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Review

Neuro-Endocrine-Immune Axis in Human Rheumatoid Arthritis

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Abstract. We present an overview of the role of neuro-endocrine-immune mechanisms in the pathophysiological responses of patients with rheumatoid arthritis (RA). In patients with RA, proinflammatory cytokines secreted by synovial cells provoke local inflammation in the joints and, simultaneously, initiate a systemic acute phase response. Thus, profound changes of the neuro-endocrine-immune axis could take place in the patients. Defects in the hypothalamus-pituitary-adrenal axis have been observed in patients with RA. Prolactin levels are often elevated and abnormal sex hormone levels have been described in RA patients. Defective neural regulation of inflammation involving neuropeptides at least partly plays a pathogenic role in RA. We and others have found that participants of the neuro-endocrine-immune interactions, such as hormones, neurotransmitters and neuropeptides, modulate RA synovial cell functions and that they are actually produced by, and their receptors are expressed on, cells within the inflammatory joint compartment. Thus, neuropeptides and hormones not only affect a systemic acute phase response of RA patients, but also modulate local inflammation directly in RA joints. These results suggest that defects in regulatory processes which are fundamental to RA may lie in the immune system, the nervous system, the endocrine system or the interactions of these. A better understanding of neuro-endocrine-immune interactions holds the promise of new approaches to the treatment of RA with the use of hormones, neurotransmitters, neuropeptides and/or their antagonists.

Key words: neuro-endocrine-immune interactions; rheumatoid arthritis; synovial cells; hypothalamus-pituitary--adrenal axis; inflammation.

Rheumatoid arthritis (RA) is characterized by synovial membrane inflammation leading to chronic polyarticular destruction[10]. The inflamed synovium shows hyperplasia of synovial lining cells and lymphocytes, macrophages and granulocytes are infiltrated into the synovium[51, 109]. Therefore, interactions between the sy-

Abbreviations used: RA – rheumatoid arthritis, MMP – matrix metalloproteinase, PRL – prolactin, CRH – Corticotropin-releasing hormone, IL – interleukin, TNF – tumor necrosis factor, CRH-BP – CRH-binding protein, DHEA – dehydroepiandrosterone, DHEAS – DHEA sulfate, SOM – somatostatin, SOMR – SOM receptor, PKA – protein kinase A, CREB – cyclic AMP responsive element binding protein, PRL – prolactin, PRLR – PRL receptor, GM-CSF – granulocyte/macrophage-colony stimulating factor, STAT – signal transduction and activation of transcription, Th – T helper, SLE – systemic lupus erythematosus, TIMP – tissue inhibitor of metalloproteinase, BRC – bromocriptine, HLA – human leucocyte antigen, CGRP – calcitonin gene-related peptide, VIP – vasoactive intestinal peptide, VIPR – VIP receptor, CGRPR – CGRP receptor, POMC – proopiomelanocortin, met-ENK – metionin-enkephalin, leu-ENK – leucine-enkephalin, END – endorphin.

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novial cells and infiltrating cells would occur in the RA synovium[25, 80]. It has been shown that synovial cells contribute to inflammatory responses by their production of proinflammatory cytokines, degrading enzymes such as matrix metalloproteinases (MMPs), and prostaglandins, all of which regulate cellular functions within the synovial compartment and cause tissue damage in patients with RA[93, 99].

Organisms, including humans, respond to a variety of environmental stresses by mounting a coordinated complex series of adaptive responses involving the immune, nervous and endocrine systems. These adaptations are aimed at restoring the homeostatic balance and returning to the steady state[52]. Each system in the immune, nervous and endocrine systems interacts with other systems to maintain the homeostatic balance. These interactions are facilitated by cytokines, hormones and neuropeptides, as well as their receptors which are endogenously expressed to the neural, immune and endocrine systems[95]. It is becoming clear that the neural, immune and endocrine systems also govern the synovial compartment[45, 54].

