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ScienceDirect
Brain, Behavior, and Immunity 21 (2007) 34–44
BRAIN, BEHAVIOR, and IMMUNITY
www.elsevier.com/locate/ybrbi

Named Series: Twenty Years of Brain, Behavior, and Immunity

Physiology of psychoneuroimmunology: A personal view

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Received 4 August 2006; received in revised form 20 September 2006; accepted 22 September 2006

Abstract

This article offers a personal view on how the concept of the existence of a network of immune–neuro–endocrine interactions has evolved in the last 30 years. The main topic addressed is the relevance of the exchange of signals between the immune, endocrine and nervous systems for immunoregulation and brain functions. Particular emphasis is given to circuits involving immune cell products, the hypothalamus–pituitary–adrenal axis and the sympathetic nervous system. The operation of these circuits can affect immune functions and the course of inflammatory, autoimmune and infectious diseases. We also discuss increasing evidence that brain-born cytokines play an important role in brain physiology and in the integration of the immune–neuro–endocrine network.

Keywords: Immunophysiology; Immune–neuro–endocrine interactions

1. Introduction: general aspects and definitions

Not long ago (at least for the time-scale in science), when we wanted to introduce an article on interactions between the immune, the endocrine and the nervous systems, we referred to an "emerging" field. This type of introduction is no longer justified since this area of research is now firmly established. The appearance of BBI 20 years ago was a great pillar for its establishment. Before, it was difficult to convince reviewers of journals that focused on a given specialty that boundaries between disciplines are relative and some times self-imposed.

We believe that this special series of BBI is important because it provides an historical perspective that may serve to influence future developments and trends. Regarding our contribution to this issue, we want to clarify that, in our view, we have been asked to cover the probably broadest aspect of the field; thus, our contribution is far from being an attempt to review the progresses in the "Physiology of Psychoneuroimmunology". We shall emphasize some aspects of Immunophysiology such as active interactions between the immune system (IS) and the hypothalamus–pituitary–adrenal (HPA) axis or the sympathetic nervous system (SNS), and the role of peripheral and central cytokines as mediators of these interactions. Interactions with other endocrine and autonomic systems are not or only marginally mentioned. Aspects such as stress, sleep and behavior are considered in other articles of this series. We have written part of this article as a sort of a "scientific autobiography" since, as "old timers", we believe that to share our own experience could be of interest for the younger generation that will certainly provide the future breakthroughs that the field needs. We apologize because, due to space limitations, the work of many colleagues is not quoted and not even mentioned. With some exceptions, references from before 1987, and also many between 1987 and 1996, are omitted. These references can be found in earlier reviews (Besedovsky and del Rey, 1996; Besedovsky and Sorkin, 1977).

We also want to clarify an aspect that makes the "essence" of Physiology. There is no doubt that the IS is a physiologic homeostatic system that, within certain limits, contributes to the constancy and integrity of the organism (preservation of self and neutralization of danger). However, a source of confusion could be that while immune responses are physiologic responses expected to be

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0889-1591/$ - see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.bbi.2006.09.008

maximally efficient during infectious/inflammatory diseases, they sometimes contribute to pathology. Thus, although the control and regulation of the different systems of an organism is at the core of Physiology, the unique condition in the case of immunoregulation is that such a physiologic process operates simultaneously and interwoven with pathological events. Another point to remark is that "Physiology" (as indicated by its etymology, derived from the Greek *Physis: nature*), is a discipline that deals with natural processes. Thus, for example, any finding of the effect of a hormone on a given immune parameter using a pharmacological approach should be followed by studies of whether such effect is also observed under natural, physiological or pathophysiological, conditions.

2. Before 1987: the "old times"

Most epistemologists agree that the acquisition of scientific knowledge is preceded and nourished by deductive intuitive views. The work on immune–neuro–endocrine interactions 35 years ago was largely based on intuition since it was based on the belief that the IS, as other physiologic systems, should also be subject to the integrative control of neuro–endocrine mechanisms. In fact, the data available was relatively scarce and based, for example, on the effect of manipulating certain brain areas and endocrine mechanisms on some immune processes that, in many cases, are not adaptive, such as acute hypersensitivity. Receptors for only few hormones, like insulin, or neurotransmitters, like adrenergic agents, were identified or suspected on immune cells. There was also some evidence showing interactions between neuro–endocrine and immune mechanisms during ontogeny, as it is the case of the effect of the thymus on the maturation of sexual functions. Nevertheless, the information available was just enough for the initial formulation of the hypothesis that immune responses are subject to a level of neuro–endocrine regulation. As with other physiologic regulations, neuro–endocrine immunoregulatory mechanisms must be based on the operation of information channels between immune cells and the nervous and endocrine systems. Because both innate and adaptive immune responses involve different cells and mediators at different stages, their extrinsic regulation should be based on well-synchronized neural and endocrine changes. Such changes should, in turn, be capable of modifying the activity of immune cells at defined step of the immune response.

