with two temporarily separate but physically linked responses, mediated by different types of cells. The first response, termed innate immunity involves neutrophils, monocytes/macrophages and dendritic cells (DCs) in the periphery and microglia in the central nervous system (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

*Correspondence to: Mario DELGADO, Instituto de Parasitologia y Biomedicina, CSIC, Avd. Conocimiento, PT Ciencias de la Salud, Granada 18100, Spain.
Tel.: 34–958-181665; Fax: 34–958-181632;
E-mail: mdelgado@ipb.csic.es

doi:10.1111/j.1582-4934.2008.00387.x

lymphocytes through specific receptors. In contrast to the innate response, adaptive immunity leads to the development of memory for a specific antigen.

The successful elimination of most pathogens requires crosstalk between the innate and adaptive arms of the immune system. The innate immune system recognizes pathogen-associated molecular patterns through pattern-recognition receptors, such as Toll-like receptors (TLRs), which induce the release of pro-inflammatory cytokines, chemokines and free radicals, recruitment of inflammatory cells to the site of infection, and lysis of infected host cells by natural killer cells and cytotoxic T lymphocytes. The inflammatory process is vital to the survival of all complex organisms, and its functions play a profound role in health and disease. Although it is a localized protective response that serves to destroy the injurious agent, once the pathogen is eliminated, cells participating in innate and adaptive immunity have to be deactivated or eliminated, to re-establish tissue homeostasis. The accumulation and subsequent activation of leucocytes are central events in the pathogenesis of virtually all forms of inflammation. Uncontrolled activation of the immune system and the sustained production of inflammatory mediators lead to serious consequences for the host, such as tissue destruction, organ failure, even death. A further damage arises from potential autoimmune responses occurring during the inflammatory response, in which the immune cells and molecules that respond to pathogen-derived antigens can also react to self-antigens. Therefore, in inflammatory/autoimmune diseases like multiple sclerosis, rheumatoid arthritis, and type 1 diabetes, the initial stages involve multiple steps that can be divided into two main phases: early events associated with initiation and establishment of autoimmunity to self-tissue components in peripheral lymphoid organs, and later events associated with the evolving immune and destructive inflammatory responses in the target tissue (Fig. 1) [1, 2]. Progression of the autoimmune response involves the development of self-reactive T helper 1 (T_H1) and T_H17 cells, their entry into the target tissue, release of pro-inflammatory cytokines and chemokines and subsequent recruitment and activation of inflammatory cells (macrophages, neutrophils and mast cells). Production of inflammatory mediators, such as cytokines, matrix-degrading enzymes and free radicals by infiltrating cells and resident cells (synovial fibroblasts, osteoclasts, microglia cells) damages self-tissues. In addition, T_H1-mediated production of autoantibodies by B cells, which form immune complexes and activate complement and neutrophils, contributes to autoimmune pathology and disease propagation [2].

How to solve the problem of excessive inflammation: to counterbalance or to resolve

It is apparently clear that safe induction of antigen-specific long-term tolerance is critical for the control of autoreactive T cells on autoimmune diseases. In addition, the inflammatory process needs to be limited since excessive responses result in severe inflammation and collateral tissue damage. In general, inflammatory responses are self-controlled by anti-inflammatory mediators secreted by host innate immune system during the ongoing process, and the ability to control an inflammatory state depends on the local balance between pro- and anti-inflammatory factors [3]. Moreover, adaptive immune system also helps to maintain immune tolerance during infection-induced immunopathology [4]. Intrinsic control of lymphocytes is exerted for example by central clonal deletion of self-reactive T cells in the thymus *via* apoptosis of immature self-reactive lymphocytes upon the exposure to self-antigen or activation-induced cell death of mature effector cells. Moreover, recent evidence demonstrate that the generation of antigen-specific regulatory T cells (Treg) with suppressive activity plays a critical role in the induction of peripheral tolerance (Fig. 1). For example, depletion of $CD4^+CD25^+$ Treg cells produces autoimmune disease in otherwise normal animals, and their reconstitution prevents disease [4, 5]. In addition, Treg cells have been shown to be deficient in patients with rheumatoid arthritis, multiple sclerosis, type-1 diabetes and other autoimmune diseases [6–8]. Moreover, numerous studies have demonstrated the therapeutic use of antigen-specific Treg cells in various models of autoimmune disorders [3–5].

From a therapeutic point of view, the fact that the appearance of exacerbated inflammatory and autoimmune diseases is a consequence of an unbalance in pro-inflammatory factors *versus* anti-inflammatory cytokines, or in self-reactive T_H1 cells *versus* Treg cells, becomes critical to the identification of agents that restore self-tolerance by regulating both unbalanced inflammatory and autoreactive responses. Non-steroidal anti-inflammatory, steroid and antihistaminic compounds, for instance, were developed on this basis. Because maintenance of immune tolerance is essential for survival, it is plausible to suppose that immune cells might produce endogenous anti-inflammatory factors during the inflammatory/autoimmune response in an attempt to maintain it under control. Numerous researchers have concentrated their efforts investigating traditional immunosuppressive cytokines, such as IL-10, IL-13 and TGF-β1 [9]. However, others have focused their search in neuropeptides and hormones, classically considered as neuroendocrine mediators, but which are also produced by immune cells, especially under inflammatory conditions [10, 11].

Similarly, the resolution phase of acute inflammation has emerged as a new terrain for drug design and resolution-directed therapeutics [12–15]. Resolution of inflammation is an active regulated program that is required for the return from inflammatory disease to health and tissue homeostasis. This event is accompanied by lipid mediator class switching from pro-inflammatory prostaglandins (PGs) and leucotrienes (LT) to the biosynthesis of anti-inflammatory agents, such as lipoxins (LXs), and the newly described pro-resolving mediators synthesized in inflammatory exudates from ω-3 polyunsaturated fatty-acid (PUFA) precursors, resolvins and protectins [12–15].

In this review, we propose a platform for the pharmacologists to develop novel therapeutics based on endogenous agents that not only simply block what initiates and drives inflammation, but also restore immune tolerance and/or resolve the problem.

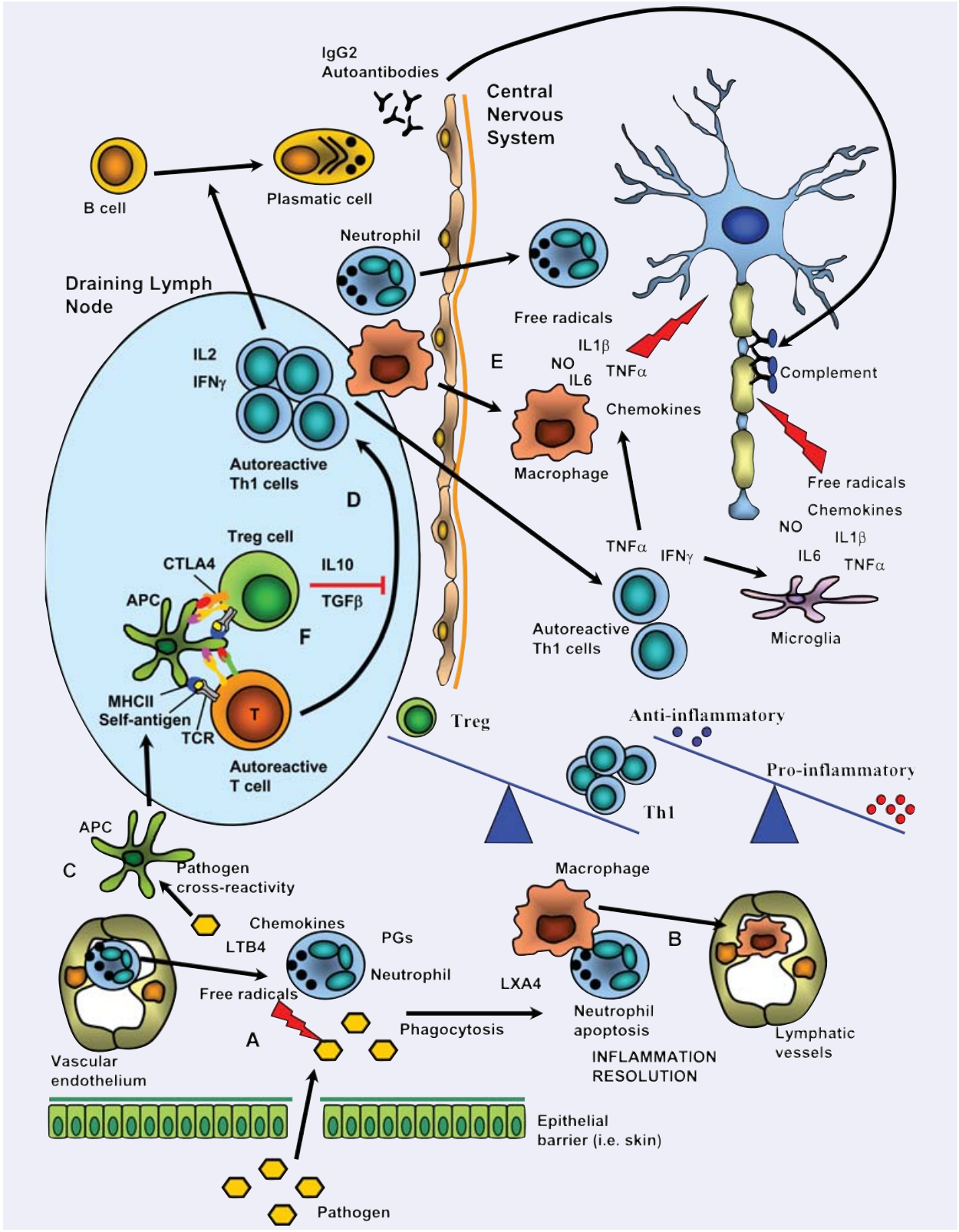

Fig. 1 Loss of immune tolerance compromises immune homeostasis and results in the onset of autoimmune disorders. Host invasion by pathogens or trauma initially stimulate prostaglandins (PGs) and initiate early events in acute inflammation. Inflammatory leucocytes migrate to the inflamed site or area of tissue damage, with neutrophils being the first cell types at the scene (**A**). Secretion of leucotrienes (LTB4) and chemokines promotes the recruitment of more phagocytes. Once pathogens are killed or phagocytosed, neutrophils are cleared from the inflamed site by returning to the circulation or suffering apoptosis and subsequent phagocytosis by newly migrated macrophages, following an active program of resolution, where lipoxins (LXA4) and other lipid mediators play a major role (**B**). If inflammation is not maintained under control or complete resolution fails, acute inflammation can lead to chronic inflammation, scarring and eventual loss of tissue function. A further damage arises from potential autoimmune responses occurring during the chronic inflammatory response, in which the antigen presenting cells (APC) and molecules that respond to pathogen-derived antigens can also cross-react to self-antigens (**C**). Progression of the autoimmune response (multiple sclerosis is shown as example) involves the development of self-reactive T helper 1 (T_H1) cells (**D**), their entry into the central nervous system (or target tissue), release of proinflammatory cytokines (tumour-necrosis factor-α (TNFα) and interferon-γ (IFNγ)) and chemokines and subsequent recruitment and activation of inflammatory cells (macrophages and neutrophils), which produce cytotoxic factors, such as cytokines, nitric oxide and free radicals (**E**). Finally, regulatory T (Treg) cells are key players in maintaining tolerance by their suppression of self-reactive T_H1 cells (**F**). Unbalance of Treg *versus* T_H1 cells, and/or of anti-inflammatory cytokines *versus* proinflammatory factors, is the cause of autoimmune disorders. Therapeutic opportunities to manage inflammatory and autoimmune disorders should be found in agents that regulate inflammation resolution, control Th1 expansion, inhibit inflammatory mediators and/or induced Treg cell generation. Interleukin-10, IL-10; transforming growth factor-β, TGFβ; cytotoxic T lymphocyte associated antigen 4, CTLA4; T cell receptor, TCR.

Anti-inflammatory neuropeptides and pro-resolving lipid mediators emerge as a new strategy to manage inflammation-based diseases.