There exist several clinical observations suggesting the involvement of the nervous system in the development and maintenance of RA lesions; RA that develops in patients who have had hemiplegia appears to have less affected the paralyzed limbs[38]; and osteoarthritis and gout may preferentially affect the nonparalyzed side in hemiplegic patients[85]. Thus, it has been hypothesized that the nervous system and neuropeptides contribute to the inflammatory reactions and pathologic processes of RA. Similarly, involvement of the endocrine system for the pathologic responses of RA has been reported, including hypothalamic-pituitary-adrenal cortisol secretion[35], prolactin (PRL) secretion[70] and the sex hormone status[71]. Furthermore, studies of animal models of arthritis have shown that defects in the neuro-endocrine-immune communication contribute to the development of arthritis[61].

In this review, we will focus on the pathogenic roles of the neuro-endocrine-immune system in patients with RA.

Hypothalamic-Pituitary-Adrenal Axis in RA

The hypothalamic-pituitary-adrenal axis plays an important role in regulating and controlling immune responses, and dysfunction of the axis has been implicated in the pathogenesis of RA[23].

Corticotropin-releasing hormone (CRH) is a major regulator of the hypothalamic-pituitary-adrenal axis and principal coordinator of the stress response[102]. As in stress, hypothalamic CRH suppresses the immune system indirectly via increased adrenal glucocorticoid production and/or sympathetic system-mediated mechanisms. During inflammatory stress, the cytokines, tumor necrosis factor α (TNF-α), interleukin1 (IL-1) and IL-6 stimulate hypothalamic CRH secretion as a way of preventing the inflammatory reaction from overreacting. On the other hand, CRH receptors are present in peripheral sites of the immune system, including acute phase inflammatory cells such as neutrophils[76], and CRH promotes several immune functions *in vitro*, such as the induction of proliferation of rat splenocytes[43]. The direct role of CRH also appears to promote the inflammatory immune process *in vivo*; CRH is secreted locally in acute carrageenin-induced inflammation in rats and has predominantly proinflammatory effects[102]; CRH is also expressed in the joints of Lewis rats with experimental arthritis[18]; *in vivo* neutralization of CRH by its specific antibody attenuates the inflammatory reactions in the rats[33].

In addition to production by immune cells, the peripheral nervous system, including the postganglionic sympathetic neurons and the sensory fibers type C, appears to contribute to CRH production in inflammatory sites[102]. The production of CRH from the postganglionic sympathetic neurons may be responsible for the stress-induced activation of allergic/autoimmune phenomena. Indeed, CROFFORD et al.[17] found that immunoreactive CRH is locally secreted in the synovium of patients with RA, suggesting that CRH functions as an autocrine and/or paracrine mediator of inflammation in humans as well.

The activity of CRH is modified by the presence of a 37 kDa binding protein (CRH-BP)[107]. WOODS et al.[107] studied CRH and CRH-BP in the synovial fluid of RA patients. They found that synovial fluid contains intact CRH-BP in molar excess to total CRH. The excess of CRH-BP may attenuate the inflammatory activity of CRH to reduce disease severity of RA patients.

CUTOLO et al.[19] assessed the hypothalamic-pituitary--adrenal axis function of sex hormone synthesis in patients with RA. They found that basal concentrations of dehydroepiandrosterone (DHEA) and its precursor, DHEA sulfate (DHEAS), which are intermediate metabolites of androgen synthesis, are significantly lower in premenopausal patients with RA than in premenopausal healthy controls. DHEA levels showed a significant negative correlation with the erythrocyte sedimentation rate, with platelet count and with the Steinbrocker class of the disease. There are several reports describing DHEA and DHEAS deficiency in patients with RA[81, 108]. Col-

lectively, it can be concluded that the abnormal androgen concentrations observed in patients with RA may support the implication of adrenal androgens in the immune/inflammatory cytokine-mediated mechanisms which are involved in the pathophysiology and clinical aspects of RA[19].