To approach the above mentioned hypothesis experimentally, it was necessary to show that: (1) the neuro–endocrine changes that occur during the immune response are not a consequence of the disease or of the stress of being sick; and (2) the immunologically induced neuro–endocrine responses can affect the functioning of the IS. Following these criteria, it was possible to demonstrate that glucocorticoid blood levels are increased in a threshold dependent manner during the course of specific immune responses to innocuous antigens. We also showed that the increase in corticosterone levels during the response to an antigen can interfere with the response to a second, unrelated antigen. This finding, which provided an explanation to the phenomenon of antigenic competition, indicated that the endocrine change observed was relevant for immunoregulation. Interestingly, while trying to induce antigenic competition *in vitro*, we also observed that low doses of glucocorticoids could increase the number of cells producing specific antibodies. The question that arose was how two distinct bodily systems, the immune and endocrine systems, can exchange information. To approach this question, we stimulated immune cells with mitogens or antigens *in vitro* and found that cell-free supernatants from these cultures contained factors capable to stimulate the HPA axis (Besedovsky et al., 1981), an effect that was mediated by the pituitary gland. We denominated this material "glucocorticoid-increasing factor" (GIF). Fortunately, we invested limited efforts to try to purify this factor, since, as it will be mentioned later, several cytokines share the capacity to stimulate the HPA axis. This finding led to the proposal of the operation of an immune-HPA axis immunoregulatory circuit. Close to the time when these studies were published, Smith and colleagues reported that glucocorticoids interfere with the production of the T cell growth factor (as IL-2 was termed at that time) and of other immune products. It was also shown that changes in endogenous levels of glucocorticoids can inhibit immunoglobulin production. Furthermore, it was found that resting immune cells are more sensitive to the inhibitory effect of glucocorticoids than activated cells. On these bases, we postulated that the immune–HPA axis circuit may have the function of preventing the excessive expansion of cells with low affinity for the antigen and of those cells that are recruited under the polyclonal influence of lymphokines. In this way, the specificity of the immune response would be preserved and even improved. It is conceivable that this circuit, by impeding a cumulative excessive expansion of lymphoid and accessory cells, plays a role in preventing autoimmune and lymphoproliferative diseases. Experimental and clinical examples showing the relevance of the HPA axis–immune circuit are mentioned later in this article. The proposed immune–HPA axis circuit that controls an over production of immune products is in line with the concept formulated by A. Munck (who was the first to characterize the glucocorticoid receptor) that the essential and general function of glucocorticoids is to control the overshoot of locally produced mediators. Around 1986, some cytokines became available in pure and recombinant form, making it possible to test their capacity to induce neuro–endocrine responses. IL-1 was the first cytokine that was shown to activate the HPA axis (Besedovsky et al., 1986). The described interconnection involving peripheral immune mechanisms and endocrine responses under brain control led to the view of "long-loop" immune–neuro–endocrine circuits (Fig. 1, blue lines) and was subject of intense investigation during the following decades.

