

Neuroimmunology of Opioids from Invertebrates to Human

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Abstract

There is today growing evidence that the nervous and the immune systems can exchange information, mainly through small molecules, either cytokines or neuropeptides. Furthermore, it appears that some so-called neurotransmitters like neuropeptides can function as endogenous messengers of the immune system, and that they most likely participate in an important part in the regulation of the various components of the immune response. In this context, it is widely accepted that all organisms have processes that maintain their state of health. Failure of these processes, such as those involving naturally occurring antibacterial peptides, may lead to pathological events. The presence of antibacterial peptides on both proenkephalin invertebrate (Leeches) and vertebrate (Human) neuropeptide precursors such like enkephalin, peptide B, further supports the hypothesis that some of neuropeptide precursors are implicated in immune response. Indeed, their peptides, with their high antibacterial activities further associate opioid peptides with immune related activities. We surmise that immune signalling molecule may lead to enhanced proenkephalin proteolytic processing by prohormone convertase freeing both opioid peptides and antibacterial peptides during innate immune response. However, because it is necessary to modulate inflammation, invertebrates like leeches are also able to synthesize panoply of messengers that modulate inflammation *e.g.* endocannabinoids, opiates and pro-opiomelanocortin derived peptides such like adrenocorticotrophin and melanostimulating hormone. This demonstrates that the equilibrium between the stimulation and the inhibition of the immune response has evolved sooner than it can be thought.

I. Introduction

It has long been thought that all physiological functions, including immune reactions were exclusively under brain control. Pioneer studies of Metchnikoff and Pasteur have unraveled the key role of the immune system that is acting as a “mobile brain”, in the organism’s defense mechanisms. It is now clear that the central nervous and the immune systems are indeed cross-talking and are both involved in immune response. Since 1980, numerous studies have demonstrated that mental variations, which are subsequent to stress or hypnosis, are modulating immune response and are implicated in the organism’s defense mechanisms. In contrast, it has also been shown that immune system modulates both the peripheral and the central nervous system and is involved in fever production which is resulting from an infection [1]. These interactions are mediated via different molecules including peptides such as CRH and ACTH, monoamines (epinephrine, norepinephrine and dopamine), glucocorticoids, free radicals, cytokines such as IL1, IL6 and TNF α , and opioid peptides and opiates [2]. In this way, Smith and Blalock have demonstrated the existence of communications between endocrine and immune systems which are mediated via intercellular messengers [3]. Moreover, they demonstrated, for the first time, that peptides such as ACTH are indeed produced by immune cells. Recipro-

cally, it has also been shown that some cytokines are synthesized in the central nervous system following peripheral or central infections, ischemy or neurodegenerative diseases [4].

These data allowed the emergence of a new research field which is called neuroimmunology or even psychoneuroimmunology and which includes endocrinology, immunology and neurobiology [5]. However, because of the complexity of nervous and immune systems in mammals, the studies of these interactions are a difficult task. Invertebrate models have been particularly useful to understand the fundamental physiological mechanisms involved in development, apoptosis, aging and immunity. Between the numerous hormonal messengers produced in immune cells [2], this review is dealing with opioid peptides, and it will compare the role of these peptides in the modulation of the immune system both in vertebrate and in invertebrate species [6–10].

II. Implication of opioid peptides in immunity

a- Structure and biosynthesis of opioids

Opioids are schematically divided into three different classes of active peptides (enkephalins, dynorphins and endorphins) which are both present in mammals [9] and invertebrates (Fig. 1). These opioids, like almost all neuropeptides, are synthesized