Tuning immune tolerance with anti-inflammatory neuropeptides

Neuropeptides as biochemical mediators in nervous-immune communication

For many years, the neuroendocrine system and the immune system have been considered as two autonomous networks functioning to maintain a balance between host and environment. The neuroendocrine system responds to external stimuli such as temperature, pain and stress, whereas the immune system responds to exposure to bacteria, viruses and tissue trauma. However, in the last 20 years, we have come to realize that both systems mount a variety of coordinated responses to danger. Acting as a 'sixth sense', the immune system signals the brain to responds to the 'danger' of pathogen infection and inflammation, resulting in the orchestration of the febrile response and its subsequent effects on behaviour, including sleep, appetite and feeding [16]. Conversely, the immune system is regulated by the CNS, in response to environmental stress, either directly by the autonomic nervous system or by way of the hypothalamus-pituitary-adrenal (HPA) axis. This intimate bi-directional network is based on the fact that the immune and neuroendocrine systems share ligands such as neuropeptides, hormones, cytokines and the respective receptors. Therefore, it is reasonable to suggest that factors produced by the neuroendocrine system could contribute to the immune tolerance maintenance. Glucocorticoids and norepinephrin are the classical examples of endogenous immunosuppressive agents produced by the HPA axis and the sympathetic nervous system, respectively, in response to stress or systemic inflammation [16, 17]. Furthermore, various neuropeptides are released at the peripheral endings of sensory and efferent nerves in close proximity to immune cells in response to various invasive and inflammatory stimuli. The antinociceptive and anti-inflammatory effect of opioids released during neurogenic inflammation is the oldest example in this field [18]. From the growing list of neuropeptides currently thought to posses immunomodulatory actions (~50), some neuropeptides have lately emerged as potential candidates to treat the unwanted immune responses that occur in inflammatory and autoimmune disorders, by tuning immune homeostasis in a cytokine-like manner. In this review, we focus on the most recent developments regarding the effects on immune tolerance of some well-known anti-inflammatory neuropeptides and we highlight the effectiveness of using these neuropeptides in treating several immune disorders. The therapeutic effects of these neuropeptides on immune disorders have been classically attributed to their dual capacity to down-regulate the inflammatory response and to inhibit antigen-specific T_H1-driven responses [12]. Furthermore, recent data suggest that some of them might facilitate immune homeostasis through a newly discovered mechanism involving the generation of Treg cells.

Vasoactive intestinal peptide (VIP), α-melanocyte stimulating hormone (αMSH), urocortin, adrenomedullin, cortistatin and ghrelin are neuropeptides belonging to different families of peptides that show no homology among them [19–24] (Table 1). Their major cellular sources and the physiological roles that they play in the organism are very different (Table 1). Apparently these neuropeptides look very different, but they share certain characteristics that make them attractive for immune tolerance. First, they are produced by immune cells (Table 1), especially under inflammatory conditions, or following antigenic stimulation [25–31]. Second, they bind to G-protein-coupled receptors (GPCRs) expressed in different immune cells, including T cells, macrophages, monocytes, DCs and neutrophils [25, 28, 29, 32–39]. Third, they mainly signal through the activation of cAMP/protein kinase A (PKA) pathway [24, 25, 29, 40–42], which is considered as an immunosuppressive signal [43, 44]. Through the elevation of intracellular

Table 1. Anti-inflammatory neuropeptides: expression and functions[a]

Neuropeptide sequence [b]	Peptide family [c]	Main source [d]	Immune source [e]	Main actions [f]	Receptor type	Receptor in immune cells [g]
VIP						
HSDAVFTDNYT RLRKQMAVKKY LNSILN-NH_2	PACAP secretin glucagon GHRH	GI, CNS, heart, lung, thyroids, kidney, genital	CD4 Th2, CD8, PMN, Mast cells	vasodilatation, ↑cardiac output, bronchodilation, hyperglycemia, smooth muscle relaxation, ↑growth, hormonal regulation, analgesia, hypertermia, neurotrophic effects, learning and behaviour, bone metabolism, GI secretion, gastric motility	VPAC1 VPAC2 PAC1	T,MΦ,Mo,DC,PMN T,MΦ (after activation) MΦ,Mo
αMSH						
SYSMEHFRWG KPV-NH_2	POMC ACTH	CNS, pituitary, skin	T, Mo, DC	skin-darkening effects, learning, attention and memory, motor effects, ↓food intake	MC1R MC3R MC5R	T,MΦ,Mo,DC,PMN,NK, B MΦ,Mo
Urocortin						
DNPSLSIDLTFHLLRTL LELADTQSQRERAQN RIIFDSV-NH_2	CRH urotensin	CNS, pituitary, GI, testis, heart, skin, kidney	T, B, MΦ, Mo, Mast cells	vasodilatation, bronchodilatation, ↑cardiac output, smooth muscle relaxation, ↓food intake, ↑ACTH secretion	CRFR2	T,MΦ,Mo,DC,PMN
Adrenomedullin						
YRQSMNNFQGLRFG [CRFGTC]TVQKLAHQ IYQFTDKDKDNVAP RNKISPQGY-NH_2	calcitonin CGRP amylin	adrenal, CNS, all peripheral tissues with the exception of thyroid	MΦ, Mo	vasodilatation, bronchodilatation, ↑cardiac output, smooth muscle relaxation,	CRLR-RAMP2/3	T,MΦ,Mo,DC
Cortistatin						
DRMP[CKNFFWKTFSSC] K-NH_2	SOM	CNS, kidney, stomach	T, Mo, MΦ	↓locomotor activity, ↑slow-wave sleep, ↓growth hormone, ↓cell proliferation	Sst1-5 5HSR	T,MΦ,Mo,DC T,MΦ,Mo
Ghrelin						
GSSFLSPEHQR VQQRKESKKPP AKLPQR-NH_2	motilin	CNS, GI, stomach, pancreas	Mo, MΦ	↑cardiac output, ↑appetite and adiposity, ↑growth hormone, vasodilatation, ↑GI secretion, ↑gastric motility	GHSR	T,MΦ,Mo,DC

[a]Abbreviations: calcitonin gene-related peptide, CGRP; proopiomelanocortin, POMC; adrenocorticotropin, ACTH; pituitary adenylate cyclase-activating polypeptide, PACAP; growth hormone-releasing hormone, GHRH; corticotropin-releasing hormone, CRH; somatostatin, SOM; central nervous system, CNS; gastrointestinal tract, GI; T cells, T; macrophage, MΦ; Monocyte, Mo; dendritic cell, DC; polymorphonuclear cell, PMN; B cells, B; melanocortin receptors, MCR; somatostatin receptors 1–5, sst1–5; ghrelin receptor, GHSR; calcitonin-related ligand receptor, CRLR; CRH receptor, CRFR; VIP/PACAP receptor, VPAC; receptor-activity-modifying proteins, RAMP; growth hormone-secretagogue receptor, GHSR.
[b]Aminoacid sequences correspond to human peptides. Disulphite bridges between cysteins on adrenomedullin and cortistatin sequences are shown in parenthesis.
[c]Family of peptides showing some homology in sequence/structure with the referenced neuropeptides.
[d]Tissues and organs producing significant levels of the different neuropeptides.
[e]Immune cells that produce anti-inflammatory neuropeptides.
[f]Major physiological roles of the neuropeptides in different tissues/organs of the body. ↓, indicates inhibition. ↑, indicates stimulation.
[g]Immune cells expressing the different neuropeptide receptor subtypes.

cAMP, these neuropeptides down-regulate the activation of several transduction pathways and transcription factors essential for the transcriptional activation of most of the inflammatory cytokines, chemokines and costimulatory factors, including the nuclear factor-κB (NFκB), mitogen-activated protein kinases (MAPK), the interferon regulatory factor 1 (IRF1) and the activator protein 1 (AP1) [25, 28, 45]. Some evidence indicate that VIP and αMSH could also affect common upstream elements located very early in the signalling of these pathways, such as inhibition of the expression of TLRs and associated proteins [46–49].

Beneficial effect of neuropeptides in inflammatory disorders

Recent studies examining these neuropeptides have shown their relevance to health, proving a potentially crucial clinical significance in inflammatory and autoimmune diseases that upset the balance of body systems (Fig. 2). Treatment with VIP, αMSH, urocortin, adrenomedullin, cortistatin or ghrelin decreases the frequency, delays the onset and reduces the severity of various experimental models of sepsis [39, 50–55], collagen-induced arthritis [56–60], inflammatory bowel disease [61–65], type I diabetes mellitus [66, 67], multiple sclerosis [68–70], Sjogren's syndrome [71], pancreatitis [72, 73] and uveoretinitis [74, 75]. The therapeutic effects of these neuropeptides are associated with the reduction of the two main phases of these diseases. They impair early events that are associated with the initiation and establishment of autoimmunity to self-tissue components, as well as later phases that are associated with the evolving immune and destructive inflammatory responses. These neuropeptides reduce the development of self-reactive T_H1 cells, their entry into the target organ, the release of pro-inflammatory cytokines and chemokines and the subsequent recruitment and activation of macrophages and neutrophils. This results in a decreased production of destructive inflammatory mediators (cytokines, nitric oxide, free radicals and matrix metalloproteinases) by infiltrating and resident (*i.e.* microglia or synoviocytes) inflammatory cells. In addition, the inhibition of the self-reactive T_H1-cell responses gives to decreased titters in IgG2a autoantibodies, an antibody subtype that activates complement and neutrophils and contributes to tissue destruction.

Neuropeptides counterbalance the inflammatory response

The anti-inflammatory action of these neuropeptides is exerted at different levels of the innate immunity (Fig. 2): (1) inhibition of phagocytic activity, free radical production, adherence and migration of macrophages [33, 36, 76]; (2) reduction in the production of inflammatory cytokines (TNFα, IL-12, IL-6 and IL-1β) and various chemokines and down-regulation of the expression of inducible nitric oxide synthase and cycloxygenase 2 (COX2) and the subsequent release of nitric oxide and prostanglandin E2 by macrophages, DCs and microglia [25, 28, 30, 35, 37, 39, 50, 51, 61, 62, 65, 77–88]; (3) stimulation of the production of anti-inflammatory cytokines such as IL-10 and IL-1Ra [39, 50, 51, 61, 89, 90]; (4) decrease in the co-stimulatory activity of antigen-presenting cells (APCs) for antigen-specific T cells by down-regulating the expression of the co-stimulatory molecules [91, 92]; (5) reduction of the secretion of late inflammatory mediator high-mobility group box 1 [93, 94]; (6) inhibition of mast cell degranulation [55]; (7) induction of apoptosis in macrophages [95] and (8) induction of the anti-inflammatory transcription factor, peroxisome proliferator-activated receptor-γ (PPARγ) [96].

Neuropeptides down-regulate Th1 responses

Although the mechanisms involved in the suppressive effects on T_H1-cell responses of most of these neuropeptides are not fully elucidated, most data coming from VIP studies have clearly demonstrated that neuropeptides regulate the T_H1/T_H2 balance through various non-excluding mechanisms involving both direct actions on differentiating T cells and indirect regulation of APC functions (Fig. 2) [67, 77, 97–102]. First, VIP inhibits the production of the T_H1-associated cytokine IL-12. Second, VIP induces CD86 expression in resting murine DCs, which is important for the development of T_H2 cells. Third, VIP has been shown to promote specific T_H2-cell recruitment by inhibiting CXC-chemokine ligand 10 (CXCL10) production and inducing CC-chemokine ligand 22 (CCL22) production, two chemokines that are involved in the homing of T_H1 cells and T_H2 cells, respectively. Fourth, VIP inhibits CD95 (FasL)- and granzyme B-mediated apoptosis of mouse T_H2 but not of T_H1 effector cells. Finally, VIP induces the T_H2 master transcription factors c-MAF, GATA-3 and JUNB in differentiating murine $CD4^+$ T cells, and inhibits T-bet, which is required for T_H1-cell differentiation.

Neuropeptides generate regulatory T cells

Finally, the generation of Treg cells has been recently found to play a major role in the beneficial effect of these neuropeptides in autoimmunity (Fig. 2). They induce the peripheral expansion of new antigen-specific $CD4^+CD25^+$ forkhead box P3 $(FOXP3)^+$ T regulatory cells, with suppressive activity on self-reactive T cells [47, 59–62, 65, 74, 103–105]. The suppressive mechanism is mediated through direct cellular contact that is mainly dependent on cytotoxic T lymphocyte-associated protein 4 (CTLA4), or through the production of the immunosuppressive cytokines IL-10 and/or TGFβ. In addition, VIP and αMSH have been found to generate DCs with a tolerogenic phenotype, characterized by their ability to induce CD4 and CD8 regulatory T cells [91, 106–108]. The involvement of Treg cells in the beneficial effect of these neuropeptides on autoimmunity is supported by the fact that the *in vivo* blockade of the Treg-cell mediators CTLA4, IL-10 and TGFβ1 significantly reversed their therapeutic action [57, 59, 60, 103, 104]. Therefore, the generation of Treg cells by neuropeptides could