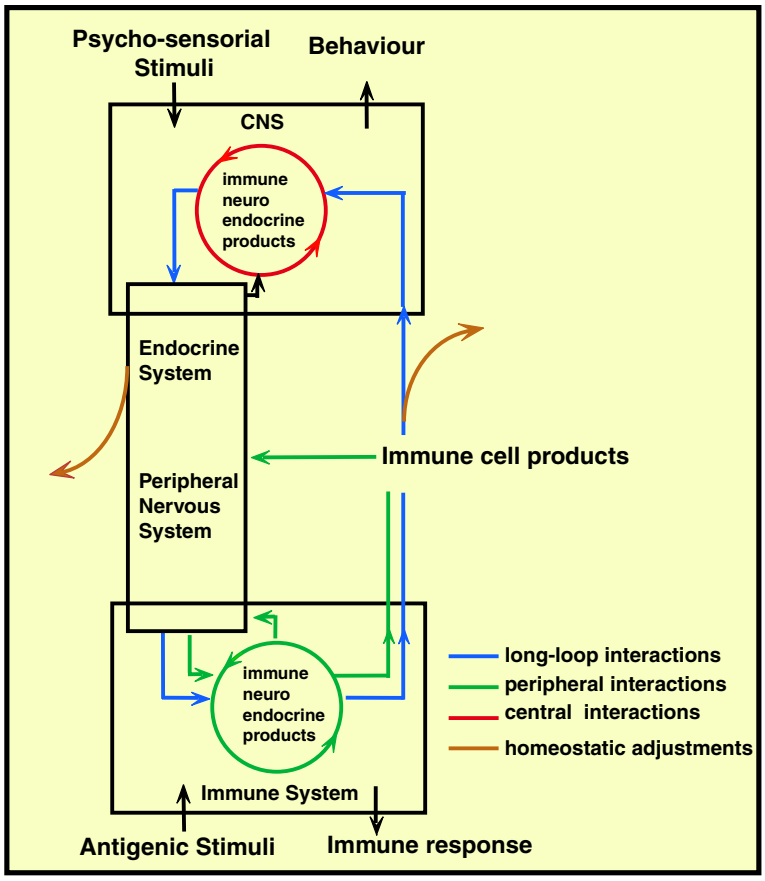

Fig. 1. The immune–neuro–endocrine network. The immune, endocrine, and nervous systems can interact at multiple levels. Schematically, these interactions can be classified as long (blue lines) and short (green lines) loops. There are also interactions within the brain (red circle). The branching arrows in brown represent other homeostatic consequences of these interactions. The operation of these interactions can be modified by stimuli acting primarily on the immune system or on the brain, for example psychosocial stimuli. The outcome of immune–neuro–endocrine interactions can result in immune and/or behavioral modulation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

In many conditions, the SNS operates in a coordinated fashion with the HPA axis. It seemed, therefore, logic to study whether the SNS also participates in immunoregulation. On this basis, we explored whether noradrenaline (NA) concentration in lymphoid organs is affected during the immune response to innocuous antigens. The results showed that a decrease in the splenic concentration, content, and turnover of NA, the main sympathetic neurotransmitter, precedes the peak of the immune response to innocuous antigens. We interpreted such neural response as a way to relieve immune cells from a tonic inhibitory effect of NA. However, this interpretation has to be taken with caution under the light of later findings (see below). Although the activity of the SNS is integrated centrally, the pioneer work of Felten and Bulloch showing that immune cells and noradrenergic nerve fibers are in close contact in lymphoid organs led us to propose the existence of short-loop immunoregulatory neural circuits (Fig. 1, green lines).

During the eighties, we also started studying the possibility that the presence of neoplastic cells, either when they are transplanted, *de novo* induced or developing spontaneously, could induce endocrine responses. Clear endocrine alterations, which occurred before the tumor became palpable and without any overt sign of disease, were noticed during tumor growth. The alterations involved not only steroid but also pituitary hormones such as prolactin and growth hormone, which, as shown by the groups of Berczi and Kelly, are known to support immune responses.

The hypothalamus controls numerous pituitary and autonomic functions. On this basis, we searched for evidence that hypothalamic neurons could change their activity during a peripheral immune response. Our studies coincided in time with the evidence provided by Ader and Cohen that immunosuppression can be behaviorally conditioned (Ader and Cohen, 1975). This finding provided an irrefutable proof that an intact brain can affect immune functions and further rationale to the search for bi-directional channels of information between the IS and the brain.

We evaluated in the same animal both the immune response and the rate of firing of individual neurons in several hypothalamic nuclei and at various intervals after injection of innocuous antigens. A clear increased in the rate of firing of neurons of the ventromedial hypothalamic nucleus was detected in parallel to the immune response to

these antigens. These results provided direct evidence that the brain receives information from the IS under non-stressful conditions in rats under narcosis, using two different non-infectious antigens and a conventional methodology to evaluate neuronal activity. It was also found that NA turnover rate in hypothalamic neurons was inhibited during the immune response to sheep erythrocytes, an effect that is likely mediated by products released by immune cells (Besedovsky et al., 1983).

The evidence showing that the immune response can, by itself, elicit neuro–endocrine responses and that products derived from immune cells can mediate these responses led us to propose that the IS acts as a peripheral receptor organ able to transmit information to the brain about responses to external or internal antigenic stimuli. At this time, Blalock and Smith discovered that immune cells can produce "pituitary hormones" (Blalock et al., 1985). Also since brain cells can produce cytokines originally described as immune products, they stressed that there was a common usage of ligands and receptors of immune and neuro–endocrine mediators, and called the IS "the sixth sense".

This period ended with a very good input to the field: a plenary symposium on immune–neuro–endocrine interactions was included for the first time at an international congress of Immunology (6th International Congress of Immunology, July 1986, Canada). Although this Symposium was held in parallel to another one dealing with the "hot" immunological finding of the MHC restriction, a large audience enthusiastically attended "ours".