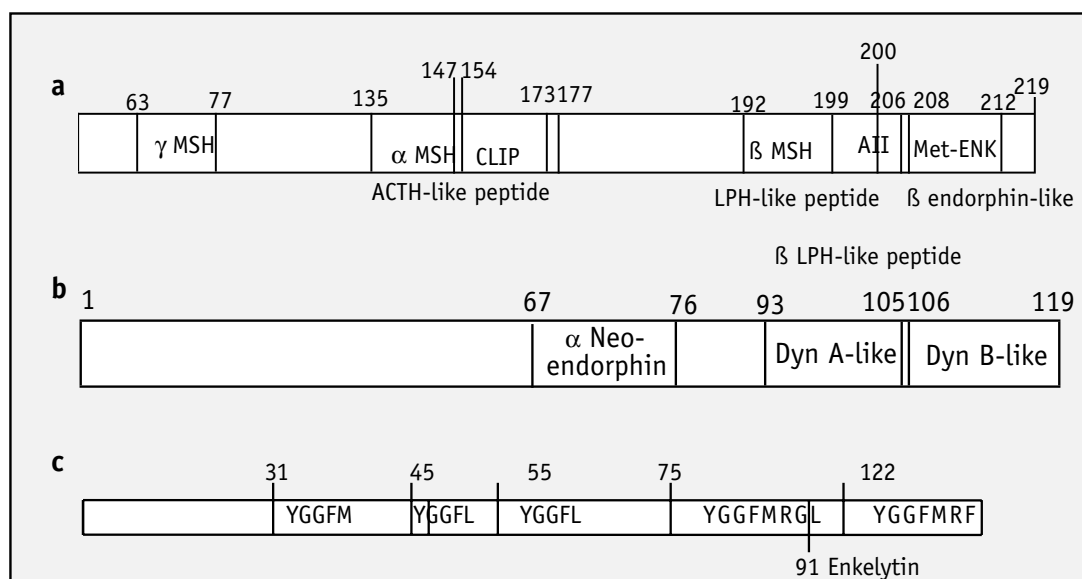


Fig. 1. Opioid precursors structure of the leech *Theromyzon tessulatum*

(a) POMC maturation leads ACTH, endorphins and the dynorphin one (b) dynorphin-like peptides. The (c) Proenkephalin generates after its processing opioid peptides like leucine-enkephalin (Leu Enk), methionine-enkephalin (Met Enk) and other active molecules like methionine enkephalin Arginine Glycine Leucine (Enk-MRGL) and the methionine enkephalin Arginine Phenylalanine (Enk-MRF).

(Methionine enkephalin (Met-Enk); Leucine enkephalin (Leu-Enk); Methionine enkephalin Arginine Glycine Leucine (Enk-MRGL); Methionine enkephalin Arginine Phenylalanine (Enk-MRF); Melanocyte Stimulating Hormone (MSH); Adrenocorticotrop Hormone (ACTH); Corticotropin Like Intermediate Lobe Peptide (CLIP); Lipotropin Hormone (LPH) et β -endorphine (β -end.). The size of the precursor are expressed in amino acid residues.

via the proteolytic processing of larger inactive precursor molecules *i.e.* proenkephalin (proEnk), prodynorphin (proDyn) and proopiomelanocortin (POMC) (Fig. 1) [7].

A protein related to proenkephalin has been recently characterized in the mollusk (*Mytilus edulis*) and in the annelid (*Theromyzon tessulatum*) [7]. Leech proenkephalin exhibits 26.2% amino acid sequence similarity with amphibian proenkephalin and *Mytilus* proenkephalin exhibits a higher one with human and guinea pig proenkephalin *i.e.* 39% and 50%, respectively. Although conservation between vertebrate and invertebrate proenkephalins is weak, peptides containing biological activities have been highly conserved during evolution. In their C-terminal domain, invertebrate proenkephalins contain molecules that exhibit 90% amino acid sequence similarity with bovine peptide B and that also possess antibacterial activities [11]. *Mytilus* proenkephalin contains Met- and Leu-enkephalin in a ratio of 3/1 and 1/2 in the leech as well as Met-enkephalin-Arg-Gly-Leu and Met-enkephalin-Arg-Phe surrounded by potential cleavage sites. In contrast, synenkephalin, an antibacterial peptide [12] present in the N-terminal part of the precursor, only shows 15% and 45% homology with the *Mytilus* and the leech counterparts, respectively. Interestingly, *mytilus* proenkephalin possesses in its sequence, a peptide having 40% sequence identity with another bovine proenkephalin peptide (80–105), the amidorphin [13].