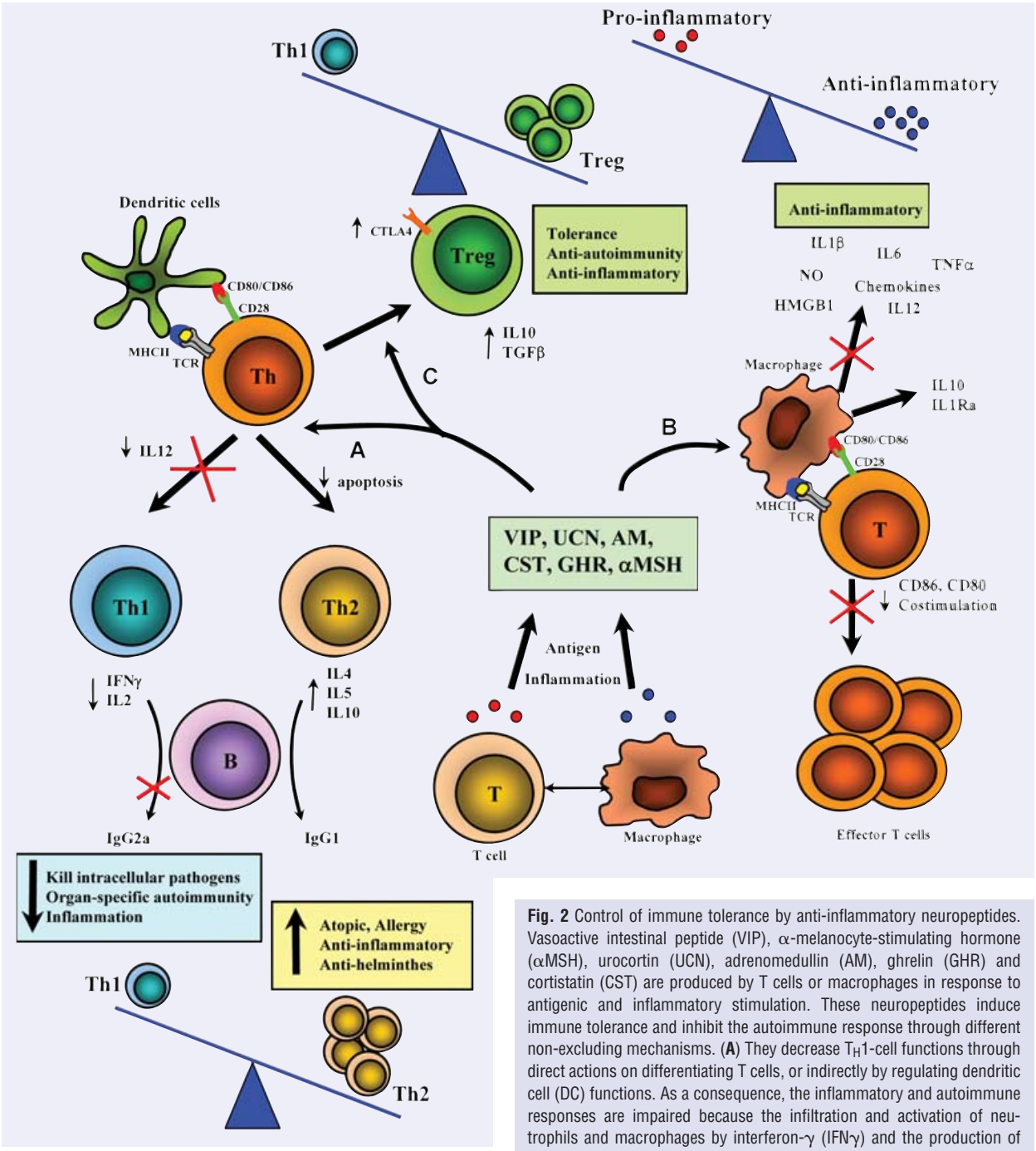

Fig. 2 Control of immune tolerance by anti-inflammatory neuropeptides. Vasoactive intestinal peptide (VIP), α-melanocyte-stimulating hormone (αMSH), urocortin (UCN), adrenomedullin (AM), ghrelin (GHR) and cortistatin (CST) are produced by T cells or macrophages in response to antigenic and inflammatory stimulation. These neuropeptides induce immune tolerance and inhibit the autoimmune response through different non-excluding mechanisms. (**A**) They decrease T_H1-cell functions through direct actions on differentiating T cells, or indirectly by regulating dendritic cell (DC) functions. As a consequence, the inflammatory and autoimmune responses are impaired because the infiltration and activation of neutrophils and macrophages by interferon-γ (IFNγ) and the production of complement-fixing IgG2a antibodies are avoided. (**B**) Neuropeptides inhibit the production of inflammatory cytokines, chemokines, free radicals (*i.e.* nitric oxide) and high-mobility group box 1 (HMGB1) by macrophages and microglia. In addition, they impair the costimulatory activity of macrophages on effector T cells, inhibiting the subsequent clonal expansion. This avoids the infiltration of leucocytes and the inflammatory response and the subsequent cytotoxicity against the target tissue. (**C**) Neuropeptides induce the new generation of regulatory T cells (Treg) that suppress activation of autoreactive T cells through a mechanism that involves production of interleukin-10 (IL-10) and transforming growth factor-β (TGFβ), and/or expression of the cytotoxic T lymphocyte-associated protein 4 (CTLA4). In addition, neuropeptides indirectly generate Treg through the differentiation of tolerogenic DCs. This effect contributes to the maintenance of an anti-inflammatory state and restores the immune tolerance. Black arrows indicate a stimulatory effect. Red crosses indicate an inhibitory effect.

explain the selective inhibition of T_H1 immune responses once T cells have completed differentiation into T_H1 effector cells, as demonstrated by the therapeutic effect of delayed administration of these neuropeptides in established arthritis, EAE and diabetes.

Are endogenous neuropeptides important for maintaining immune tolerance in the body?

Of physiological relevance is the presence of these neuropeptides in barrier organs like skin and mucosal barriers of the gastrointestinal, genital and respiratory tracts suggests that they may be key components of the innate immune system (Table 1). Indeed, (MSH, ghrelin and adrenomedullin have shown antimicrobial properties [94, 109, 110]. The relevance of these neuropeptides as natural anti-inflammatory factors is also supported by results obtained in several inflammatory models performed in animals that are deficient for any of these neuropeptides or their receptors. For example, mice that lack VIP or its receptor show higher systemic inflammatory responses and are more susceptible to die by septic shock [111, 112], and VIP receptor-deficient mice have increased T_H1-type responses (*i.e.* delayed-type hypersensitivity), whereas mice that overexpress VIP receptors show eosinophilia, high levels of IgE and IgG1 and increased cutaneous anaphylaxis (typical T_H2-type responses) [113–115]. Moreover, an altered expression of VIP receptors in T cells has been related to aberrant T_H1 immunity in patients with multiple sclerosis and rheumatoid arthritis [116–118]. In addition, it has been recently found in a genetic association between multiple-marker haplotypes of VIP receptor and rheumatoid arthritis susceptibility [117]. Finally, the significant reduced levels of VIP found in patients with lupus and autoimmune thyroditis have been related to the existence of high amounts of autoantibodies with VIPase activity [119]. These findings support the notion that reduced levels of neuropeptides, and deficiencies and/or mutations in their receptors and signalling make us more susceptible to suffer inflammatory and autoimmune diseases.

Resolution of inflammation by endogenous lipid mediators

Human beings depend on the nutritional supply of two types of essential PUFAs, ω-3 and ω-6, which are enriched in fish oils. They are precursors of lipid mediators critical for a variety of cellular functions, including inflammation, nociception, renal function, reproductive activity, haemodynamics and blood clotting [120]. Most of the studies in the past were focused on the ω-6 PUFA arachidonic acid (AA) as a classic precursor of bioactive PGs and LTs locally produced after tissue injury, microbial infection and surgical trauma through pathways involving cycloxygenases (COX-1 and COX-2) and lipoxygenases (LOX). AA-derived lipid mediators such as PGE2, PGD2 and LTs generated in the initial phase of inflammation have been classically involved in pro-inflammatory signalling and implicated in the pathogenesis of various inflammatory diseases [120, 121]. However, recent studies in animal models have proposed that they can also stimulate circuits to resolution of inflammation.

Lipoxins

Both PGE2 and PGD2 activate the expression of 15-LOX in neutrophils, which switches the mediator profile of these cells from LTB4 to lipoxins [122]. Lipoxins were the first mediators identified to have both anti-inflammatory and pro-resolving activities [123]. Lipoxins (LXA4 and LXB4) are synthesized by transcellular metabolism of AA by LOX/LOX interaction of infiltrating neutrophils with endothelial cells, fibroblasts and platelets localized in the inflammatory exudate (Fig. 3). In addition, lipoxins can be synthesized in an alternative and interesting way. Acetylation of COX-2 by aspirin modifies the enzyme so that it can act as a LOX, which synthesizes the lipoxin precursor 15-hydroxyeicosatetraenoic acid (15-HETE) from AA. By the action of leucocyte 15-LOX, 15-HETE is then transformed in the so-called aspirin-triggered lipoxins (ATLs) 15-epi-lipoxin A4 or 15-epi-lipoxin B4, which show even more potent anti-inflammatory effects than LXA4 (Fig. 3).

Lipoxins and ATLs promote the resolution of inflammation by selectively stopping the entry of new PMNs to sites of inflammation that includes inhibition of chemotaxis of PMNs and their adhesion to and transmigration through endothelial cells and reduction of vascular permeability [122–125]. At the same time, lipoxins and ATLs stimulate the recruitment of monocytes by stimulating their chemotaxis and adherence without causing release of reactive oxygen species [126–128], and promote the non-phlogistic phagocytosis of apoptotic neutrophils by macrophages [129, 130]. This is supported by a shift to an anti-inflammatory response by these mediators, because lipoxin, ATLs and their stable analogues inhibit the production and action of chemokines (*i.e.* IL-8) and TNFα, whereas stimulate the anti-inflammatory cytokine TGFβ [128, 131]. These effects seem to be mediated through the GPCR, LXA4 receptor (ALXR) also referred to as formyl peptide receptor-like 1 (FPRL1) and the subsequent inactivation of the transactivating pathways NFkB and AP1 [127, 129].

As a consequence, these lipid mediators have been proven effective in various models of inflammatory diseases such as ischemia/reperfusion injury, inflammatory bowel disease, glomerulonephritis, allergic pleural edema, periodontitis, dermal inflammation and cystic fibrosis [131, 132]. Importantly, some of the beneficial effects ascribed to aspirin in various human diseases should be a consequence of the generation of ATL and subsequent promotion of inflammation resolution.

Resolvins and protectins

Recent evidence have demonstrated that AA is not the only fatty-acid substrate that can be transformed by COXs and LOXs to bioactive mediators with roles in anti-inflammation and resolution. The

