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# Immune-Neuroendocrine Interactions: Evolution, Ecology, and Susceptibility to Illness

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The integration between immune and neuroendocrine systems is crucial for maintaining homeostasis from invertebrates to humans. In the first, the phagocytic cell, *i.e.*, the immunocyte, is the main actor, while in the latter, the principle player is the lymphocyte. Immunocytes are characterized by the presence of pro-opiomelanocortin (POMC) peptides, CRH, and other molecules that display a significant similarity to their mammalian counterparts regarding their functions, as both are mainly involved in fundamental functions such as immune (chemotaxis, phagocytosis, cytotoxicity, etc.) and neuroendocrine (stress) responses. Furthermore, the immune-neuroendocrine system provides vital answers to ecological and immunological demands in terms of economy and efficiency. Finally, susceptibility to disease emerges as the result of a continuous dynamic interaction between the world within and the world outside. New fields such as ecological immunology study the susceptibility to pathogens in an evolutionary perspective while the field of neuro-endocrine-immunology studies the susceptibility from a more immediate perspective.

**MeSH Keywords:** **Immune System • Invertebrates • Neuroendocrinology**

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## Background

Cells communicate with each other through highly specific chemical signals. The organisms that developed this form of communication during evolution seem to have greater opportunities for survival and thus the transmission of the new trait to their offspring. A conceivable reason underlying the appearance of this complex type of communication could have been driven by the presence of many synaptic molecules and hormones that promote intercellular communication.

The immune system and the central nervous system (CNS) “talk to each other” in order to maintain bodily homeostasis [1]. Different studies in mammals have demonstrated a bidirectional communication between these two systems [2,3].

In the course of evolution, the complexity of neuro-endocrine immune communication has increased reaching its highest degree of sophistication in mammals. However, the individual aspects of this dialogue must be traced back to the defense mechanisms of invertebrates. In this view, the innate response of the vertebrate appears to be a composed of a mosaic of various invertebrate immune mechanisms aimed to deal with pathogens [4].

In this review we summarize the three main emergent aspects in an evolutionary context, immunity, stress, and their relation to inflammation. First, a common pool of molecules exists that mediates these phenomena. Second, these molecules seem to have been highly conserved, suggesting that the immune and neuroendocrine systems have a common origin [3,4]. Third, a unique cell type endowed with phagocytic activity, called immunocytes, emerges above all others as the cell type best able to sustain immune responses, stress, and inflammation [5].

## The Neuroendocrine Role of Vertebrate Lymphocytes and Invertebrate Immunocytes

The role of lymphocytes in the regulation of neuroendocrine functions in vertebrates is well-documented. Neuroendocrine hormones such as adrenocorticotrophic hormone (ACTH) and endorphins are expressed by human lymphocytes [6]. The lymphocytes from different species (e.g., anuran amphibian, reptiles, birds, and mice) express ACTH [7-9]. ACTH and endorphins are generated by cleavage of the product of the pro-opiomelanocortin (POMC) gene [10,11]. Different cell types express and post-translationally process POMC protein into distinct sets of peptide products [12]. In mammals, the POMC gene consists of 3 exons, which in the pituitary gland are entirely transcribed to form the POMC mRNA. However, in extrapituitary sites cells express shorter transcripts of the POMC gene [13,14]. The biological activity of POMC-derived peptides is regulated by additional

post-translational mechanisms including glycosylation, phosphorylation, amidation, sulfation, and acetylation [12,15–20]. More importantly, ACTH and endorphins produced in lymphocytes are almost indistinguishable from those produced by the pituitary [21]. Furthermore, lymphocytes display different peptidic hormones such as corticotropin-releasing hormone (CRH), thyrotropin hormone (TSH), growth hormone (GH), vasoactive intestinal peptide (VIP), somatostatin, vasopressin, and oxytocin [3]. Moreover, human peripheral blood lymphocytes, monocytes, and lymphoid cell lines express receptors for ACTH,  $\beta$ -endorphin, VIP, and GH [2,3,22]. Lastly, the major mediators of immune system activity, the cytokines, play a special role in interactions between the immune and neuroendocrine systems [23].