GUTIERREZ et al.[35] have reported recently that the basal serum levels of cortisol in a RA group were not significantly different from those of a healthy control group. The hypoglycemia stimulation-specific "delta" cortisol response was significantly reduced for the 30 to 45 min interval in patients with RA compared with controls. They concluded that active RA is associated with a subtle dysfunction of the hypothalamic-pituitary-adrenal glucocorticoid function[35]. Nonetheless, current studies suggest partial defects in the hypothalamic-pituitary-adrenal axis. The significance of the defects on RA pathogenesis has also been elusive. It should be clarified whether abnormal function of the hypothalamic-pituitary-adrenal axis is the primary cause of the disease or the secondary event to the systemic inflammation of RA. Future studies should thus focus on the relationship between the modulated functions of the hypothalamic-pituitary-adrenal axis and the subsequent pathophysiologic responses of RA.

Somatostatin

Somatostatin (SOM) is a small peptide which was isolated originally from the hypothalamus and which inhibits the hypophysial release of several hormones[7]. It is widely distributed throughout the central and peripheral nervous systems[97]. SOM is described as an anti-proliferative molecule and an inhibitor of exocrine or endocrine secretion from a variety of tissues including the pancreas, the gastro-intestinal tract and the central and peripheral nervous systems[32].

SOM also elicits inhibitory effects on both immune responses and inflammatory responses[34, 46]. SOM inhibits immunoglobulin synthesis by human B lymphocytes[49] and proliferation of human T lymphocytes[75]. It has been reported that the anti-inflammatory actions of glucocorticoid are partly mediated by increased secretion of SOM[47].

The inhibitory potential of SOM or its analogues is regarded to be applicable for the treatment of clinical conditions ranging from human cancers to Alzheimer's and Parkinson's diseases. Indeed, SOM has been shown to have analgesic properties in cluster headache[86] and in cancer[15]. SOM was applied for inducing remission of arthralgia in patients with psoriatic arthritis[65] and also in patients with RA[66]. All the data suggest that SOM may have anti-rheumatic effects.

SOM regulates synovial cell functions of RA patients

We have conducted experiments using *in vitro* cell cultures to elucidate the effects of SOM on RA synovial cell functions. We first measured the proliferation of RA synovial cells stimulated with serum and/or TNF-α in the presence or absence of SOM. We found that SOM inhibits the proliferative responses of RA synovial cells and the proliferation of both purified RA fibroblast-like synovial cells and macrophage-like synovial cells.

We next turned our attention onto the effects of SOM on proinflammatory cytokine production, including IL-6 and IL-8 by RA synovial cells. RA synovial cells produced a large quantity of IL-6 and IL-8 in response to TNF-α stimulation; treatment of RA synovial cells with SOM suppressed IL-6 and IL-8 mRNA expression and the protein production significantly. However, SOM did not inhibit TNF-α and IL-1β production.

We further tested whether SOM inhibits the production of MMP, important molecules for joint destruction, by RA synovial cells. SOM treatment of the RA synovial cells significantly reduced MMP-1, MMP-2 and MMP-9 mRNA expression. In addition, SOM treatment reproducibly reduced MMP protein synthesis and their enzymatic activities. These results suggest beneficial effects of SOM on the pathological responses of RA. We have confirmed that these effects of SOM are not due to its direct cytotoxic mechanism (unpublished observation). There is an important report that SOM treatment induces apoptosis of some tumor cell types[13]. Taken together, SOM would induce apoptotic cell death of RA synovial cells, thereby causing suppression of their functions[100]. SOM may thus be a desirable therapeutic strategy for treating patients with RA.