3. 1987–1996

An almost explosive increase in the number of scientists committed to research in immune–neuro–endocrine interactions occurred during the decade 1987–1996. BBI was launched not only as consequence of the broad interest in this field but also contributed to its growth.

The understanding of the immune–HPA axis circuit was deepened by studies showing that many cytokines share the capacity to stimulate this axis. IL-1, IL-2, IL-3, IL-6, IL-8, IL-11, IL-12, TNF, INFγ, and GM-CSF are among the cytokines that can integrate such glucocorticoid-mediated feedback. The site of action of the cytokines was a matter of discussion. It is now clear that the acute effect of cytokines is basically exerted at the level of the hypothalamus via the release of CRH and to some extend also AVP. However, during prolonged situations effects at pituitary and adrenal levels were also observed (for review Besedovsky and del Rey, 1996; Turnbull and Rivier, 1999). The understanding of the relevance of the HPA axis–immune feed back circuit during disease begun during the period 1987–1996 with three remarkable findings. It was reported by different groups that: (1) the stimulation of the HPA axis induced by LPS is to large extent mediated by cytokines. Although it was known that LPS administration results in increased glucocorticoid levels, this effect was considered a consequence of the septic shock induced by the endotoxin; (2) the immune–HPA axis loop is altered in chickens that develop autoimmune thyroiditis spontaneously (Schauenstein et al., 1987); and (3) Lewis rats, which are prone to inflammatory diseases such as rheumatoid arthritis, have a deficient corticosterone response to IL-1 (Sternberg et al., 1989). We shall come back to this issue in the next section. It should also be mentioned that it was found that, besides the HPA axis, other endocrine systems are affected during the immune response (for review Besedovsky and del Rey, 1996).

The cellular levels at which the SNS can exert regulatory actions were further characterized during this period (for review Heijnen and Kavelaars, 1999). The main cells target of noradrenergic neurotransmitters appear to be immature and mature thymocytes, thymic epithelial cells, T lymphocytes, macrophages, mast cells, plasma cells, and enterochromaffin cells. Evidence was found that NA and adrenaline, by stimulating the β_2-adrenoreceptor-cAMP-protein kinase A pathway, inhibit the production of type 1/proinflammatory cytokines, e.g. IL-12, TNFα and IFNγ. Stimulation of β-adrenergic receptors inhibits antigen-presenting cells and Th1 cells, but stimulate the production of type 2/anti-inflammatory cytokines such as IL-10 and TGFβ. Thus, endogenous catecholamines may cause a selective suppression of Th1-mediated inflammation and cellular immunity, favoring in this way humoral immunity and also protect the host from the detrimental effects of proinflammatory cytokines and other products of activated macrophages (for review and references Elenkov et al., 2000; Sanders, 2006). Synergistic effects of glucocorticoids and catecholamines were found (Elenkov et al., 2000).

There were some controversies about the effect of surgical sympathetic denervation and chemical depletion of NA stores on immune responses. We are personally biased in this matter because we found that both procedures result in an increased immune response to sheep erythrocytes, an effect that agrees with other reports using different antigens. However, studies using 6-hydroxy-dopamine to deplete catecholamines showed, in some cases, immunosuppressive effects when tested shortly after the administration of the neurotoxin. Our interpretation is that the acute NA release caused by nerve terminal damage and the marked increase in glucocorticoid levels induced by this procedure exert an inhibitory effect on immune cells. It is, however, clear to us that denervation is a very extreme procedure that, although reveling tonic effects of sympathetic neurotransmitters on immune cell activation, differentiation and survival, does not allow definitive conclusions regarding immune responses that are phasic, dynamic, and stage-depending processes. For more information on sympathetic immunoregulation obtained during 1987–1997 see reviews (Elenkov et al., 2000; Sanders, 2006). Although cholinergic innervation was found in the thymus but not in peripheral lymphoid organs such as the spleen, effects of parasympathetic mediators on immune processes were reported. For example, Rinner and Schauenstein

observed an inhibitory effect of the cholinergic agonist carbachol on an *in vitro* induced immune response to SRBC.

Peptidergic innervation was also found to integrate potential immunoregulatory short-loop circuits. Most lymphoid organs also receive sensory peptidergic innervation that is mainly confined to the parenchyma, and the most abundant peptides found are tachykinins (substance P, neurokinin A), calcitonin gene-related peptide (CGRP), and vasoactive intestinal polypeptide/peptide histidine isoleucine (VIP/PHI). In addition, evidence was found that some of these peptides co-exist with catecholamines, and that there is a close spatial relationship between peptidergic nerve fibers and mast cells, T cells and macrophages. Peptidergic nerves also appear to be sparse in pure B cell regions. Neuron-mast cell contacts are relatively often seen in all lymphoid organs, with the exception of the spleen (Weihe et al., 1991).