In invertebrates, molluscs, and leeches, the presence of POMC-products, cytokines, biologically active peptides [24–30], glucocorticoids [24,31–33], and biogenic amines [34] has been demonstrated using different technical approaches (e.g., radioimmunoassay (RIA), immunocytochemistry, flow cytometry, and *in situ* hybridization).

## Immune-Neuroendocrine Response in Vertebrates and Invertebrates

All organisms, in order to survive, must activate defenses both at the molecular as well as the genetic level. Furthermore, they must cope with the environment in which they live, in addition to a multitude of diseases. Thus, they must be able to provide answers to all the stressors with which they come in contact in order to preserve bodily homeostasis.

In this context, previous studies have shown that in invertebrates and vertebrates, the same or similar “defense molecules” are present [21,35]. In these higher forms of life, the function of these molecules remains fundamentally alike. Nevertheless, nature has apparently made new uses of these old molecules, while at the same time evolving towards more complicated and centralized functions and organs.

With respect to immune function, cell shape changes, chemotaxis, and phagocytosis play a central role and are therefore of particular importance.

### Cell shape changes and chemotaxis

The incubation of the hemolymph of the bivalve mollusc *Mytilus galloprovincialis* with ACTH (1-24) induced changes in the cell shape (expression of cell motility) of immunocytes via the cAMP pathway, as well as by protein kinase C activity [36]. Such conformational cell modifications were detected using computer-assisted microscopy image analysis and were evaluated as a shape factor that represents the degree of deviation of the cell

from round (inactive state) to amoeboid (active state). This value was determined by measurements of the cellular area and its perimeter and is mathematically expressed using the shape factor formula of the American Innovision Analysis System [37].

Also, opioid neuropeptides are involved in adherence and chemotaxis in invertebrates (the mollusc *M. edulis* and the insect *Leucophaea maderae*) and in human immunocytes [38,39]. Moreover, cytokines such as PDGF-AB, TGF- $\beta$ 1, and IL-8 provoke cell motility. These molecules trigger the cells by using different transduction signaling pathways. PDGF-AB and TGF- $\beta$ 1 transduce extracellular signals along the phosphoinositide signaling pathway. The action of PDGF-AB, extracellularly, is  $\text{Ca}^{2+}$ -independent, while that of TGF $\beta$ 1 is  $\text{Ca}^{2+}$ -dependent [40,41]. IL-8 induces cell motility via protein kinase A and C pathways [42].

The complete ACTH molecule (1-39) and its fragments (1-24), (1-4), (4-0), (1-13), (11-24), CRH [43], and the entire endorphin molecule (1-31) all exert a chemotactic action on the molluscan immunocyte [44], stressing the fact that chemotaxis is the expression of non-random cell locomotion [45]. Similar behavior was observed for cytokines such as IL-1 $\alpha$ , TNF- $\alpha$ , IL-8, PDGF-AB, and TGF- $\beta$ 1 [29].

### Phagocytosis

This phenomenon is the first and vital defense mechanism of all living organisms. It was observed for the first time in the larvae of starfish by Metchnikoff in 1882 [46]. Experiments performed on molluscan immunocytes in the presence of ACTH fragments, CRH and cytokines, revealed a significant increase in the phagocytic activity of bacteria in controls, while no effect was observed in the presence of  $\beta$ -endorphin [47]. More importantly, the phagocytic action of ACTH (1-24) has been maintained as humans transitioned from lower vertebrates such as urodelan amphibians [9].

From these studies on chemotaxis and phagocytosis in invertebrates emerges the general assumption that these 2 phenomena are correlated, but this is not true [48].

### Stress response

The phenomenon of stress was identified and conceptualized by the physiologist Hans Selye around the 1930s. Indeed, in 1936, he published the paper entitled: "A syndrome produced by diverse noxious agents", in the journal Nature. Subsequently, Selye defined his concept of stress more articulately as "a non-specific reaction exhibited by the body when it is faced with a need or adapt to a new situation". This, then, suggests that all living beings must be equipped appropriately in order to survive.