SOM-producing cells and SOM receptor-expressing cells in RA joints

Because we found that SOM reduces hyperfunctions of RA synovial cells, we next studied whether SOM is present within the affected joints of RA patients. It is especially important to clarify which cell type(s) is responsible for the SOM present within RA joints; SOM is delivered by the peripheral circulation or is actively produced by some cell types in the joints.

We found SOM mRNA expression by fibroblast-like synovial cells in patients with RA. We have confirmed that SOM protein is actually produced by the

synovial cells in patients with RA. In contrast, RA synovium-infiltrating lymphocytes, RA peripheral blood lymphocytes and normal peripheral blood lymphocytes neither expressed SOM mRNA nor produced SOM peptide. Thus, it is evident that SOM produced by RA fibroblast-like synovial cells acts on synovial cells to modulate their own function.

SOMRs were identified in synovial tissues displaying histologic changes compatible with active RA using classic ligand-binding techniques, *in vitro* autoradiography and *in vivo* scintigraphy[79, 98]. The SOMRs are members of the seven transmembrane segment receptor superfamily and molecular cloning studies have identified 5 subtypes[5]. We have characterized SOMR subtypes expressed on RA synovial cells. We found that subtypes 1 and 2 SOMR are expressed on RA fibroblast-like synovial cells. Of importance is the fact that expression of subtype 2 SOMR is up-regulated when synovial cells are stimulated with TNF-α. The results suggest the existence of an autoinhibitory circuit in the synovium, where an inhibitory signal of SOM could be augmented by enhanced SOMR expression in response to proinflammatory cytokine stimulation.

Molecular mechanism of SOM governing synovial cell functions in patients with RA

It has been shown that accumulation of intracellular cyclic AMP (cAMP) induces activation of the catalytic subunit of the phosphorylating enzyme, protein kinase A (PKA). Activation of PKA leads to the phosphorylation of ser-133 of cAMP-responsive element-binding protein (CREB); the phosphorylated CREB acts as a transcription factor to turn on the transcription and subsequent protein synthesis in certain cell types[37]. It is reported that SOM inhibits adenylate cyclase activity in many tissues[74]. Thus, it is likely that SOM inhibits adenylate cyclase activity and then cAMP accumulation, followed by deficient PKA activity in RA synovial cells[78]. This process may lead to deficient transcription factor CREB activity in the nucleus and reduced protein synthesis[37].

We have further found that CREB is importantly involved in the proinflammatory cytokine and MMP production by the RA synovial cells[99]. Indeed, we found that nuclear translocation of cytoplasmic CREB is induced by TNF-α stimulation in RA fibroblast-like synovial cells and that this translocation of CREB is inhibited by SOM treatment of the RA synovial cells. SOM thus inhibits RA synovial cell function by reducing CREB activity.

SOM analogues exert direct anti-proliferative actions in various tumor cells *in vitro*[88]. However, the mechanism of the anti-proliferative action may be distinct from that of RA synovial cells, because the tumor cells express SOMR 5, whereas RA synovial cells express SOMR 1 and 2. The intracellular signaling cascade of SOM in different cell types expressing a distinct SOMR subtype needs to be clarified.

Recent study has shown the involvement of cAMP and a cAMP-responsive element (CRE) in the up-regulation of IL-1β synthesis[57]. This may be the case for the inhibition by SOM of the production of proinflammatory cytokines and MMPs by RA synovial cells; up-regulation of IL-6 and collagenase synthesis may involve CREB and CRE in the promoter/enhancer regions of the relevant genes.