Following the first molecular data obtained on the marine worm *Nereis diversicolor* demonstrating the existence of a prodynorphin precursor related to the vertebrates ones [14], a mammalian prodynorphin-derived peptide, α -neoendorphin, has been purified from the central nervous system of *T. tessulatum* [15]. Subsequently leech prodynorphin has been characterized and it exhibits 28.8 % sequence homology with rat, and 22% with the human and pig [16]. In leech prodynorphin, α -neoendorphin is found at position 67–76 and it exhibits 100% sequence identity with the mammalian peptide. Dynorphin A-like material (position 93–105) and dynorphin B-like peptide (position 106–117) respectively exhibit 50% and 76.6% homology with their mammalian counterparts [17]. *Mytilus* prodynorphin is distinguished the leech one in that the N-terminus is longer. Additionally, by sequence comparison, the presence of an orphanin FQ-like peptide, exhibiting 50% sequence similarity with that found in mammals, has been detected. Although this molecule is not flanked by dibasic amino acid residues and not yet found in the hemolymph of the animal, the authors speculate that this peptide could act as a protective molecule during injury of pathogen infections to alarm the nervous system by producing pain. This peptide could act in conjunction with the Met-

enkephalin and the Met-enkephalin-Arg-Phe known to trigger, via the vagal nerve, a central action to release the substance P [18].

The first demonstration of the presence of β -endorphin and a POMC-related gene transcribed in an invertebrate has been achieved in *Schistosoma mansoni* [19]. It has been demonstrated, using human POMC probe, that mollusk (*M. galloprovincialis*, *Viviparus alter* and *Planorbarius corneus*) hemocytes express POMC mRNA [20–22]. Same results were obtained in the protozoan tetrahymena by Renaud *et al.* (1995) [23]. In 1994, Salzet *et al* isolated for the first time a peptide related to the melanostimulating hormone from leech brain [24]. Recently, a mammalian-like POMC has been characterized from leech immunocytes [25]. At the same time Stefano *et al* [26] demonstrated the existence of such a precursor in a non-parasitic animal, the mollusk *M. edulis*. The leech POMC contains six of its derived peptides, including adrenocorticotropin (ACTH) and melanocyte stimulating hormone (MSH). Of the six peptides, three showed high sequence similarity to their mammalian counterparts, namely, Met-enkephalin, α -MSH and ACTH (100, 84.6 and 70% respectively) whereas γ -MSH, β -endorphin and γ -LPH exhibited only 45, 20 and 10% sequence identity. In accordance to that has been observed in mammals, leech α -MSH is flanked at its C-terminus by the Gly-Arg-Lys amidation signal. The coding region of leech POMC was also reported by RT-PCR using degenerated oligonucleotide primers [25]. Furthermore, immunocytochemistry using a panel of antisera mapping the POMC confirms the both presence in brain and immunocytes of POMC [27]. *Mytilus edulis* hemocytes also contain a mammalian-like POMC [26]. Of the six peptides found in this opioid precursor, Met-enkephalin, γ -MSH, α -MSH and ACTH exhibited 100, 90, 80 and 74% sequence identity, respectively with *Xenopus* molecule. The β -endorphin-like and γ -LPH-like molecules exhibit only 25 and 10% sequence identity. Dibasic amino acid residues are found at the C-terminus of MSH and ACTH, indicating cleavage sites.

b- Opioid precursor processing and peptide metabolism

Neuropeptide precursors processing usually occur at single arginine (Arg) residue or at dibasic sites. Different enzymatic activities capable of cleaving precursor molecules after basic residues have been identified. Among these enzymes, prohormone convertases (PCs), which have been characterized both in mammals and invertebrates appear to be the best candidates to mediate this proteolytic activation step. PCs are calcium dependant serine proteinases belonging to the bacterial subtilisin superfamily. To date, seven members of the PC family have been cloned in mam-