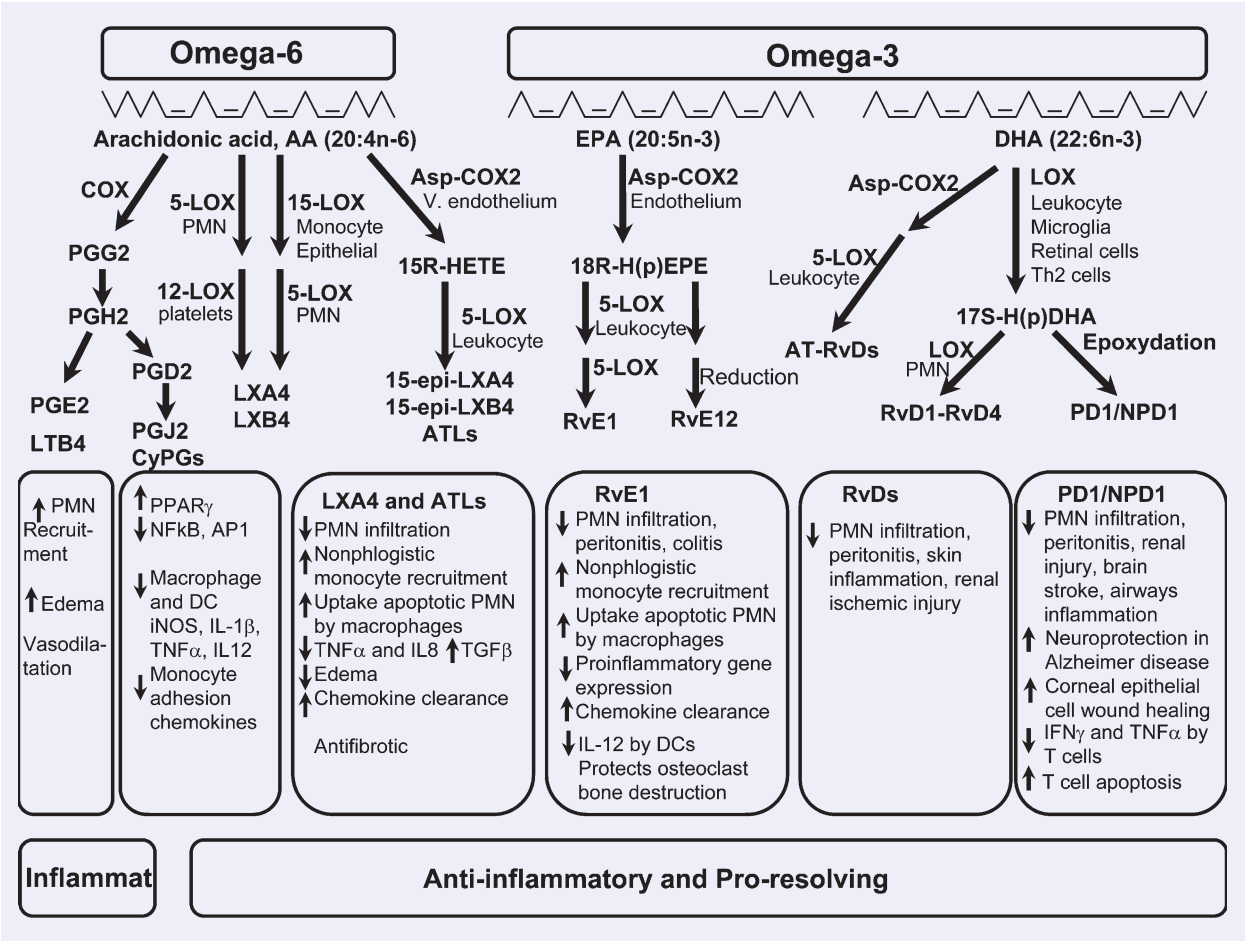

Fig. 3 Role of new PUFA-derived lipid mediators in the progression and resolution of acute inflammation. A schematic of the biosynthetic pathways for lipid mediators derived from ω-6 (AA, arachidonic acid) and ω-3 (EPA, eicosapentanoic acid; DHA, docosahexaenoic acid) with key enzymes is shown. AA is metabolized by cycloxygenase 1 (COX1) or COX2 to prostaglandin (PG) G2 and then PGH2, which in turn serves as a substrate for a series of downstream synthases to give rise to the PGs. PGs (such as PGE2) and leucotrienes (LTB4) participate in the initiation of the inflammatory response. PGH2 is also metabolized to PGD2 and then broken down to PGJ2 and the cyclopentenone PGs (cyPGs), which act as anti-inflammatory factors. After the initiation of acute inflammation by PGs and LTs, a class switching occurs with time towards pro-resolving lipid mediators that start with the generation of lipoxins (LXs) from AA through three distinct biosynthetic routes. First, AA is sequentially metabolized in a transcellular manner by 5-lipoxygenase (5-LOX) in polymorphonuclear cells (PMNs) and platelet 12-LOX to lypoxin A4 (LXA4) and LXB4. Second, AA can be transformed *via* sequential actions of the 5-LOX in monocytes or epithelial cells and the 5-LOX in PMNs yielding an epoxide intermediate that is converted to LXA4 and LXB4 by leucocyte epoxide hydrolases. Third, aspirin acetylates the active site of COX2 (Asp-COX2) that now is able to metabolize AA to 15(R)-hydroxyeicosatetraenoic (15R-HETE), which when released from endothelial and epithelial cells is converted by leucocyte 5-LOX to the called aspirin-triggered LXs (ATLs), 15-epi-LXA4 and 15-epi-LXB4. Once AA is metabolized to ATLs, it is substituted by the ω-3 EPA and DHA as substrates of the E-series and D-series resolvins and protectins. Vascular endothelial Asp-COX2 converts EPA to 18R-hydroxyperoxy-EPE (18R-H(p)EPE), which is further sequentially metabolized by leucocyte 5-LOX to lead the formation of resolving E1 (RvE1). Microbial P-450s may also convert EPA in RvE1. 5-LOX can also generate RvE2 from 18R-H(p)EPE and further reduction. DHA is transformed by the leucocyte LOX to 17S-H(p)DHA, which is rapidly converted by PMN LOX into two epoxide intermediates that finally lead the formation of the bioactive products 17S-resolvin D series (RvD1 to RvD4). Alternatively, Asp-COX2 can metabolize DHA to a 17R-H(p)DHA, which in turn generate the 17R-resolvin D series (AT-RvDs) by action of the leucocyte 5-LOX. Finally, by action of the 15-LOX in microglia, brain leucocytes, retinal cells or Th2 cells, DHA is converted to 17S-H(p)DHA, which following further enzymatic epoxidation and hydrolysis form protectin 1 (PD1), or neuroprotectin 1 (NPD1) if formed in the brain. LXA4, ATLs, resolvins and PD1 share some anti-inflammatory and pro-resolving actions, although they have distinct roles within the induction of resolution. dendritic cells, DC; inducible nitric oxide synthase, iNOS; peroxisome proliferator-activating receptor γ, PPARγ; transforming growth factor, TGF.

beneficial roles of the ω-3 PUFAs, eicosapentoaneoic acid (EPA) and docosahexaenoic acid (DHA), in health and organ function were known from almost one century ago [133]. Both DHA and EPA are held to be beneficial in a wide range of human inflammatory diseases, including rheumatoid arthritis, Alzheimer's disease, cardiovascular diseases, inflammatory bowel disease and lung fibrosis [134–139]. Until very recently, the established hypotheses about the mechanisms that govern these beneficial effects were that these ω-3 PUFAs prevent conversion of AA to pro-inflammatory eicosanoids (PGs and LTs) or serve as an alternative substrate generating less-potent products. However, the group leadered by C.N. Serhan dramatically changed the scenario in this field, since they uncovered a series of oxygenated derivatives of ω-3 PUFAs, named Resolvins (Rvs) and Protectins (PDs), which possess potent anti-inflammatory and pro-resolving actions [122, 140–147]. Thus, they found that EPA generates the 18R E-series resolvins (resolvin E1 and resolvin E2) in the presence of aspirin during the spontaneous resolution phase of acute inflammation where specific cell–cell interactions occur (Fig. 3). They also elucidated that DHA generates the 17S D-series resolvins (resolvins D1 to D6) and protectin D1 (PD1) through interaction with leucocyte LOX, and alternatively, DHA forms the 17R D-series resolvins (AT-RvDs) in the presence of aspirin-acetylated COX2 (Fig. 3).

RvE1 inhibits PMN transendothelial migration *in vitro*, reduces leucocyte infiltration *in vivo*, and inhibits DC migration and IL-12 production [140, 141, 147–150]. Moreover, RvE1 improved the survival of mice with colitis by reducing PMN recruitment and the expression of pro-inflammatory genes [151]. RvE1 can interact with the LTB4 receptor (BLT1) in leucocytes and attenuate LTB4-stimulated signals, acting as a partial agonist or antagonist [152]. In addition, RvE1 seems to bind to the GPCR receptor ChemR23 and inhibit NFkB signalling through a mechanism that involves phosphorylation rather than mobilization of calcium or cAMP [152]. The other resolvins, especially RvD1, inhibit TNFα-induced IL-1β production by microglia, reduce PMN infiltration into inflamed brain, skin and peritoneum, and are protective in a model renal ischemic injury [141, 142, 153].

PD1, named as neuroprotectin D1 when produced in the neural tissue, also attenuates neutrophil transmigration *in vitro* and reduces *in vivo* PMN infiltration in models of peritonitis and cerebral stroke [142, 143]. Attending to its name, PD1 has shown neuroprotective effects, promotes corneal epithelial cell wound healing and repair, and protects from lung damage by reducing airway inflammation and from kidney injury [153–155]. PD1 is the only lipid mediator derived from ω-3 PUFAs that showed direct immunomodulatory effects on T-cell function. PD1 inhibits the production of IFNγ and TNFα by activated T cells and induces T cell apoptosis, suggesting that is down-regulating Th1-mediated responses [156]. Of note is the fact that PD1 is produced by Th2-skewed human peripheral blood mononuclear cells through a LOX-dependent mechanism [157]. This finding correlated with the early studies reporting that DHA modulates T-cell functions to favour a Th2 phenotype [157, 158], suggesting that PD1 is a major DHA conversion product that is biosynthesized during Th2 polarization and promotes anti-inflammatory responses.

On the other hand, RvE1, PD1 and LXA4 show an interesting mechanism to facilitate chemokine removal during resolution, consisting in the up-regulation of CCR5 expression on late apoptotic leucocytes, which sequestrate the CCR5 ligands and are then engulfed by resolving macrophages in a non-phlogistic manner [159]. Finally, an elegant and recent work has demonstrated that RvE1, PD1 and an ATL analogue promote inflammation resolution in a murine model of peritonitis, not only by reducing the recruitment (influx) of leucocytes to the inflammatory exudate (considered as anti-inflammation), but also promoting phagocyte removal (considered as pro-resolution) by stimulating macrophage ingestion of apoptotic neutrophils and microbial products and enhancing the efflux of these phagocytes from inflamed peritoneum to draining lymph nodes and spleen [160].

These findings outline the potential application of these lipid mediators in acute inflammatory diseases. However, their relevance in autoimmune disorders is still unknown, since research on the effect of lipid mediators in T-cell activity is scarce. This is probably an emerging field and further studies will determine whether we can extend their therapeutic use to autoimmune disorders. Similar to neuropeptides, the induction of immune tolerance by lipid mediators should be a critical point.

Therapeutic perspectives: rationale for using endogeneous neuropeptides and lipid mediators in immune disorders

Our body responds to an exacerbated inflammatory response by increasing the production of endogenous anti-inflammatory neuropeptides and lipid mediators, in an attempt to restore the immune homeostasis [161–167]. The findings reviewed above indicate that these neuropeptides act in a pleiotropic and in many cases in a redundant manner to regulate the balance between pro-inflammatory and anti-inflammatory factors, and between autoreactive T_H1 cells and Treg cells. Lipid mediators such as LXs, Rvs and PDs each play an active role in controlling and programming resolution of inflammation and stimulating endogenous anti-inflammatory and pro-resolving pathways. Based in these characteristics, anti-inflammatory neuropeptides and pro-resolving lipid mediators could represent feasible therapeutic agents in the treatment on immune diseases, such as rheumatoid arthritis, multiple sclerosis or Crohn's disease, characterized by a double component, inflammatory and autoimmune.

Advantages of using endogenous neuropeptides and lipids

From a therapeutic point of view, the wide spectrum of action of these endogenous mediators represents an advantage *versus* agents directed only against one component of these diseases. In

addition, the promotion of an active program of inflammation resolution by lipid mediators is therapeutically attractive because the goal is restoration of tissue homeostasis without significantly affecting key components of the inflammatory response (*i.e.* cytokines). However, although both neuropeptides and lipids have revealed their clinical therapeutic possibilities, most studies were performed in animal models, and precautions should be taken to extend them to human diseases. This will depend on the dosage of these factors and expression of specific receptors in the different cell types participating in the immune response. In addition, we should also to consider the potential side effects as a consequence of the other effects of these neuropeptides and lipid mediators in the body, such as hypotension, gut motility and endocrine disorders, reproductive activity, diarrhoea and circadian rhythm and memory alterations. It is important to note that some of these neuropeptides have been already tested in human beings for the treatment of sepsis and other disorders [168–171], without such complications. This suggests that they should be well tolerated in human beings in doses similar to those that are able to prevent immunological diseases in animals. Therefore, as compared to existing anti-inflammatory drugs, neuropeptides are not associated with dramatic side effects, because as physiological compounds they are intrinsically non-toxic. In addition, neuropeptides are rapidly cleared from the body through natural hepatic detoxification mechanisms and renal excretion. Moreover, other cytokines, neuropeptides and hormones often counterbalance their actions, meaning that the homeostasis of normal tissues should not be excessively perturbed. Regarding lipid mediators, lipoxins, PD1 and Rvs are rapidly generated in response to stimuli, act locally and then are rapidly inactivated by metabolic enzymes (*i.e.* LAX4/PGE 13, 14-reductase/LTB4 12-hydroxydehydrogenase). Therefore, no side effects should be expected for them.

In addition to their wide spectrum of action, the molecular structure and size of the neuropeptides and lipid mediators make them attractive compounds to treat excessive inflammation. As small molecules, they possess excellent permeability properties that permit rapid access to the site of inflammation. This is critical for neuroinflammatory disorders, where the blood-brain barrier is partially disturbed. The second advantage owes to their high-affinity binding to specific receptors, thus making them very potent in exerting their actions. Finally, the *in vitro* synthesis of neuropeptides and lipid mediators is straightforward and permits easy modification if necessary.