Application of SOM on arthritides

MATUCCI-CERINIC et al.[64] studied the antiinflammatory effect of intra-articular SOM in experimental arthritis in rabbits. SOM treatment induced a reduction of knee joint swelling. This effect was shorter than that produced by triamcinolone acetonide; however, the antiinflammatory activity elicited by successive doses of triamcinolone acetonide declined both in extent and duration, while the effects of SOM remained unchanged for each successive treatment. They suggested that SOM exerts an antiinflammatory effect in experimental arthritis and may represent an alternative to corticosteroids for intra-articular therapy of arthritis[64]. Following that COARI et al.[16] studied the effects of intra-articular injections of SOM on the arthritis of RA patients. The efficacy of SOM was evaluated by determining acute phase parameters (erythrocyte sedimentation rate and C-reactive protein), the thickness of the synovial membrane measured by ultrasound technique, and clinical assessment and telethermography. They clearly showed that the intra-articular injection of SOM was able to reduce the thickness of synovial membrane and to improve other clinical parameters in patients with RA, and thus indicated that SOM may directly reduce synovitis[16]. SOM was also applied to patients with painful shoulder and athletes with joint diseases, and was found effective in both studies[83, 87]. They concluded that SOM may be considered useful for the treatment of articular and tendineous phlogistic diseases. A large-scale controlled study of SOM is needed to clarify its effectiveness in patients with RA.

Prolactin

Prolactin (PRL) is a mammotropic neuropeptide produced by the pituitary and extrapituitary cells exist-

ing as several isoforms[77]. The secretion of pituitary PRL is under hypothalamic control, and the cytokines IL-1, IL-2 and IL-6 stimulate pituitary PRL production, while interferon-γ (INF-γ) and endothelin-3 (ET-3) inhibit the production[14].

PRL exerts its effects via PRL receptors (PRLR) which exist as three isoforms. PRL regulates the reproduction, osmoregulation and behavior and has potent immunomodulatory effects[14]. PRL is structurally related to members of the cytokine/hematopoietic growth factor family such as erythropoietin, granulocyte/macrophage-colony-stimulating factor (GM-CSF), growth hormone and IL-2 to IL-7. The PRLR is a member of the cytokine/hematopoietic growth factor receptor family. Interaction of PRL with PRLR activates the Jak kinases which phosphorylates latent signal transduction and activation of transcription (STAT) proteins, resulting in the activation of transcription[14].

It should be emphasized that PRL has immunoregulatory potential[14, 84]. Activated lymphocytes produce PRL[84], and not only lymphocytes but also monocytes in the circulation express PRLR[82]. Moreover, PRL stimulates B cells to produce immunoglobulin and induces T cell proliferation[36, 84]. It is quite interesting that PRL counteracts the effects of corticosteroids by enhancing T helper (Th) 1 cell responses, and excessive Th1 cell response has been assigned an important pathogenic role in patients with RA[14].

Several investigators have demonstrated clear relationships between circulating PRL concentrations and incidence of autoimmune diseases, including systemic lupus erythematosus (SLE)[42, 69], autoimmune thyroid diseases[27] and autoimmune Addison's disease[55].

There are numerous reports describing elevated circulating PRL levels and its role in the pathogenesis of patients with RA[30, 71]; the plasma level of PRL has been shown to be significantly elevated in patients with RA compared with patients with osteoarthritis and osteomyelitis[44, 62]; PRL aggravated collagen-induced arthritis in mice when given during the immunization phase[63].

We have recently found accumulation of PRL-producing lymphocytes and PRLR-bearing synovial cells in RA synovial tissue[68]. We also found that *in vitro* treatment with PRL induces enhanced proliferation of RA synovial cells. PRL treatment provoked excessive production of proinflammatory cytokines and tissue destructive proteolytic enzymes, MMP, by RA synovial cells. In addition, PRL inhibited the tissue inhibitor of metalloproteinase1 (TIMP-1) production by the synovial cells. Thus, total collagenase activity in the joints may be up-regulated in the case of excessive PRL secretion in the joints. The finding that PRL-producing lymphocytes accumulate in the RA synovial tissue and fibroblast-like synovial cells express PRLR supports the importance of T cell-synovial cell interactions for RA pathogenesis.