mals: PC1, also called PC3; PC2; furin, also known as PACE; PACE4; PC4; PC5, also called PC6; and PC7, also known as LPC or C8 [28,29]. Recent results have demonstrated that all these convertases, with however the exception to PC2 and PC4, which is exclusively present in ovary and testis, are synthesized by immune cells [30,31]. Between these enzymes, only PC1 and PC2 have been shown to be the major enzymes cleaving peptide hormones and neuropeptides. Based on tissue distribution and co-expression studies, it has been shown that PC1 usually produces high molecular weight intermediate forms whereas PC2 is implicated in the generation of smaller peptides. For example, numerous studies, using co-expression, antisense and homologous recombination strategies, have shown that PC1 cleaves POMC molecule into ACTH and that the coordinate action of PC1 and PC2 produces α MSH (ACTH₁₋₁₃) [32,33]. The role of PC1 and PC2 in rodent Prodynorphin processing has also been investigated. PC2 alone, without a previous requirement of PC1, is able to generate small opioid peptides such as Dyn A 1-17, Dyn B 1-13, alpha-neo-endorphin but also Dyn A 1-8 and small amount of Leu-enkephalin [34]. Finally, it has been recently demonstrated that the generation of small opioid peptides from intermediates is mediated almost entirely by PC2, since the amounts of three mature enkephalins were depleted by more than three quarters in the brain of PC2 knock-out mice [35]. In mammals immunocytes, the presence and the co-localization of PC3 and proenkeph-

alin in macrophages allow to sustain that such a mechanism also occurs in these cells [31]. We recently confirm their modulation during bacteria challenge [10]. Moreover, opioid precursors have been found in the animals hemolymph, indicating that part of their processing may also occur outside the immunocytes (Fig. 2) [9, 10] like in vertebrates [2]. Thus, having the active opioid peptides imbedded in their respective precursors may serve as a process to protect them from proteolytic attack [36]. However, in the vicinity of a high concentration of the respective enzymes, i.e., immunocytes, they are processed to their active derived peptides. Among the enzymes present at the level of the immunocytes membranes and in fluid in vertebrates and invertebrates, ACE and NEP [37] suggesting their potential involvement in peptide biosynthesis.

c- Presence, regulation and physiological role of opioids in immune system

In mammals opioid peptides are implicated in neural, neuroimmune and autoimmunoregulatory signaling [2]. These phenomena have been supported by studies documenting the presence of stereospecific opioid receptors on specific leukocytes, i.e., granulocytes, and nerve cells [10, 38]. Additionally, these cells express the actual signaling molecules used for this chemical signals including the expression of mRNA [39]. Opioid peptides induce immunocyte chemotaxis as well as they initiate the release of cytokines [38, 40] and Met-enkephalin can be now considered as a