Cell-based therapy

An important aspect for translational medicine is the induction of Treg cells by neuropeptides. This has not only been crucial to a better understanding of the immunomodulatory action of neuropeptides, but has also supposed the proposal of a new cell-based strategy for the treatment of immune disorders where tolerance restoration is needed. Considerable effort is recently focused on the use of antigen-specific Treg cells generated *ex vivo* to treat autoimmune diseases, transplantation and asthmatic disorders [4]. The ability to translate important biological findings about Treg cells to the clinic has been limited by several issues, including the low frequency of these cells and the potential for pan immunosuppression. The potential solution for this problem should consist in expanding them and making them antigen-specific using selected antigens and peptides. However, although Treg cells replicate relatively efficiently *in vivo*, they are anergic and refractory to stimulation *in vitro* [4, 5]. Therefore, protocols that efficiently expand Treg populations *in vitro* while maintaining their immunoregulatory properties *in vivo* should be based in the conditions that allow their expansion *in vivo*, including TCR occupancy, crucial co-stimulatory signals and selective growth factors. Neuropeptides could be one of these endogenous growth factors involved in the generation/expansion of Treg cells. In fact, neuropeptides induce the generation of self-peptides-specific Treg cells from otherwise conventional T cells *in vitro*. These cells prevent very efficiently the progression of experimental autoimmune diseases by suppressing the systemic autoantigen-specific T- and B-cell responses and the tissue-localized inflammatory response. On the other hand, the capacity of certain classes DCs to induce Treg cells makes them attractive for the expansion/generation of antigen-specific Treg cells *ex vivo*, or alternatively, for their use *in vivo* as therapeutic cells that restore immune tolerance by inducing Treg cells in the host [172, 173]. In this sense, VIP-induced tolerogenic DCs pulsed with self-antigens have been shown to ameliorate the progression of rheumatoid arthritis, EAE and inflammatory bowel disease [106, 174]. This effect is mainly mediated through the generation of antigen-specific Treg cells in the treated animal. Probably, the most important issue that the cell-based therapy with neuropeptide-induced Treg cells or tolerogenic DCs needs to resolve is to determine the necessity of antigen specificity. Whereas polyclonal Treg cells might function in allograft transplantation and autoimmunity in lymphopaenic (*i.e.* systemic lupus eritematous) or inflammatory bowel disease settings, in other autoimmune disorders antigen-specific Treg cells are most effective [4]. In this sense, etiology and self-antigens in most human autoimmune disorders are mostly unknown, reducing the therapeutic efficiency and applicability of Treg cells. In addition, the proposed cell-based therapy will require an *ex vivo* manipulation of the blood cells of patients. Therefore, it is necessary to determine whether neuropeptides *in vitro* are able to generate Treg cells or tolerogenic DCs with the same efficiency, reliability, homing capacity and survival *in vivo* compared to those obtained from animals or healthy individuals, since in contrast to mouse models, in patients there exists considerable variability. In any case, what this is an individualized therapy, which will involve procedures that are likely to be expensive, but which will be indicated to patients that are non-responsive to established treatments.

Pitfalls: how to solve them?

Despite the potential advantages of using neuropeptides on immune disorders, several obstacles stand between translating

neuropeptide based-treatment into viable clinic therapies. Due to their natural structural conformation, neuropeptides are very unstable and extremely sensitive to peptidases present in most tissues. Several methodological strategies have been developed to increase the neuropeptide half-life, especially in long-term treatments. For example, modifications or substitutions of certain amino acids in the sequence or cycling the structure increase the stability of these peptides [175, 176]. Perhaps even more important is work towards improving neuropeptide delivery to target tissues and cells while protecting it against degradation. Different strategies being tested under experimental conditions include neuropeptide gene delivery or the insertion of VIP into micelles or nanoparticles [66, 71, 177–180]. Other methods include combining neuropeptide treatment with inhibitors of neutral endopeptidases to reduce the degradation of the peptide in the circulation. Alternatively, administration of serum-specific neuropeptide binding proteins (*i.e.* adrenomedullin binding protein-1) would protect them from peptidases and enhance their delivery in the proximity of their receptors in the inflamed tissue [96]. Other combinatory treatments aim to take advantage of the fact that activation of the cAMP/PKA pathway appears to be the major signal involved in their immunomodulatory effect. Thus, combining neuropeptides with inhibitors of phosphodiesterases (enzymes involved in the degradation of cAMP) has been found to be therapeutically attractive in the treatment of some inflammatory diseases [181–183].

However, the definitive approach by the pharmaceutical companies as a prerequisite for successful clinical applications is the development of metabolically stable analogues. Understanding of the structure/function relationship of these neuropeptides and their specific receptors, including receptor signalling, internalization and homo/heterodimerization, will facilitate the development of novel pharmacologic agents for translational medicine. However, in the case of the type 2 GPCRs (*i.e.* receptors for VIP, urocortin, αMSH and adrenomedullin), the pharmaceutical industry has so far failed to generate effective non-peptide-specific agonists [184]. Even where synthetic agonists were designed specifically for VIP receptors, they were less effective than the natural peptide as anti-inflammatory agents [25]. In the case of the type 1 GPCRs, the generation of several somatostatin agonists offered new therapeutic opportunities for the treatment of acromegaly and endocrine tumours [185]. However, compared to cortistatin, some sst agonists (*i.e.* octreotide) show much less effectiveness, if any, reducing inflammation and autoimmunity [10, 11, 185]. It remains to be investigated whether recently isolated non-peptide ghrelin-receptor agonists share some therapeutic actions with ghrelin. Regarding pro-resolving lipid mediators, screening for non-LX agonists of the LXA4 receptor ALX/FPRL-1 resulted in the identification of orally active anti-inflammatory agents in animal models [15, 186], although their efficiency in human diseases remains untested. In any case, the focus on the use of natural peptides in therapy is not new, and may be a case of history repeating itself, since naturally occurring human compounds have often proved to have striking therapeutic value (*e.g.* insulin and cortisone). Elucidation of the molecular mechanisms of the reported beneficial actions of ω-3 PUFAs has supposed an important challenge for molecular and translational medicine.

Finally, it is important to take in account that many drugs currently used in inflammatory diseases were developed without an appreciation of their potential impact in resolution. Thus, the widely used COX-2 inhibitors have been proven to be resolution toxic, whereas other agents such as glucocorticoids or aspirin can posses pro-resolving actions [15]. Although neuropeptides inhibit the infiltration of leucocytes to the inflamed tissue, it is still unknown whether they are able to actively promote programs of inflammation resolution similarly to lipid mediators. However, two recent studies have reported that VIP and the structurally related pituitary adenylate cyclize-activating polypeptide (PACAP) bind to the ALX/FPRL-1 in monocytes [187, 188], suggesting that both neuropeptides could mimic LXA4-mediated resolving actions. In any case, combinatory treatment with both anti-inflammatory neuropeptides and pro-resolving lipid mediators emerges as an attractive therapy for many immune disorders that course with exacerbated inflammatory responses.

Acknowledgements

This work was supported by grants from the Spanish Ministry of Health and Junta de Andalucia.

References

1. **Firestein GS.** Evolving concepts of rheumatoid arthritis. *Nature.* 2003; 423: 356–61.
2. **Goodnow CC.** Multistep pathogenesis of autoimmune diseases. *Cell.* 2007; 130: 25–35.
3. **Nathan C.** Points of control in inflammation. *Nature.* 2002: 420: 846–52.
4. **Mills KHG.** Regulatory T cells: friend or foe in immunity to infection? *Nat Rev Immunol.* 2004; 4: 841–55.
5. **Bluestone JA.** Regulatory T-cell therapy: is it ready for the clinic? *Nat Rev Immunol.* 2005; 5: 343–9.
6. **Ehrenstein MR, Evans JG, Singh A, Moore S, Warnes G, Isenberg DA, Mauri C.** Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFα therapy. *J Exp Med.* 2004; 200: 277–85.
7. **Lindley S, Dayan CM, Bishop A, Roep BO, Peakman M, Tree TI.** Defective suppressor function in $CD4^+CD25^+$ T-cells from patients with type 1 diabetes. *Diabetes.* 2005; 54: 92–9.
8. **Viglietta V, Baecher-Allan C, Weiner HL, Hafler DA.** Loss of functional suppression by $CD4^+CD25^+$ regulatory T cells in patients with multiple sclerosis. *J Exp Med.* 2004; 199: 971–9.
9. **Wan YY, Favell RA.** The roles for cytokines in the generation and maintenance of regulatory T cells. *Immunol Rev.* 2006; 212: 114–30.

10. **Gonzalez-Rey E, Chorny A, Delgado M.** Regulation of immune tolerance by anti-inflammatory neuropeptides. *Nat Rev Immunol.* 2007; 7: 52–63.
11. **Gonzalez-Rey E, Delgado M.** Anti-inflammatory neuropeptide receptors: new therapeutic targets for immune disorders? *Trends Pharmacol Sci.* 2007; 28: 482–91.
12. **Ariel A, Serhan CN.** Resolvins and protectins in the termination program of acute inflammation. *Trends Immunol.* 2007; 28: 176–83.
13. **Gilroy DW, Lawrence T, Perretti M, Rossi AG** Inflammatory resolution: new opportunities for drug discovery. *Nat Rev Drug Discov.* 2004; 3: 401–16.
14. **Serhan CN, Yacoubian S, Yang R.** Anti-inflammatory and proresolving lipid mediators. *Annu Rev Pathol.* 2008; 3: 279–312.
15. **Serhan CN, Chiang N.** Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus. *Br J Pharmacol.* 2008; 153: S200–15.
16. **Blalock JE.** The immune system as the sixth sense. *J Intern Med.* 2005; 257: 126–38.
17. **Sternberg EM.** Neural regulation of innate immunity: a coordinated nonspecific host response to pathogens. *Nat Rev Immunol.* 2006; 6: 318–28.
18. **Molina PE.** Opioids and opiates: analgesia with cardiovascular, haemodynamic and immune implications in critical illness. *J Intern Med.* 2006; 259: 138–54.
19. **Said SI, Mutt V.** Polypeptide with broad biological activity: isolation from small intestine. *Science.* 1970; 169: 1217–18.
20. **Hadley ME, Haskell-Luevano C.** The proopiomelanocortin system. *Ann NY Acad Sci.* 1999; 885: 1–21.
21. **Oki Y, Sasano H.** Localization and physiological roles of urocortin. *Peptides.* 2004; 25: 1745–9.
22. **Hinson JP, Kapas S, Smith DM.** Adrenomedullin, a multifunctional regulatory peptide. *Endocr Rev.* 2000; 21: 138–67.
23. **Spier AD, De Lecea L.** Cortistatin: a member of the somatostatin neuropeptide family with distinct physiological functions. *Brain Res Rev.* 2000; 33: 228–41.
24. **Van der Lely AJ, Tschop M, Heiman ML, Ghigo E.** Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. *Endocr Rev.* 2004; 25: 426–57.
25. **Delgado M, Pozo D, Ganea D.** The significance of vasoactive intestinal peptide in immunomodulation. *Pharmacol Rev.* 2004; 56: 249–90.
26. **Delgado M, Ganea D.** Cutting Edge: is vasoactive intestinal peptide a type 2 cytokine? *J Immunol.* 2001; 166: 2907–12.
27. **Blalock JE.** Proopiomelanocortin and the immune-neuroendocrine connection. *Ann NY Acad Sci* 1999; 885: 161–72.
28. **Lipton JM, Catania A.** Anti-inflammatory actions of the neuroimmunomodulator alpha-MSH. *Immunol Today.* 1997; 18: 140–5.
29. **Gravanis A, Margioris AN.** The corticotropin-releasing factor (CRF) family of neuropeptides in inflammation: potential therapeutic applications. *Curr Med Chem.* 2005; 12: 1503–12.
30. **Kubo A, Minamino N, Isumi Y, Katafuchi T, Kangawa K, Dohi K, Matsuo H.** Production of adrenomedullin in macrophage cell line and peritoneal macrophage. *J Biol Chem.* 1998; 273: 16730–8.
31. **Dalm VA.** Cortistatin rather than somatostatin as a potential endogenous ligand for somatostatin receptors in the human immune system. *J Clin Endocrinol Metab.* 2003; 88: 270–6.
32. **Pozo D.** VIP- and PACAP-mediated immunomodulation as prospective therapeutic tools. *Trends Mol Med.* 2003; 9: 211–7.
33. **Neumann Andersen G, Nagaeva O, Mandrika I, Petrovska R, Muceniece R, Mincheva-Nilsson L, Wikberg JE.** MC(1) receptors are constitutively expressed on leucocyte subpopulations with antigen presenting and cytotoxic functions. *Clin Exp Immunol.* 2001; 126: 441–6.
34. **Becher E, Mahnke K, Brzoska T, Kalden DH, Grabbe S, Luger TA.** Human peripheral blood-derived dendritic cells express functional melanocortin receptor MC-1R. *Ann NY Acad Sci.* 1999; 885: 188–95.
35. **Taherzadeh S, Sharma S, Chhajlani V, Gantz I, Rajora N, Demitri MT, Kelly L, Zhao H, Ichiyama T, Catania A, Lipton JM.** Alpha-MSH and its receptors in regulation of tumor necrosis factor-alpha production by human monocyte/macrophages. *Am J Physiol.* 1999; 276: R1289–94.
36. **Catania A, Rajora N, Capsoni F, Minonzio F, Star RA, Lipton JM.** The neuropeptide alpha-MSH has specific receptors on neutrophils and reduces chemotaxis in vitro. *Peptides.* 1996; 17: 675–9.
37. **Getting SJ, Gibbs L, Clark AJ, Flower Rj, Perretti M.** POMC gene-derived peptides activate melanocortin type 3 receptor on murine macrophages, suppress cytokine release, and inhibit neutrophil migration in acute experimental inflammation. *J Immunol.* 1999; 162: 7446–53.
38. **Dalm VA.** Expression of somatostatin, cortistatin, and somatostatin receptors in human monocytes, macrophages, and dendritic cells. *Am J Physiol Endocrinol Metab.* 2003; 285: E344–53.
39. **Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, Lillard JW Jr, Taub DD.** Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J Clin Invest.* 2004; 114: 57–66.
40. **Poyner DR, Sexton PM, Marshall I, Smith DM, Quirion R, Born W, Muff R, Fischer JA, Foord SM.** International Union of Pharmacology. XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin, and calcitonin receptors. *Pharmacol Rev.* 2002; 54: 233–46.
41. **Hauger RL, Grigoriadis DE, Dallman MF, Plotsky PM, Vale WW, Dautzenberg FM.** International union of pharmacology. XXXVI. Current status of the nomenclature for receptors for corticotropin-releasing factor and their ligands. *Pharmacol Rev.* 2003; 55: 21–6.
42. **Harmar AJ, Arimura A, Gozes I, Journot L, Laburthe M, Pisegna JR, Rawlings SR, Robberecht P, Said SI, Sreedharan SP, Wank SA, Waschek JA.** International Union of Pharmacology. XVIII. Nomenclature of receptors for vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. *Pharmacol Rev.* 1998; 50: 265–70.
43. **Banner KH, Trevethick MA.** PDE4 inhibition: a novel approach for the treatment of inflammatory bowel disease. *Trends Pharmacol Sci.* 2004; 25: 430–6.
44. **Lugnier C.** Cyclic nucleotide phosphodiesterase (PDE) superfamily: a new target for the development of specific therapeutic agents. *Pharmacol Ther.* 2006; 109: 366–8.
45. **Yoon SW, Goh SH, Chun JS, Cho EW, Lee MK, Kim KL, Kim JJ, Kim CJ, Poo H.** Alpha-Melanocyte-stimulating hormone inhibits lipopolysaccharide-induced tumor necrosis factor-alpha production in leukocytes by modulating protein kinase A, p38 kinase, and nuclear factor kappa B signaling pathways. *J Biol Chem.* 2003; 278: 32914–20.
46. **Taylor AW.** The immunomodulating neuropeptide alpha-melanocyte-stimulating hormone (α-MSH) suppresses

LPS-stimulated TLR4 with IRAK-M in macrophages. *J Neuroimmunol.* 2005; 162: 43–50.