FIGUEROA et al.[28] studied the immunological and clinical effects of PRL suppression in RA patients with active disease. They treated the patients for 3 months with bromocriptine (BRC), an inhibitor of PRL secretion. They concluded that BRC treatment induces a significant depression of the *in vitro* immune function in RA patients and that these changes are related to parameters of disease activity[29]. Similarly, BRC has been beneficial in treating animal models of RA[104]. However, there are controversial reports regarding this issue[21, 73]. We have studied the effects of BRC in *in vitro* culture of RA synovial cells. We found that BRC potently reduces proliferation of, and proinflammatory cytokine and MMP production by, RA synovial cells.

It is well known that the only consistent genetic association with RA is for genes encoded in the human leukocyte antigen (HLA) complex, particularly HLA-DR4[103]. The PRL gene is in close proximity to the HLA region on the short arm of chromosome six. Thus, BRENNAN et al.[8, 9] found that there may be a linkage disequilibrium between HLA-DRB1 disease susceptibility alleles and microsatellite markers close to the PRL gene in women with RA. This suggests the possibility of extended haplotypes encoding for HLA-DRB1 susceptibility and for high PRL production, which contribute to susceptibility to RA. It is thus intriguing to compare PRL production by synovium-infiltrating lymphocytes and HLA haplotypes.

Calcitonin gene-related peptide vasoactive intestinal peptide

Articular joints are innervated by nociceptive nerve fibers, which are capable of secreting vasodilatory neuropeptides, substance P and calcitonin gene-related peptide (CGRP) [41]. The phenomenon, called neurogenic inflammation, encompasses the inflammatory response of connective tissue and immune cells to neuropeptides, neurotransmitters and vasoactive amines[50]. Several studies suggest a close association between the development of arthritis and the nervous system in the joints[106]. Originally, substance P was reported to have proinflammatory effects on collagen-induced arthritis in rats. This is also true for patients with RA; substance P is importantly involved in the exacerbation of inflammation of the RA joints[58]. ANICHINI et al.[3] assayed serum substance P in RA patients to evaluate whether neurogenic inflammation with substance P release is

significant in RA. They found that the mean serum substance P level is significantly higher in RA patients than in controls, suggesting that neurogenic inflammation with substance P release may contribute significantly to the pathogenesis of RA.

CGRP is a 37-amino-acid neuropeptide derived from the calcitonin/CGRP gene by alternative RNA splicing[2] which has a potent vasodilatory property[6]. It has also been shown that CGRP has an immune-modulating activity[31, 101]. In healthy humans, CGRP is present in the serum at low concentrations but is found to be elevated in several disease conditions, such as thyroid carcinoma, cluster headache and leukemia[26, 40, 91]. In addition, it has been reported that CGRP and vasoactive intestinal peptide (VIP) concentrations are elevated in the synovial fluids of RA patients[4, 59], though their pathological significance in RA remained obscure.

CALZA et al.[12] conducted an immunocytochemical and *in situ* hybridization study of neuropeptides on rats with adjuvant arthritis. They quantitatively investigated peptide- and peptide mRNA-expression in the sensory circuit at the spinal level; 1) a decrease in substance P- and CGRP-immunoreactivities in dorsal root ganglia was observed 5 days after adjuvant injection, with recovery (CGRP) or even an increase (substance P) after 21 days; 2) CGRP and substance P peptide levels were increased in dorsal root ganglia after 21 days; 3) opioid peptide (enkephalin and dynorphin) and substance P mRNAs were strongly up-regulated in dorsal horn neurons after 21 days; and 4) a dramatic decrease in CGRP mRNA levels was found in motor neurons in the ventral horn after 21 days. A straightforward interpretation of the complicated results seems difficult. Nonetheless, these data indicate that peptide expression in dorsal root ganglia and the spinal cord is markedly influenced by severe inflammation with distinct and individual temporal patterns, which are also related to the severe rearrangement of joint structure during polyarthritis.