The majority of studies in this area concern vertebrates, particularly mammals, and only since the 1990s have researchers focussed their attention on invertebrates.

These studies demonstrated that the stress response occurs in vertebrates and invertebrates using the same molecules, following the same order and pattern, *such as* CRH > ACTH > biogenic amines [49]. However, they substantially differ in the location where such molecules are produced. In vertebrates, the areas of production are the hypothalamus, pituitary, and adrenal glands, which are organs that do not exist in invertebrates. In the latter, the above-mentioned molecules are secreted by the immunocytes. It is important to appreciate that in this invertebrate scenario the same actors are involved in a different and simpler scenario.

## Eco-Immune-Neuroendocrinology

From an ecological point of view, the immune response should provide the optimal response with minimum cost [50]. The activation of a common pathway involving immune and neuroendocrine components is a classic example used by living organisms to cope with a series of endogenous and exogenous molecules and to combat dangers that may alter their body homeostasis. Indeed, as previously reported, these 2 systems share a whole series of mediators or defensive molecules from lower to higher organisms.

Bow ties, a theoretical framework [51,52], may be considered another example in favor of ecological requirements. The proposed model consists of a large "fan in" (many inputs) and a relatively small "knot", composed of a small number of elements, for processes of control and elaboration, and a large "fan out" of products that may exert a feedback type of control. The "knot" is able to integrate a wide variety of stimuli. In this way, the immune system can be considered as a network of bow ties working at different levels of immune response.

In vertebrates, the thymus and bursa of Fabricius (in chicken) are crucial in acquired immunity and are responsible for B and T lymphocyte maturation. These 2 organs represent another example that emphasizes an evolutionary strategy of re-using pre-existing material [53]. Both organs display involution and remodeling of the cellular microenvironment and the presence of POMC-derived peptides and cytokines [54–56], involved in thymic education and positive selection [57,58].

On the whole, an increased number of POMC-products emerge, together with a decline in the area of lymphoid tissue observed during aging of the 2 organs, suggest a survival role of these molecules in the maintenance of the thymus and bursa of Fabricius.

Finally, epigenetic mechanisms influence the immune-neuroendocrine responses regulating gene expression [59]. LPS injection in the apple snail, *Pomacea canaliculata*, provokes the

induction of phospho-acetylation of histone H3 in the ganglia within 3 h after immune challenge, whereas 6 h later the values were close to those of control snails. Moreover, these findings were correlated with an increase in c-Fos protein levels.

## Susceptibility to Illness

To conclude, a proper dialogue between immune and neuroendocrine functions plays a fundamental part in the balance of living organisms [3,60,61]. Occasional unbalances in 1 of the 2 systems are absorbed by the compensatory actions of the other. To date, it is very difficult to give priority to 1 of the 2 systems in regulating health or illness. Indeed, it is much more useful and probably more appropriate to talk about equilibrium and continuous dialogue.

Numerous data in the literature show that functional alterations in the activity of the immune system can cause profound alteration in both the nervous and endocrine systems [61–65]. Similarly, changes in the neuroendocrine system profoundly modify the activities and functionality of the immune system [66–68].

From this emerges that these 2 systems, historically studied and considered separate, affect each other, and this reciprocal influence has fundamental repercussions regarding health or disease. Consequently, the relative certainty that separate systems act by using independent mechanisms and mediators belonging to different parts of the organism has been replaced by now firmly rooted knowledge that emphasizes that the major mediators of immune system activity, the cytokines, play a special role in the functioning of the neuroendocrine system [68–73]. In humans and rodents, the activation of immune responses by the administration of LPS induces massive immune activation that culminates in a complex molecular, systemic, and behavioral response called sickness behavior [73–77]. This complex framework is induced and maintained mainly by cytokines (e.g., IL-1, IL-6, and IL-18) [73]. Many cytokines, especially the pro-inflammatory ones, are produced by CNS cells and released under the influence of specific stimuli [73]. These molecules are capable of binding their receptors to distinct central cellular populations, including neurons and microglia cells, by activating highly specific signaling pathways [75–78]. The levels of these cytokines and the activity of the associated signal transduction systems modify extremely complex functions such as, mood, psychosis, anxiety, and the control of food intake [79]. In rodents, the ablation of the gene coding for the pro-inflammatory cytokine IL-18 produces an obese phenotype, while the direct application of the recombinant cytokine to the bed nucleus of the stria terminalis (BST) has a potent anorexigenic effect. This led us to propose that the immune mediator IL-18 directly contributes to the loss of appetite observed during sickness [80].