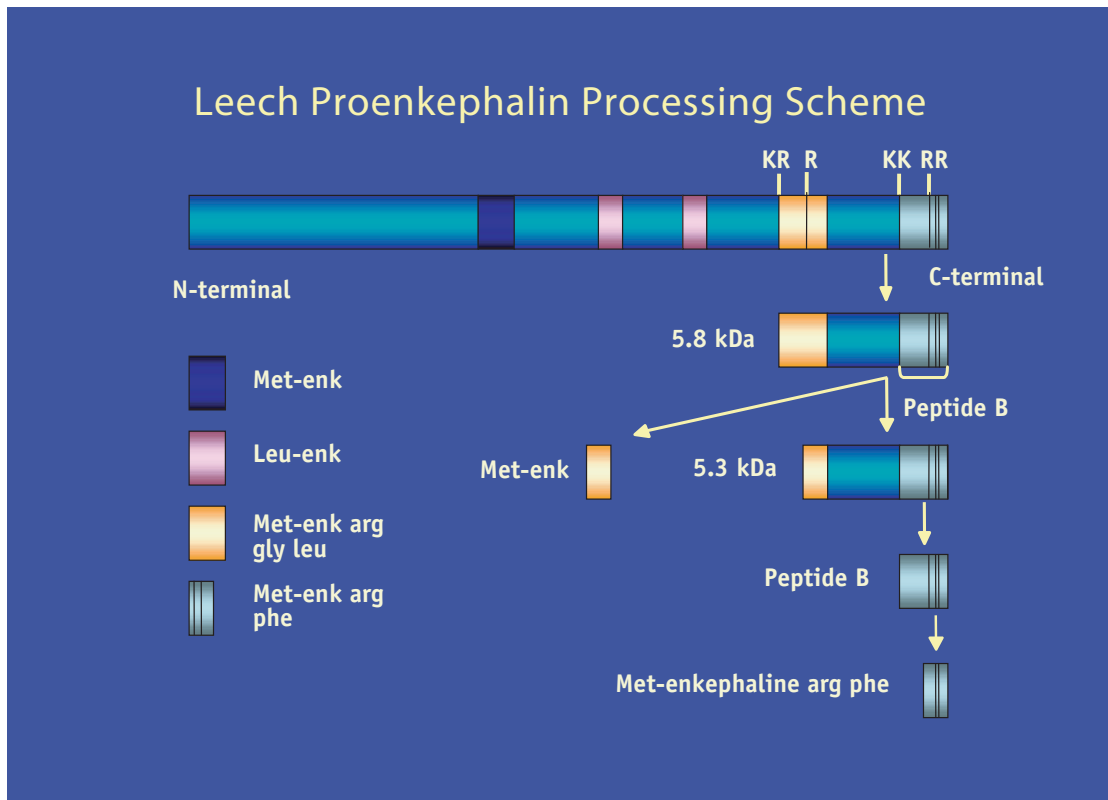


Fig.2. Leech proenkephalin processing

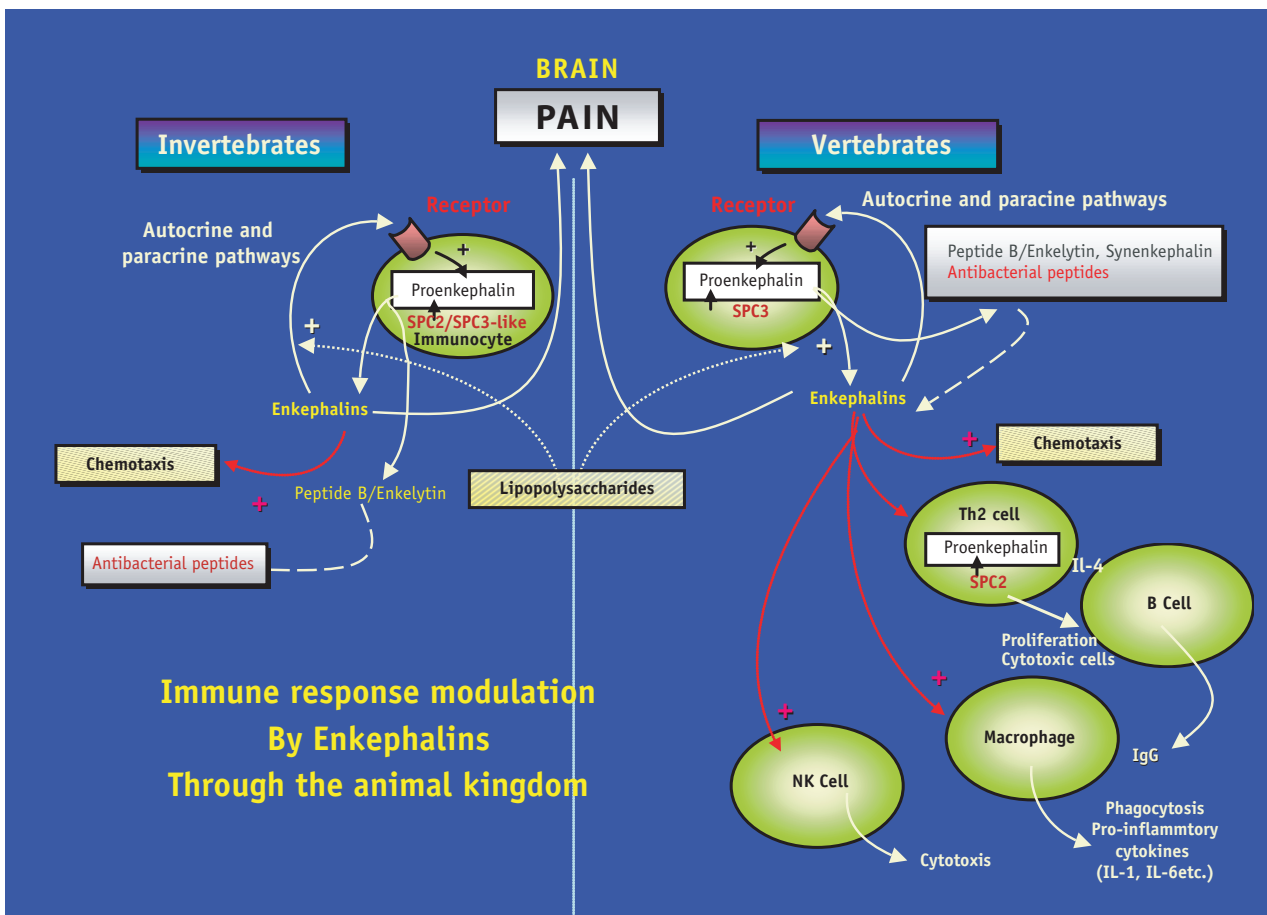


Fig. 3. Comparison of the neuroimmune processes in vertebrates and invertebrates of proenkephalin derived peptides after lipopolysaccharide injection.

Immune level: An infection done experimentally provokes the enkephalins synthesis by SPCs attack on neuropeptide precursor to lead neuropeptide derived peptides. Enkephalins induce immunocyte chemotaxis and the release of other signaling molecules (i.e. cytokines), whereas peptideB/enkephalins exert an antibacterial action. Within minutes enkephalins are processed to yield [Met]enkephalin-Arg-Phe that further augments the immunocyte response. Enke-

phalins also stimulate the Th2 lymphocyte responses via CD3, coupled to Ca²⁺ intracellular release that conducted to IL4 release. Thus, enkephalins act as immune messengers, so called cytokines. It also stimulates cathelicidin and defensin precursor processing in order to rise antimicrobial peptides in a systemic response. At the present time processing enzymes are unknown. *Peripheral and central nervous system (CNS) actions of enkephalins:* In peripheral tissues, immunocytes expressing the proenkephalin molecule process and release its derived peptides upon appropriate stimulation (see above). Enkephalins seem also implicated

in the release of signaling molecules that promote nociception (e.g. histamine, tachykinins). Using nerve endings and pain fibers they carry the message to the spinal cord where substance P is released and further gives the 'sensation' into appropriate brain areas. We speculate that a negative feedback control could be done by central enkephalergic neurons. In fact when they are stimulated, substance P release is inhibited, halting the transmission of this signal. (with permission from Trends in Neurosciences [10]).

cytokine [41]. In invertebrates, these same peptides induce chemotaxis and the release of mammalian-like cytokines (Stefano *et al.*, 1996), including interleukin-1, -6 and tumor necrosis factor- α . Furthermore, invertebrate immunocytes contain both δ_1 and δ_2 opioid receptors, which also occur on human granulocytes [6,38], supporting their presence and significance in immune signaling in invertebrates.

The presence of antibacterial peptides on both proenkephalin invertebrate and vertebrate precursors such like enkephalins and peptide B [18] further supports the hypothesis that these molecules first evolved in simpler animals. Indeed, these antibacterial peptides, with their high antibacterial activi-

ties further associate opioid peptides with immune related activities. We surmise, that immune signaling may lead to enhanced proenkephalin proteolytic processing freeing both opioid peptides and antibacterial peptides [18]. In this scenario, the opioid peptides would stimulate immunocyte chemotaxis and phagocytosis as well as the secretion of classical cytokines. During this process, the simultaneously liberated proenkephalin fragments having antibacterial activities would attack bacteria immediately, allowing time for the immune stimulating capabilities of opioid peptides to manifest itself. This hypothesis is further supported by the presence of specific Met-enkephalin receptors.