We therefore decided to study the effects of the neuropeptides on the RA synovial cell functions *in vitro*, because this is the most straightforward way to assign their pathological significance for RA. It has been shown that CGRP and substance P are costored and released together[11, 105]. Synovial tissue proper and juxta-articular bone have been shown to be innervated by substance P- and CGRP-containing nerves[53]. In addition to substance P, CGRP has a vasodilatation effect[6], suggesting involvement of CGRP for the increase in the vascular permeability of RA lesions. Thus, it has been postulated that CGRP and substance P are associated with the pathophysiologic responses of RA. Furthermore, neutralization utilizing systemic administration of antibodies to substance P and/or CGRP has shown the alleviation of both neurogenic inflammation and arthritis[53]. Thus, it is hypothesized that CGRP is involved in the development and/or exacerbation of inflammation, in synergy with substance P in patients with RA[53]. Chemical denervation by the neurotoxin capsaicin[56] and surgical denervation[1] significantly reduce the occurrence of sensory neuropeptides, substance P and CGRP. Nevertheless, neither denervation could prevent the development of arthritis in rats. So far, it remains uncertain to what extent CGRP contributes to joint inflammation in humans as well as in rats.

Our *in vitro* study has clearly shown that CGRP and VIP inhibit proliferation of, and secretion of proinflammatory cytokines and MMP production by, RA synovial cells. Thus, the unmyelinated sensory afferent fibers may attenuate the pathological responses associated with RA through the release of CGRP. In this regard, it has been suggested that some of the sensory afferent fibers contain both substance P and CGRP, whereas the others contain CGRP alone[53]. Our study suggests that CGRP inhibits pathological responses of RA and has rather beneficial effects on RA pathophysiology. It has been shown that CGRP inhibits bone resorption, further supporting the beneficial effects of CGRP on RA pathophysiology[22].

There are two subtypes of CGRP receptor (CGRPR) and VIP receptor (VIPR) reported so far. We found that subtype 1 CGRPR and subtype 2 VIPR are constitutively expressed on, and are functional in, RA fibroblast-like synovial cells. The affinity of the respective receptor to the relevant ligand is quite high by radioreceptor binding assays, suggesting the CGRP and VIP binding to the synovial cells is authentic.

This result also suggests the importance of the parasympathetic system in controlling joint inflammation through release of VIP[38]. In this sense, it has been suggested that cathecholamines, mediators of the sympathetic system, exacerbate the pathologic responses of RA. Thus, the sympathetic system exacerbates, whereas the parasympathetic system attenuates the pathologic responses of RA. All the data support the importance of the two neuropeptides, CGRP and VIP, for the regulation of inflammation in RA joints.

CGRP and VIP inhibit adenylate cyclase activity in many tissues[77]. Thus, it is likely that CGRP and VIP inhibit adenylate cyclase activity and then cAMP accumulation, followed by deficient PKA activity, as does SOM[77]. This process may lead to the deficient transcription factor CREB activity in the nucleus[93]. Indeed,

we found that nuclear translocation of cytoplasmic CREB and phosphorylation of ser-133 of the CREB are induced by TNF-α stimulation in RA fibroblast-like synovial cells; the translocation and phosphorylation of CREB are efficiently inhibited by CGRP and VIP treatment[92].

TANABE et al.[94] have suggested that CGRPR stimulation reduces substance P-induced O2- production probably in a cAMP-associated fashion in neutrophils. Similar inhibitory responses may occur in the RA synovial cells. The CGRPR and VIPR have been found in activated lymphocytes and monocytes, thus it is likely that CGRP and VIP released from nerve endings also modulate functions of synovium-infiltrating lymphocytes[72, 96]. Thus it is possible that CGRP and VIP exert their inhibitory effects on synovium-infiltrating lymphocytes as well[72, 96].