47. **Sarkar A, Sreenivasan Y, Manna SK.** Alpha-Melanocyte-stimulating hormone inhibits lipopolysaccharide-induced biological responses by downregulating CD14 from macrophages. *FEBS Lett.* 2003; 553: 286–94.
48. **Gomariz RP, Arranz A, Abad C, Torroba M, Martinez C, Rosignoli F, Garcia-Gómez M, Leceta J, Juarranz Y.** Time-course expression of Toll-like receptors 2 and 4 in inflammatory bowel disease and homeostatic effect of VIP. *J Leukoc Biol.* 2005; 78: 491–502.
49. **Delgado M, Leceta J, Abad C, Martinez C, Ganea D, Gomariz RP.** Shedding of membrane-bound CD14 from lipopolysaccharide-stimulated macrophages by vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. *J Neuroimmunol.* 1999; 99: 61–71.
50. **Gonzalez-Rey E, Chorny A, Varela N, Robledo G, Delgado M.** Urocortin and adrenomedullin prevent lethal endotoxemia by downregulating the inflammatory response. *Am J Pathol.* 2006; 168: 1921–30.
51. **Gonzalez-Rey E, Chorny A, Robledo G, Delgado M.** Cortistatin, a new anti-inflammatory peptide with therapeutic action in lethal endotoxemia. *J Exp Med.* 2006; 203: 463–71.
52. **Chiao H, Foster S, Thomas R, Lipton J, Star RA.** Alpha-melanocyte-stimulating hormone reduces endotoxin-induced liver inflammation. *J Clin Invest.* 1996; 97: 2038–44.
53. **Delgado M, Martinez C, Pozo D, Calvo JR, Leceta J, Ganea D, Gomariz RP.** 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.* 1999; 162: 1200–5.
54. **Wu R.** Ghrelin improves tissue perfusion in severe sepsis via downregulation of endothelin-1. *Cardiovasc Res.* 2005; 68: 318–26.
55. **Tuncel N, Tore F, Sahinturk V, Ak D, Tuncel M.** Vasoactive intestinal peptide inhibits degranulation and changes granular content of mast cells: a potential therapeutic strategy in controlling septic shock. *Peptides.* 2000; 21: 81–9.
56. **Delgado M, Abad C, Martinez C, Leceta J, Gomariz RP.** Vasoactive intestinal peptide prevents experimental arthritis by downregulating both autoimmune and inflammatory components of the disease. *Nat Med.* 2001; 7: 563–8.
57. **Gonzalez-Rey E, Chorny A, Varela N, Del Moral, RG, Delgado M.** Therapeutic effect of cortistatin on experimental arthritis by downregulating inflammatory and TH-1 responses. *Ann Rheum Dis.* 2007; 66: 582–8.
58. **Ceriani G, Diaz J, Murphree S, Catania A, Lipton JM.** The neuropeptide alpha-melanocyte-stimulating hormone inhibits experimental arthritis in rats. *Neuroimmunomodulation.* 1994; 1: 28–32.
59. **Gonzalez-Rey E, Chorny A, Varela N, O'Valle F, Delgado M.** Therapeutic effect of urocortin on collagen-induced arthritis by downregulating inflammatory and Th1 response and inducing regulatory T cells. *Arthritis Rheum.* 2007; 56: 531–43.
60. **Gonzalez-Rey E, Chorny A, O'Valle F, Delgado M.** Adrenomedullin protects from experimental arthritis by downregulating inflammation and Th1 response and inducing regulatory T cells. *Am J Pathol.* 2007; 170: 263–71.
61. **Gonzalez-Rey E, Varela N, Sheibanie AF, Chorny A, Ganea D, Delgado M.** Cortistatin, a new anti-inflammatory peptide with therapeutic action in inflammatory bowel disease. *Proc Natl Acad Sci USA.* 2006; 103: 4228–33.
62. **Gonzalez-Rey E, Fernandez-Martin A, Chorny A, Delgado M.** Therapeutic effect of urocortin and adrenomedullin in a murine model of Crohn´s disease. *Gut.* 2006; 55: 824–32.
63. **Abad C, Martinez C, Juarranz MG, Arranz A, Leceta J, Delgado M, Gomariz RP.** Therapeutic effects of vasoactive intestinal peptide in the trinitrobenzene sulfonic acid mice model of Crohn's disease. *Gastroenterology.* 2003; 124: 961–71.
64. **Rajora N, Boccoli G, Catania A, Lipton JM.** Alpha-MSH modulates experimental inflammatory bowel disease. *Peptides.* 1997; 18: 381–5.
65. **Gonzalez-Rey E, Chorny A, Delgado M.** Therapeutic action of ghrelin in a mouse model of colitis. *Gastroenterology.* 2006; 130: 1707–20.
66. **Herrera JL, Fernandez-Montesinos R, Gonzalez-Rey E, Delgado M, Pozo D.** Protective role for plasmid DNA-mediated VIP gene transfer in non-obese diabetic mice. *Ann NY Acad Sci.* 2006; 1070: 337–41.
67. **Rosignoli F, Roca V, Meiss R, Leceta J, Gomariz RP, Perez Leiros C.** VIP and tolerance induction in autoimmunity. *Ann NY Acad Sci.* 2006; 1070: 525–30.
68. **Poliak S, Mor F, Conlon P, Wong T, Ling N, Rivier J, Vale W, Steinman L.** Stress and autoimmunity: the neuropeptides corticotropin-releasing factor and urocortin suppress encephalomyelitis *via* effects on both the hypothalamic-pituitary-adrenal axis and the immune system. *J Immunol.* 1997; 158: 5751–6.
69. **Li H, Mei Y, Wang Y, Xu L.** Vasoactive intestinal polypeptide suppressed experimental autoimmune encephalomyelitis by inhibiting T helper 1 responses. *J Clin Immunol.* 2006; 26: 430–7.
70. **Gonzalez-Rey E, Fernandez-Martin A, Chorny A, Martin J, Pozo D, Ganea D, Delgado M.** Therapeutic effect of vasoactive intestinal peptide on experimental autoimmune encephalomyelitis: downregulation of inflammatory and autoimmune responses. *Am J Pathol.* 2006; 168: 1179–88.
71. **Lodde BM, Mineshiba F, Wang J, Cotrim AP, Afione S, Tak PP, Baum BJ.** Effect of human vasoactive intestinal peptide gene transfer in a murine model of Sjörgre's disease. *Ann Rheum Dis.* 2006; 65: 195–200.
72. **Kojima M, Ito T, Oono T, Hisano T, Igarashi H, Arita Y, Kawabe K, Coy DH, Jensen RT, Nawata H.** VIP attenuation of the severity of experimental pancreatitis is due to VPAC1 receptor-mediated inhibition of cytokine production. *Pancreas.* 2005; 30: 62–70.
73. **Dembinski A, Warzecha Z, Ceranowicz P, Tomaszewska R, Stachura J, Konturek SJ, Konturek PC.** Ghrelin attenuates the development of acute pancreatitis in rat. *J Physiol Pharmacol.* 2003; 54: 561–73.
74. **Nishida T, Taylor AW.** Specific aqueous humor factors induce activation of regulatory T cells. *Invest Ophthalmol Vis Sci.* 1999; 40: 2268–74.
75. **Keino H, Kezuka T, Takeuchi M, Yamakawa N, Hattori T, Usui M.** Prevention of experimental autoimmune uveoretinitis by vasoactive intestinal peptide. *Arch Ophthalmol.* 2004; 122: 1179–84.
76. **Gonzalez-Rey E, Varela N, Chorny A, Delgado M.** Therapeutic approaches of vasoactive intestinal peptide as a pleiotropic immunomodulator. *Curr Pharm Des.* 2007; 13: 1113–39.
77. **Delgado M, Munoz-Elias EJ, Gomariz RP, Ganea D.** VIP and PACAP inhibit IL-12 production in LPS-stimulated macrophages. Subsequent effect on IFNγ synthesis by T cells. *J Neuroimmunol.* 1999; 96: 167–81.
78. **Hernanz A, Tato E, De La Fuente M, De Miguel E, Arnalich F.** Differential effects of

gastrin-releasing peptide, neuropeptide Y, somatostatin and vasoactive intestinal peptide on interleukin-1 beta, interleukin-6 and tumor necrosis factor-alpha production by whole blood cells from healthy young and old subjects. *J Neuroummunol.* 1997; 71: 25–30.