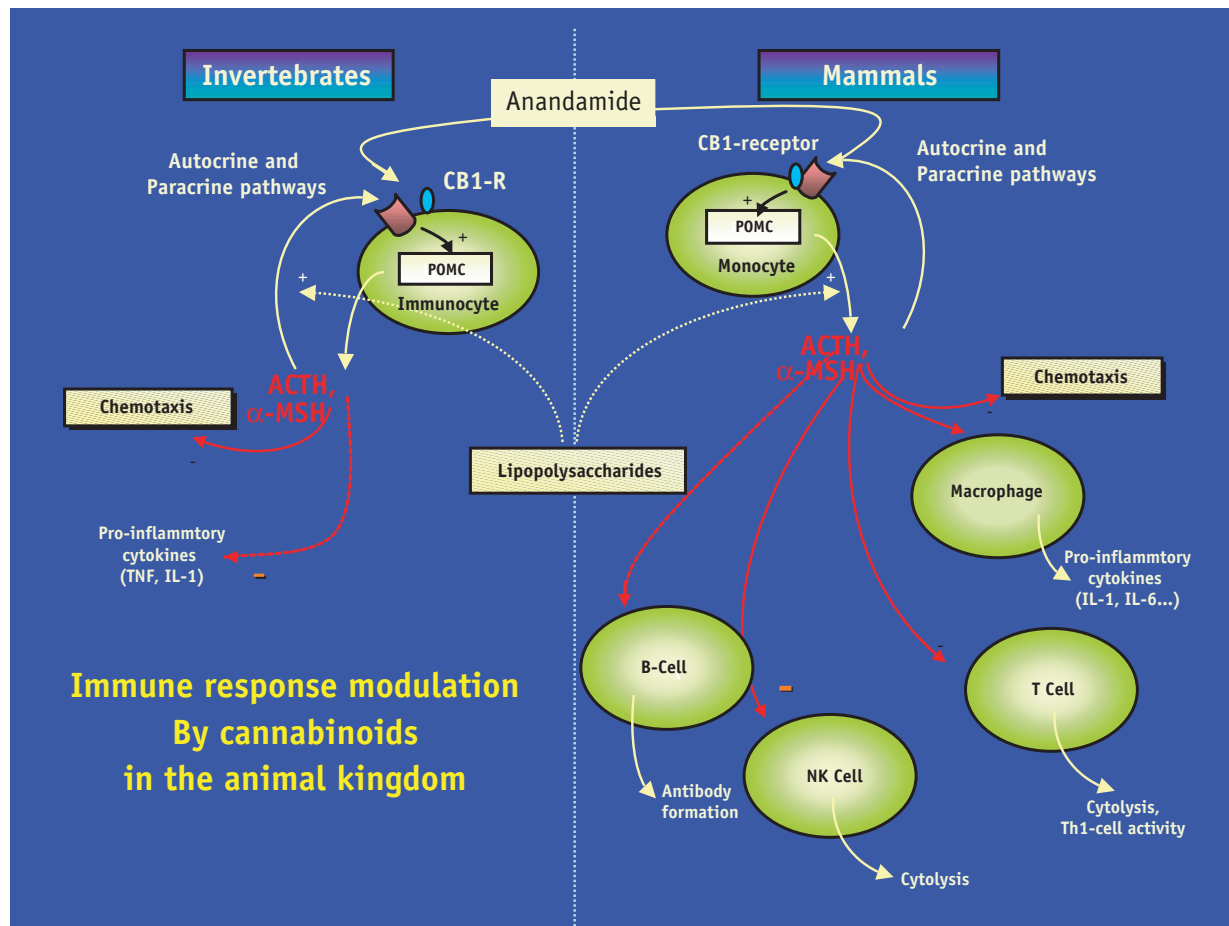


Fig. 4. Comparison between immune response modulation in vertebrates and in mammals: implication of opioid/ POMC-derived peptides.

In mammals, THC and anandamide inhibit T lymphocyte proliferation and Th1 activity. They also stimulate IgE production but inhibit the IgG production. THC and anandamide block cytolysis and phagocytosis of

natural killer cells and macrophages, respectively. Moreover, both monocytes and invertebrate immunocytes contain POMC, pro-hormone convertase genes and cannabinoid receptors. After either cognitive stress or pathogen infections, and through autocrine, paracrine or endocrine pathways, these cascade events lead to ACTH and α MSH release in both animal kingdoms.