Opioid

Opioid peptides contribute to the control of pain transmission by interacting with opioid receptors within the central nervous system[60]. Opioid peptides are encoded by three genes: the proopiomelanocortin (POMC) gene, the preproenkephalin gene and the prodynorphin gene[20]. They all give rise to precursor proteins, which are processed in a tissue-specific manner into small biologically active peptides[20]. Methionine--enkephalin (met-ENK) and leucine-enkephalin (leu-ENK), which have immunoregulatory effects on immune and inflammatory cells, are derived from pro-enkephalin A[39, 67]. β-endorphin (END) is a neuroendocrine peptide with morphine-like effects produced by the anterior pituitary, cells of the immune system, and synovial cells. END is synthesized and processed within various types of immune cells under pathological conditions[90]. Lymphocytes and other immune cells residing in inflamed tissue contain opioid peptides, which eventually lead to the inhibition of pain[89].

Several studies have been suggested the involve opioid peptides in the pathophysiologic responses of RA[24, 48]. We found that END and ENK inhibited proliferation of RA synovial cells. END and ENK also inhibited proinflammatory cytokine production by the synovial cells. TNF-α and IL-1β production were profoundly reduced, whereas IL-6 and IL-8 production as marginally affected. These results suggest that END and ENK act selectively on macrophage-like synovial cells. Indeed, macrophage-like synovial cells expressed the μ type receptor of opioid peptides, whereas fibroblast-like synovial cells did not express the opioid peptide receptor. This finding is in striking contrast to CGRP, VIP and SOM, which inhibit IL-6 and IL-8, products of fibroblast-like synovial cells.

RA fibroblast-like synovial cells and macrophage--like synovial cells produce ENK and END. Thus, END and ENK act as autocrine and paracrine mediators of RA synovial cell functions. ELBEIALY et al. [24] found that plasma β-END levels in patients with severe RA were significantly lower than those in patients with mild RA. The mean serum level of β-END was also significantly lower in the RA group than that of normal controls. The depressed plasma β-END in patients with RA may be associated with the hyperactivity of RA synovial cells and excessive production of proinflammatory cytokines in the joints.

Tabel 1. Neuropeptides involved in the regulation of local inflammation of RA

	Opioid	SOM	CGRP/VIP	Substance P	Prolactin
Production or secretion by	Macrophage-like synovial cells Fibroblast-like synovial cells	Fibroblast-like synovial cells	Nerve ending	Nerve ending	Infiltrating lymphocytes Macrophage-like synovial cells
Targets	Macrophage-like synovial cells	Fibroblast-like synovial cells	Fibroblast-like synovial cells	Fibroblast-like synovial cells	Macrophage-like synovial cells Fibroblast-like synovial cells
Effects	Proliferation ↓ TNF-α ↓ IL-1β ↓ MMP-9 ↓ IL-6 → IL-8 →	Proliferation ↓ IL-6 ↓ IL-8 ↓ MMP-1 ↓ MMP-2 ↓ MMP-9 ↓ TNF-α → IL-1β →	Proliferation ↓ IL-6 ↓ IL-8 ↓ MMP-2 ↓ TNF-α → IL-1β →	Proliferation ↑ IL-6 ↑ IL-8 ↑	Proliferation ↑ TNF-α ↑ IL-6 ↑ IL-8 ↑ MMP-2 ↑ MMP-3 ↑ MMP-8 ↑ MMP-9 ↑ TIPM-1 ↓

Conclusion

There have been several studies which suggest malfunctions of the neuro-endocrine-immune interactions in patients with RA. However, how the defective interactions contribute to the development of RA remains largely obscure. We found that several neuropeptides actually affect synovial cell function and, thus, aberrant expression of the neuropeptides leads to the exacerbation and/or attenuation of pathophysiologic responses in patients with RA. Table 1 summarizes the effects of the neuropeptides we have studied in patients with RA. The next step we have to do is to elucidate the precise mechanisms of the neuropeptides which affect the synovial cell function and the development of clinical application of the neuropeptides for treating patients with RA.

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