79. **Delgado M, Pozo D, Martinez C, Leceta J, Calvo JR, Ganea D, Gomariz RP.** Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit endotoxin-induced TNF production by macrophages: *in vitro* and *in vivo* studies. *J Immunol.* 1999; 162: 2358–67.
80. **Delgado R, Carlin A, Airaghi L, Demitri MT, Meda L, Galimberti D, Baron P, Lipton JM, Catania A.** Melanocortin peptides inhibit production of proinflammatory cytokines and nitric oxide by activated microglia. *J Leukoc Biol.* 1998; 63: 740–5.
81. **Delgado M, Jonakait GM, Ganea D.** Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit chemokine production in activated microglia. *Glia.* 2002; 39: 148–61.
82. **Delgado M, Leceta J, Ganea D.** Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide inhibit the production of proinflammatory mediators by activated microglia by down-regulating NFkB. *J Leukoc Biol.* 2003; 73: 155–64.
83. **Martínez C, Delgado M, Pozo D, Leceta J, Calvo JR, Ganea D, Gomariz RP.** Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide modulate endotoxin-induced IL-6 production by murine peritoneal macrophages. *J Leukoc Biol.* 1998; 63: 591–601.
84. **Delgado M, Muñoz-Elías EJ, Gomariz RP, Ganea D.** Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide prevent inducible nitric oxide synthase transcription in macrophages by inhibiting NF-kB and IFN regulatory factor 1 activation. *J Immunol.* 1999; 162: 4685–96.
85. **Agnello D, Bertini R, Sacco S, Meazza C, Villa P, Ghezzi P.** Corticosteroid-independent inhibition of tumor necrosis factor production by the neuropeptide urocortin. *Am J Physiol Endocrinol Metab.* 1998; 275: E757–62.
86. **Delgado M, Ganea D.** Inhibition of endotoxin-induced macrophage chemokine production by vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide *in vitro* and *in vivo*. *J Immunol.* 2001; 167: 966–75.
87. **Wang MJ, Lin SZ, Kuo JS, Hang HY, Tzeng SF, Liao CH, Chen DC, Chen WF.** Urocortin modulates inflammatory response and neurotoxicity induced by microglial activation. *J Immunol.* 2007; 179: 6204–14.
88. **Delgado M, Gonzalez-Rey E.** Vasoactive intestinal peptide inhibits cycloxygenease 2 expression in activated macrophages, microglia and dendritic cells. *Brain Behav Immun.* 2008; 22: 35–41.
89. **Bhardwaj RS, Schwarz A, Becher E, Mahnke K, Aragane Y, Schwarz T, Luger TA.** Pro-opiomelanocortin-derived peptides induce IL-10 production in human monocytes. *J Immunol.* 1996; 156: 2517–21.
90. **Delgado M, Muñoz-Elías EJ, Gomariz RP, Ganea D.** Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide enhance IL-10 production by murine macrophages: *in vitro* and *in vivo* studies. *J Immunol.* 1999; 162: 1707–16.
91. **Luger TA, Scholzen TE, Brzoska T, Bohm M.** New insights into the functions of alpha-MSH and related peptides in the immune system. *Ann NY Acad Sci.* 2003; 994: 133–40.
92. **Delgado M, Reduta A, Sharma V, Ganea D.** VIP/PACAP oppositely affect immature and mature dendritic cell expression of CD80/CD86 and the stimulatory activity of $CD4^+$ T cells. *J Leukoc Biol.* 2004; 75: 1122–30.
93. **Chorny A, Delgado M.** Neuropeptides rescue mice from lethal sepsis by down-regulating the secretion of the late-acting inflammatory mediator high mobility group box 1. *Am J Pathol.* 2008; 172: 1297–307.
94. **Chorny A, Anderson P, Gonzalez-Rey E, Delgado M.** Ghrelin protects against experimental sepsis by inhibiting high-mobility group box 1 release and by killing bacteria. *J Immunol.* 2008; 180: 8369–77.
95. **Tsatsanis C, Androulidaki A, Dermitzaki E, Charalampopoulos I, Spiess J, Gravanis A, Margioris AN.** Urocortin 1 and urocortin 2 induce macrophage apoptosis via CRFR2. *FEBS Lett.* 2005; 579: 4259–64.
96. **Miksa M, Wu R, Cui W, Dong W, Das P, Simms HH, Ravikuman TS, Wang P.** Vasoactive hormone adrenomedullin and its binding protein: anti-inflammatory effects by up-regulating peroxisome proliferator-activating receptor-γ. *J Immunol.* 2007; 179: 6263–72.
97. **Delgado M, Leceta J, Gomariz RP, Ganea D.** VIP and PACAP stimulate the induction of Th2 responses by upregulating B7.2 expression. *J Immunol.* 1999; 163: 3629–35.
98. **Jiang X, Jing H, Ganea D.** VIP and PACAP down-regulate CXCL10 (IP-10) and up-regulate CCL22 (MDC) in spleen cells. *J Neuroimmunol.* 2002; 133: 81–94.
99. **Delgado M, Gonzalez-Rey E, Ganea D.** VIP/PACAP preferentially attract Th2 versus Th1 cells by differentially regulating the production of chemokines by dendritic cells. *FASEB J.* 2004; 18: 1453–5.
100. **Sharma V, Delgado M, Ganea D.** Granzyme B, a new player in activation-induced cell death, is down-regulated by vasoactive intestinal peptide in Th2 but not Th1 effectors. *J Immunol.* 2006; 176: 97–110.
101. **Delgado M, Leceta J, Ganea D.** Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide promote *in vivo* generation of memory Th2 cells. *FASEB J.* 2002; 16: 1844–6.
102. **Voice J, Donnelly S, Dorsam G, Dolganov G, Paul S, Goetzl EJ.** c-Maf and JunB mediation of Th2 differentiation induced by the type 2 G protein-coupled receptor (VPAC2) for vasoactive intestinal peptide. *J Immunol.* 2004; 172: 7289–96.
103. **Gonzalez-Rey E, Fernandez-Martin A, Chorny A, Delgado, M.** Vasoactive intestinal peptide induces $CD4^+CD25^+$ regulatory T cells with therapeutic effect on collagen-induced arthritis. *Arthritis Rheum.* 2006; 54: 864–76.
104. **Fernandez-Martin A, Gonzalez-Rey E, Chorny A, Ganea D, Delgado M.** Vasoactive intestinal peptide induces regulatory T cells during experimental autoimmune encephalomyelitis. *Eur J Immunol.* 2006; 36: 318–26.
105. **Delgado M, Chorny A, Gonzalez-Rey E, Ganea D.** Vasoactive intestinal peptide generates $CD4^+CD25^+$ regulatory T cells *in vivo*. *J Leukoc Biol.* 2005; 78: 1327–38.
106. **Chorny A, Gonzalez-Rey E, Fernandez-Martin A, Pozo D, Ganea D, Delgado M.** Vasoactive intestinal peptide induces regulatory dendritic cells with therapeutic effects on autoimmune disorders. *Proc Natl Acad Sci USA.* 2005; 102: 13562–7.
107. **Delgado M, Gonzalez-Rey E, Ganea D.** The neuropeptide vasoactive intestinal peptide generates tolerogenic dendritic cells. *J Immunol.* 2005; 175: 7311–24.
108. **Gonzalez-Rey E, Chorny A, Fernandez-Martin A, Ganea D, Delgado M.** Vasoactive intestinal peptide generates human tolerogenic dendritic cells that induce CD4 and CD8 regulatory T cells. *Blood.* 2006; 107: 3632–8.

109. **Cutuli M, Cristiani S, Lipton JM, Catania A.** Antimicrobial effects of alpha-MSH peptides. *J Leukoc Biol.* 2000; 67: 233–9.
110. **Allaker RP, Zihni C, Kapas S.** An investigation into the antimicrobial effects of adrenomedullin on members of the skin, oral, respiratory tract and gut microflora. *FEMS Immunol Med Microbiol.* 1999; 23: 289–93.
111. **Martinez C, Abad C, Delgado M, Arranz A, Juarranz MG, Rodriguez-Henche N, Brabet P, Leceta J, Gomariz RP.** Anti-inflammatory role in septic shock of pituitary adenylate cyclase-activating polypeptide receptor. *Proc Natl Acad Sci USA.* 2002; 99: 1053–8.
112. **Szema AM, Hamidi SA, Lyubsky S, Dickman KG, Mathew S, Abdel-Razek T, Chen JJ, Waschek JA, Said SI.** Mice lacking the VIP gene show airway hyperresponsiveness and airway inflammation, partially reversible by VIP. *Am J Physiol Lung Cell Mol Physiol.* 2006; 291: 880–6.
113. **Voice JK, Dorsam G, Lee H, Kong Y, Goetzl EJ.** Allergic diathesis in transgenic mice with constitutive T cell expression of inducible VIP receptor. *FASEB J.* 2001; 15: 2489–96.
114. **Goetzl EJ, Voice JK, Shen S, Dorsam G, Kong Y, West KM, Morrison CF, Harmar AJ.** Enhanced delayed-type hypersensitivity and diminished immediate-type hypersensitivity in mice lacking the inducible VPAC(2) receptor for VIP. *Proc Natl Acad Sci USA.* 2001; 98: 13854–9.
115. **Voice J, Donnelly S, Dorsam G, Dolganov G, Paul S, Goetzl EJ.** Roles of vasoactive intestinal peptide (VIP) in the expression of different immune phenotypes by wild-type mice and T cell-targeted type II VIP receptor transgenic mice. *J Immunol.* 2003; 170: 308–14.
116. **Juarranz Y, Gutierrez-Canas I, Santiago B, Carrion M, Pablos JL, Gomariz RP.** Differential expression of vasoactive intestinal peptide and its functional receptors in human osteoarthritic and rheumatoid synovial fibroblasts. *Arthritis Rheum.* 2008; 58: 1086–95.
117. **Delgado M, Robledo G, Rueda B, Varela N, O´Valle F, Hernandez-Cortes P, Caro M, Orozco G, Gonzalez-Rey E, Martin J.** Genetic association of vasoactive intestinal peptide with rheumatoid arthritis. Altered expression and signal in immune cells. *Arthritis Rheum.* 2008; 58: 1010–9.
118. **Sun W, Hong J, Zang YC, Liu X, Zhang JZ.** Altered expression of vasoactive intestinal peptide receptors in T lymphocytes and aberrant Th1 immunity in multiple sclerosis. *Int Immunol.* 2006; 18: 1691–700.
119. **Bangale Y, Karle S, Planque S, Zhou YX, Taguchi H, Nishiyama Y, Li L, Kalaga R, Paul S.** VIPase autoantibodies in Fas-defective mice and patients with autoimmune disease. *FASEB J.* 2003; 17: 628–35.
120. **Moncada S, Ferreira SH, Vane JR.** Prostaglandins, aspirin-like drugs and the oedema of inflammation. *Nature.* 1973; 246: 217–9.
121. **Tilley SL, Coffman TM, Koller BH.** Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J Clin Invest.* 2001; 208: 15–23.
122. **Levy BD, Clish DB, Schmidt B, Gronert K, Serhan CN.** Lipid mediator class switching during acute inflammation: signals in resolution. *Nat Immunol.* 2001; 2: 612–9.
123. **Serhan CN.** Lipoxins and novel aspirin-triggered 15-epi-lipoxins (ATL): a jungle of cell-cell interactions or a therapeutic opportunity? *Prostaglandins.* 1997; 53: 107–37.
124. **Serhan CN, Takano T, Clish CB, Gronert K, Petasis N.** Aspirin-triggered 15-epi-lipoxin A4 and novel lipoxin B4 stable analogs inhibit neutrophil-mediated changes in vascular permeability. *Adv Exp Med Biol.* 1999; 469: 287–93.
125. **Papayianni A, Serhan CN, Brady HR.** Lipoxin A4 and B4 inhibit leukotriene-stimulated interactions of human neutrophils and endothelial cells. *J Immunol.* 1996; 156: 2264–72.
126. **Maddox JF, Serhan CN.** Lipoxin A4 and B4 are potent stimuli for human monocyte migration and adhesion: selective inactivation by dehydrogenation and reduction. *J Exp Med.* 1996; 183: 137–46.
127. **Maddox JF, Hachicha M, Takano T, Petasis NA, Fokin VV, Serhan CN.** Lipoxin A4 stable analogs are potent mimetics that stimulate human monocytes and THP-1 cells via a G-protein-linked lipoxin A4 receptor. *J Biol Chem.* 1997; 272: 6972–8.
128. **József L, Zouki C, Petasis NA, Serhan CN, Filep JG.** Lipoxin A4 and aspirin-triggered 15-epi-lipoxin A4 inhibit peroxynitrite formation, NF-kappa B and AP-1 activation, and IL-8 gene expression in human leukocytes. *Proc Natl Acad Sci USA.* 2002; 99: 13266–71.
129. **Godson C, Mitchell S, Harvey K, Petasis NA, Hogg N, Brady HR.** Cutting edge: lipoxins rapidly stimulate nonphlogistic phagocytosis of apoptotic neutrophils by monocyte-derived macrophages. *J Immunol.* 2000; 164: 1663–7.
130. **Mitchell S, Thomas G, Harvey K, Cottell D, Reville K, Berlasconi G, Petasis NA, Erwig L, Rees AJ, Savill J, Brady HR, Godson C.** Lipoxins, aspirin-triggered epi-lipoxins, lipoxin stable analogues, and the resolution of inflammation: stimulation of macrophage phagocytosis of apoptotic neutrophils *in vivo*. *J Am Soc Nephrol.* 2002; 13: 2497–507.
131. **Serhan CN.** Resolution phase of inflammation: novel endogenous anti-inflammatory and proresolving lipid mediators and pathways. *Annu Rev Immunol.* 2007; 25: 101–37.
132. **Yacoubian S, Serhan CN.** New endogenous anti-inflammatory and proresolving lipid mediators: implications for rheumatic diseases. *Nat Clin Pract Rheumatol.* 2007; 3: 570–9.
133. **Burr GO, Burr MM.** A new deficiency disease produced by the rigid exclusion of fat from the diet. *J Biol Chem.* 1929; 82: 345–67.
134. **Camuesco D, Galvez J, Nieto A, Comalada M, Rodriguez-Cabezas ME, Concha A, Xaus J, Zarzuelo A.** Dietary olive oil supplemented with fish oil, rich in EPA and DHA (n-3) polyunsaturated fatty acids, attenuates colonic inflammation in rats with DSS-induced colitis. *J Nutr.* 2005; 135: 687–94.
135. **Billman GE, Kang JX, Leaf A.** Prevention of sudden cardiac death by dietary pure ω-3 polyunsaturated fatty acids in dogs. *Circulation.* 1999; 99: 2452–7.
136. **Marchioli R, Barzi F, Bomba E, Chieffo C, Di Gregorio D, Di Mascio R, Franzosi MG, Geraci E, Levantesi G, Maggioni AP, Mantini L, Marfisi RM, Mastroginseppe G, Mininni N, Nicolosi GL, Santini M, Schweiger C, Tavazzi L, Tognoni G, Tucci C, Valagussa F, GISSI-Prevenzione Investigators.** Early protection against sudden death by n-3 polyunsaturated fatty acids after myocardial infarction: time-course analysis of the results of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-Prevenzione. *Circulation.* 2002; 105: 1897–1903.
137. **Holub DJ, Holub BJ.** Omega-3 fatty acids from fish oils and cardiovascular disease. *Mol Cell Biochem.* 2004; 263: 217–25.
138. **Engler MM, Engler MB, Malloy M, Chiu E, Besio D, Paul S, Stuehlinger M, Morrow JD, Ridker PM, Rifai N, Mietus-Snyder M.** Docosahexaenoic acid restores endothelial function in children with hyperlipidemia: results from the EARLY study. *Int J Clin Pharmacol Ther.* 2004; 42: 672–9.