More importantly, such effects are generated by high levels of cytokines produced by cerebral cells or cytokines formed by immune cells that pass the blood-brain barrier [35]. This constitutes one of the most convincing demonstrations of a tight link between these systems in support of the existence of a single, highly intertwined, and interdependent immune-neuroendocrine system. Because this is now firmly accepted and demonstrated, the role of the nervous and endocrine system in controlling immune function was next questioned. The resulting studies are now historical and form the cornerstone of the stress response literature [3]. Currently, the clearest and best-studied example accepted in support of a single intricate system is the activity, functionality, and pathophysiological role of the hypothalamus-pituitary-adrenal axis. This system is considered to be one of the main controllers of mammalian activity [81,82], especially as it relates to stress and the stress response in relation to susceptibility to disease. Its activation or shutdown is under strict control of limbic areas [81]. Changes in the activity of areas of the limbic system, caused by endogenous or exogenous stimuli, are crucial in determining complex functions such as fight or flight reactions and thus the survival of individuals. In addition, the end-product of HPA axis activity, cortisol in humans or corticosterone in other species, profoundly regulates the functionality and efficiency of the immune response. Chronic high levels of circulating steroid hormones cause a state of widespread inflammation and powerful immunosuppression. Under these conditions, the immune system loses its ability to respond properly to pathogens or to exercise appropriate immunosurveillance [69]. In humans, major depression, which is the most prominent psychiatric illness, is associated with the deregulation of HPA axis feedback mechanisms that are major contributors to the clinical picture of the disease. The therapeutic response is often mediated by normalization of HPA axis activity. These data have been confirmed by studies in animal models, where genetic alterations in the genes that control HPA axis function generate a pathophysiological condition very similar to the neuroendocrine dysfunctions associated with major depression observed in patients [83]. In this regard, it is essential that we relinquish the erroneous idea that different systems play functionally and spatially separate roles, and consider these systems as a single highly integrated system in which, depending on contexts and situations, one system controls the other or vice versa. Moreover, this determines that, depending on the place or situation a system find its self in, it can adapt by performing actions normally not attributed to it.

Currently, immunology is used to study disease susceptibility and the neural and neuroendocrine structural and functional architecture that mediate these intrinsic as well as extrinsic factors, among which are the role of parasites and pathogens in sickness behavior and the susceptibility to somatic and well as mental disease [81].

The more we learn about the interaction among the nervous, endocrine, and immune systems, the more we see that the susceptibility to disease and illness is controlled or influenced by biological as well as behavioral characteristics of the organism, which are characteristics that go both ways. On the one hand, we need to understand the relevance of the environmental context, made up by factors [4,68,81,82] such as temperature, altitude, and photoperiod, but also crowding, urbanization, the presence of parasites and pathogens, drought, and famine. On the other hand, we should consider intrinsic or innate factors such as changes in hormonal and neurotransmitter communication, network formation, and the direct or indirect interference with the neurons and brain regions that mediate behavioral expression. These factors, in combination with the genetic predisposition to stress and the physiological

and the behavioral response to stress, eventually determine who is healthy and who is not [81].

## Conclusions

Consequently, we need an interdisciplinary approach that examines interactions among host physiology and disease susceptibility in an ample set of environmentally relevant contexts, studying the appropriate animals or animal systems from the simpler ones to the most complex, while combining different methods and approaches that include fields as evolution, ecology, and life history theory with endocrinology, neuroscience, molecular biology, and, ultimately, behavior.

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