These peptides, like THC and anandamide, are known to inhibit T cell proliferation, IgE production, macrophage phagocytosis and NK cell-mediated cytolysis. Hence, these substances inhibit in synergy the immune response. IL, interleukin; TNF-α, tumor necrosis factor-α.

In mammals, the presence of both opioid precursors mRNA and derived peptides in many types of immune cells has been clearly demonstrated *e.g* proenkephalin has been found in T lymphocytes and monocytes [40]. POMC is present in lung macrophages and in spleen [42]. In rodents, using northern blot and *in situ* hybridization techniques, the expression of proenkephalin was detected in fetal thymocytes but also in normal B and T lymphocytes. The expression of proenkephalin mRNA was markedly enhanced after a short incubation with lipopolysaccharide in B cells whereas in the thymocytes the presence of the messenger RNA was exclusively dependent upon mitogenic stimulus (Concanavalin-A). Kowalski (1998) [43] demonstrated that the Met-enkephalin stimulates the B and T cell proliferation and it has also been shown that Leu-enkephalin and its degrading fragments increase the number of T helper and T cytotoxic cells [44]. Met-enkephalin like β-endorphin is able to stimulate *in vitro* the migration of monocytes,

lymphocytes and neutrophils in direction of the site of injection [2]. Synenkephalin and its derived fragments are released by human lymphocytes after phytohemagglutinine injection [45] and its fragments stimulate leucocytes proliferation. Peptide B, enkelytin, of human proenkephalin are released from leucocytes during coronary cardiology by-pass. [46]. Kamphuis *et al.* (1998) [40] have shown an increase of pro-enkephalin mRNA in human circulatory monocytes by Th2 cell cytokines, confirming the presence of both opioids and cytokines at the inflammatory site. Met-enkephalin and its analogs like Leu-enkephalin, Met-Enkephalin-Arg-Phe stimulate the release of pro-inflammatory cytokines such as interleukine-6 (IL-6) [39]. Moreover, proenkephalin mRNA level of peripheral human blood monocytes is increased in presence of IL-6 [40]. So, in regards to the proenkephalin derived peptides activities in immune system, we suggest that these molecules, in general, serve as early proinflammatory signals in vertebrates and invertebrates (Fig.

3). However, high concentration of Met-Enkephalin inhibits the inflammatory response like do the POMC derived peptides such as adrenocorticotropin hormone (ACTH (1–39) and melanostimulating hormone (MSH:ACTH (1–13)) after anandamide or morphine application (Fig.4). In contrast to ACTH (1–39) and MSH which are immunomodulators [47], ACTH (1–24) or ACTH(1–16) are immunostimulators, reflecting the important role of peptidases in the immune response balance (Fig. 4).

Taken together, in invertebrates and vertebrates, opioid peptides are initiating factors of the innate response. Moreover, they stimulate and participate to the specific immune response. The immune system represents equilibrium between the activation of the immune response (after the recognition of the non-self and/or the entrance of pathogens) and its feedback control (i.e. its inhibition by molecules such like MSH or glucocorticoids). The disruption of this equilibrium conducts to diseases that are often due to a lack of feed-back control e.g. autoimmune disease or in contrary to its exacerbation e.g. AIDS, parasitism [48].

III. Conclusion

There is a growing interest on studies that, based on detailed analysis of the immunity mechanisms in invertebrates, are directly used in vertebrates. Two examples reflect such remark. The first one is the panoply of anti-bacterial peptides found in invertebrates and in vertebrates that constitute the major element of the innate immunity. Some peptides that are present in vertebrates and in invertebrates are also found in plants e.g. the defensins. These molecules seem to be well adapted to offer a first defense line against pathogens. The second one is illustrated by the recent work of Medzhitov *et al.* (1997) [49] who have demonstrated, in human, the presence of a Toll receptor, initially discovered in Insects [50]. Thus the discovering of such receptor implicated in the initiation of the innate response reflects the usefulness of simple models. Such results have suggested that the innate response is conserved in the course of evolution and that this type of immunity would allow combating pathogens before the specific immune response takes place. This will allow producing different signals informing the immune cells of the presence of pathogens on the organisms. Such chemical signaling molecules include opioids playing the bidirectional information exchange (Figs. 3, 4).

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