139. **Lim GP, Calon F, Morihara T, Yang F, Teter B, Ubeda O, Salem N Jr, Frautschy SA, Cole GM.** A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. *J Neurosci.* 2005; 25: 3032–40.
140. **Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K.** Novel functional sets of lipid-derived mediators with anti-inflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal anti-inflammatory drugs and transcellular processing. *J Exp Med.* 2000; 192: 1197–204.
141. **Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, Moussignac RL.** Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter pro-inflammation signals. *J Exp Med.* 2002; 196, 1025–37.
142. **Hong S, Gronert K, Devchand P, Moussignac RL, Serhan CN.** Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood and glial cells: autacoids in anti-inflammation. *J Biol Chem.* 2003; 278: 14677–87.
143. **Marcheselli VL, Hong S, Lukiw WJ, Tian XH, Gronert K, Musto A, Hardy M, Gimenez JM, Chiang N, Serhan CN, Bazan NG.** Novel docosanoids inhibit brain ischemia-reperfusion-mediated leukocyte infiltration and proinflammatory gene expression. *J Biol Chem.* 2003; 278: 43807–17.
144. **Mukherjee PK, Marcheselli VL, Serhan CN, Bazan NG.** Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. *Proc Natl Acad Sci USA.* 2004; 101: 8491–6.
145. **Lukiw WJ, Cui JG, Marcheselli VL, Bodker M, Botkjaer A, Gotlinger K, Serhan CN, Bazan NG.** A role for docosahexaenoic acid-derived neuroprotectin D1 in neural cell survival and Alzheimer disease. *J Clin Invest.* 2005; 115: 2774–83.
146. **Serhan CN, Gorlinger K, Hong S, Lu Y, Siegelman J, Baer T, Yang R, Colgan SP, Petasis NA.** Anti-inflammatory actions of neuroprotectin D1/protectin D1 and its natural stereoisomers: assignments of dihydroxy-containing docosatrienes. *J Immunol.* 2006; 176: 1848–59.
147. **Arita, M. Bianchini F, Aliberti J, Sher A, Chiang N, Hong S, Yang R, Petasis NA, Serhan CN.** Stereochemical assignment, anti-inflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. *J Exp Med.* 2005; 201: 713–22.
148. **Bannenberg GL, Chiang N, Ariel A, Arita M, Tjonahen E, Gotlinger KH, Hong S, Serhan CN.** Molecular circuits of resolution: formation and actions of resolvins and protectins. *J Immunol.* 2005; 174: 4345–55.
149. **Hasturk H, Kantarci A, Ohira T, Arita M, Ebrahimi N, Chiang N, Petasis NA, Levy BD, Serhan CN, Van Dyke TE.** RvE1 protects from local inflammation and osteoclast- mediated bone destruction in periodontitis. *FASEB J.* 2006; 20: 401–3.
150. **Campbell EL, Louis NA, Tomassetti SE, Canny GO, Arita M, Serhan CN, Colgan SP.** Resolvin E1 promotes mucosal surface clearance of neutrophils: a new paradigm for inflammatory resolution. *FASEB J.* 2007; 21: 3162–70.
151. **Arita M, Yoshida M, Hong S, Tjonahen E, Glickman JN, Petasis NA, Blumberg RS, Serhan CN.** Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2,4,6-trinitrobenzene sulfonic acid-induced colitis. *Proc Natl Acad Sci USA.* 2005; 102: 7671–6.
152. **Arita M, Ohira T, Sun YP, Elangovan S, Chiang N, Serhan CN.** Resolvin E1 selectively interacts with leukotriene B4 receptor BLT1 and ChemR23 to regulate inflammation. *J Immunol.* 2007; 178: 3912–7.
153. **Duffield JS, Hong S, Vaidya VS, Lu Y, Fredman G, Serhan CN, Bonventre JV.** Resolvin D series and protectin D1 mitigate acute kidney injury. *J Immunol.* 2006; 177: 5902–11.
154. **Gronert K, Maheshwari N, Khan N, Hassan IR, Dunn M, Laniado Schwartzman M.** A role for the mouse 12/15-lipoxygenase pathway in promoting epithelial wound healing and host defense. *J Biol Chem.* 2005; 280: 15267–78.
155. **Levy BD, Kohli P, Gotlinger K, Haworth O, Hong S, Kazani S, Israel E, Haley KJ, Serhan CN.** Protectin D1 is generated in asthma and dampens airway inflammation and hyperresponsiveness. *J Immunol.* 2007; 178: 496–502.
156. **Ariel A, Li PL, Wang W, Tang WX, Fredman G, Hong S, Gotlinger KH, Serhan CN.** The docosatriene protectin D1 is produced by TH2 skewing and promotes human cell apoptosis via lipid raft clustering. *J Biol Chem.* 2005; 280: 43079–86.
157. **Arrington JL, Chapkin RS, Switzer KC, Morris JS, McMurray DN.** Dietary n-3 polyunsaturated fatty acids modulate purified murine T-cell subset activation. *Clin Exp Immunol.* 2001; 125: 499–507.
158. **Chapkin RS, Arrington JL, Apanasovich TV, Carroll RJ, McMurray DN.** Dietary n-3 PUFA affect TcR-mediated activation of purified murine T cells and accessory cell function in co-cultures. *Clin Exp Immunol.* 2002; 130: 12–8.
159. **Ariel A, Fredman G, Sun YP, Kantarci A, Van Dyke TE, Luster AD, Serhan CN.** Apoptotic neutrophils and T cells sequester chemokines during immune response resolution through modulation of CCR5 expression. *Nat Immunol.* 2006; 7: 1209–16.
160. **Schwab JM, Chiang N, Arita M, Serhan CN.** Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature.* 2007; 447: 869–74.
161. **Ueda S, Nishio K, Minamino N, Kubo A, Akai Y, Kangawa K, Matsuo H, Fujimura Y, Yoshioka A, Masui K, Doi N, Murao Y, Miyamoto S.** Increased plasma levels of adrenomedullin in patients with systemic inflammatory response syndrome. *Am J Respir Crit Care Med.* 1999; 160: 132–6.
162. **Brandtzaeg P, Oktedalen O, Kierulf P, Opstad PK.** Elevated VIP and endotoxin plasma levels in human gram-negative septic shock. *Regul Pept.* 1989; 24: 37–44.
163. **Delgado M, Abad C, Martinez C, Lecta J, Gomariz RP.** Vasoactive intestinal peptide in the immune system: potential therapeutic role in inflammatory and autoimmune diseases. *J Mol Med.* 2002; 80: 16–24.
164. **Yudoh K, Matsuno H, Kimura T.** Plasma adrenomedullin in rheumatoid arthritis compared with other rheumatic diseases. *Arthritis Rheum.* 1999; 42: 1297–8.
165. **Torpy DJ, Webster EL, Zachman EK, Aguilera G, Chrousos GP.** Urocortin and inflammation: confounding effects of hypotension on measures of inflammation. *Neuroimmunomodulation.* 1999; 6: 182–6.
166. **Peracchi M, Bardella MT, Caprioli F, Massironi S, Conte D, Valenti L, Ronchi C, Beck-Peccoz P, Arosio M, Piodi L.** Circulating ghrelin levels in patients with inflammatory bowel disease. *Gut.* 2006; 55: 432–3.
167. **Serhan NC, Savill J.** Resolution of inflammation: the beginning programs the end. *Nat Immunol.* 2005: 6: 1191–7.
168. **Nagaya N, Satoh T, Nishikimi T, Uematsu M, Furuichi S, Sakamaki F, Oya H, Kyotani S, Nakanishi N, Goto Y, Masuda Y, Miyatake K, Kangawa K.** Hemodynamic, renal, and hormonal effects of adrenomedullin infusion in

patients with congestive heart failure. *Circulation.* 2000; 101: 498–503.

169. **Davis ME, Pemberton CJ, Yandle TG, Lainchbury JG, Rademaker MT, Nicholls MG, Frampton CM, Richards AM.** Effect of urocortin 1 infusion in humans with stable congestive cardiac failure. *Clin Sci.* 2005; 109: 381–8.
170. **Petkov V, Mosgoeller W, Ziesche R,. Raderer M, Stiebellehner L, Vonbank K, Funk GC, Hamilton G, Novotny C, Burian B, Block LH.** Vasoactive intestinal peptide as a new drug for treatment of primary pulmonary hypertension. *J Clin Invest.* 2003; 111: 1339–46.
171. **Broglio F, Benso A, Gottero C, Prodam F, Gauna C, Filtri L, Arvat E, van der Lely AJ, Deghenghi R, Ghigo E.** Ghrelin secretion is inhibited by either somatostatin or cortistatin in humans. *J Clin Endocrinol Metab.* 2002; 87: 4829–32.
172. **Hackstein H, Thomson AW.** Dendritic cells: emerging pharmacological targets of immunosuppressive drugs. *Nat Rev Immunol.* 2004; 4: 24–34.
173. **Rutella S, Danese S, Leone G.** Tolerogenic dendritic cells: cytokine modulation comes of age. *Blood.* 2006; 108: 1435–40.
174. **Gonzalez-Rey E, Delgado M.** Therapeutic treatment of experimental colitis with regulatory dendritic cells generated with vasoactive intestinal peptide. *Gastroenterology.* 2006; 131: 1799–1811.
175. **Gotthardt M, Boermann OC, Behr TM, Behe MP, Oyen WJ.** Development and clinical application of peptide-based radiopharmaceuticals. *Curr Pharm Des.* 2004; 10: 2951–63.
176. **Bolin DR, Michalewsky J, Wasserman MA, O'Donnell M.** Design and development of a vasoactive intestinal peptide analog as a novel therapeutic for bronchial asthma. *Biopolymers.* 1995; 37: 57–66.
177. **Onyuksel H, Ikezaki H, Patel M, Gao XP, Rubinstein I.** A novel formulation of VIP in sterically stabilized micelles amplifies vasodilation *in vivo*. *Pharm Res.* 1999; 16: 155–60.
178. **Sethi V, Onyuksel H, Rubinstein I.** Liposomal vasoactive intestinal peptide. *Methods Enzymol.* 2005; 391: 377–95.
179. **Kato K, Yin H, Agata J, Yoshida H, Chao L, Chao J.** Adrenomedullin gene delivery attenuates myocardial infarction and apoptosis after ischemia and reperfusion. *Am J Physiol Heart Cir Physiol.* 2003; 285: H1506–14.
180. **Delgado M, Toscano MG, Benabdellah K, Cobo M, O'Valle F, Gonzalez-Rey E, Martın F.** *In vivo* delivery of lentiviral vectors expressing vasoactive intestinal peptide complementary DNA as gene therapy for collagen-induced arthritis. *Arthritis Rheum.* 2008; 58: 1026–37.
181. **Sedo A, Duke-Cohan JS, Balaziova E, Sedova LR.** Dipeptidyl peptidase IV activity and/or structure homologs: contributing factors in the pathogenesis of rheumatoid arthritis? *Arthritis Res Ther.* 2005; 7: 253–69.
182. **Foey AD, Field S, Ahmed S, Jain A, Feldmann M, Brennan FM, Williams R**. Impact of VIP and cAMP on the regulation of TNF-alpha and IL-10 production: implications for rheumatoid arthritis. *Arthritis Res Ther.* 2003; 5: 317–28.
183. **Moffatt, JD, Lever, R.** and Page, CP Activation of corticotropin-releasing factor receptor-2 causes bronchorelaxation and inhibits pulmonary inflammation in mice. *FASEB J.* 2006; 20: 1877–9.
184. **Blakeney JS, Fairlie DP.** Nonpeptide ligands that target peptide-activated GPCRs in inflammation. *Curr Med Chem.* 2005; 12: 3027–42.
185. **Weckbecker G, Lewis I, Albert R, Schmid HA, Hoyer D, Bruns C.** Opportunities in somatostatin research: biological, chemical and therapeutic aspects. *Nat Rev Drug Disc.* 2003; 2: 999–1017.
186. **Bürli RW, Xu H, Zou X, Muller K, Golden J, Frohn M, Adlam M, Plant MH, Wong M, McElvain M, Regal K, Viswanadhan VN, Tagari P, Hungate R.** Potent hFPRL1 (ALXR) agonists as potential anti-inflammatory agents. *Bioorg Med Chem Lett.* 2006; 16: 3713–8.
187. **Kim Y, Lee BD, Bae YS, Lee T, Suh PG, Ryu SH.** Pituitary adenylate cyclase-activating polypeptide 27 is a functional ligand for formyl peptide receptor-like 1. *J Immunol.* 2006; 176: 2969–75.
188. **El Zein N, Badran B, Sariban E.** VIP differentially activates beta2 integrins, CR1, and matrix metalloproteinase-9 in human monocytes through cAMP/PKA, EPAC, and PI-3K signaling pathways via VIP receptor type 1 and FPLR1. *J Leukoc Biol.* 2008; 83: 972–81.