Although some evidence was provided earlier, it became well established during 1987–1997 that immune cytokines influence complex mechanisms that involve a variety of neuronal circuits such as thermoregulation, food intake, sleeping patterns and behavior. These effects will not be discussed here and references can be found in specific reviews. We shall only mention a few examples. It became clear that there is not a single endogenous pyrogen since several endogenous substances, such as IL-1, IL-6, IL-8, IFNγ, IFNβ and GM-CSF can induce fever. Also several cytokines can inhibit food intake, among them are IL-1, IL-6, IL-8 and TNFα. The capacity to increase slow wave sleep is also shared by different cytokines such as IL-1, IL-2, IFNγ, and TNFα, an effect that is moderated by IL-4, IL-10 and IL-13. Several cytokines are known to exert profound effects on behavior, e.g. learning and explorative and avoidance behavior. Some of these actions are likely to occur at CNS levels and mediated by IL-1 since i.c.v. administration of IL-1ra blocks such effects (Kent et al., 1992). Many of the mechanisms integrated at brain levels are under control of catecholaminergic and serotonergic brain neurons. Simultaneously with A. Dunn, we reported that IL-1 stimulates NA turnover rate in the brain. In the case of noradrenergic neurons the effect was not only restricted to the brain but was also detected at low spinal cord levels, suggesting a neural pathway for IL-1 effects in the CNS. There was also evidence of the relevance of the stimulation of central noradrenergic neurons for the effect of IL-1 on the HPA axis and fever (for review Besedovsky and del Rey, 1996; Turnbull and Rivier, 1999).

An aspect that raised great interest during this period was to understand how immune signals can reach the brain and affect brain functions. The evidence showed that humoral and neural pathways are involved. Humoral pathways can convey information to the brain either via circumventricular organs or via the endothelial/ glial interphase in brain vessels. The groups of Dantzer, Maier and Watkins, and Blatteis showed the existence of a neural pathway by demonstrating the important role of vagal afferences in the transmission of cytokine signals to the brain (for review Dantzer, 2004; Maier et al., 1998). In general, the data available indicated that the humoral route may be followed during immune processes that result in high levels of cytokines in the circulation while the neural route seems to predominate when these mediators are released locally in tissues with vagal innervation. In addition, both routes could operate simultaneously or sequentially during immune responses. Towards the end of the period 1987–1996, an important event for the field of brain-immune interactions was the demonstration originally derived from the laboratories of Bartfai and Dantzer that activation of peripheral immune cells by LPS induces cytokine production in the brain. Initially, we had some concerns about these results because the dose of LPS used can disrupt the blood–brain barrier or cause brain alterations as consequence of the endotoxic shock. However, when the studies were done using a dose of LPS that does not affect the blood–brain barrier and does not cause overt symptoms of sickness behavior, we also detected cytokine gene expression in the brain (Pitossi et al., 1997), thus confirming and extending previous work. The expression of the genes for IL-1β, IL-6, TNFα, and IFNγ is increased following peripheral administration of LPS. The onset of transcription and the peak of mRNA accumulation depend on the cytokine and the brain region studied. IL-1β and IL-6 expression is preferentially increased in the hypothalamus and hippocampus, while TNFα expression is more marked in the thalamus–striatum. In the conditions mentioned, these cytokines are less inducible in the brain cortex. No correlation between cytokine gene expression and the density of vascular structures in a given brain area was detected, neither was a preferential cytokine expression observed in brain areas that include the circumventricular organs.

In our view, the neuro–endocrine responses triggered by activated immune cells involve powerful hormones and neurotransmitters that can affect not only immune mechanisms but that also contribute to homeostatic adjustments necessary during diseases in which the IS is activated. In the following, we shall refer to the effect of cytokines in controlling blood flow in lymphoid organs and in setting the conditions for adequate provision of energy to immune cells.

A main physiologic function of the SNS is the control of blood pressure and tissue blood flow. This process is relevant for the effectiveness of the immune response. Indeed, immune cells need to circulate to reach the sites where inflammatory and infectious processes occur. The coupled blood and lymphatic circulatory systems provide the routes for immune cell circulation while adhesion molecules, chemokines, integrins and other locally produced mediators control their homing and mobilization. In addition, the spleen is inserted in the circulatory system in a way that favors the clearance, uptake and retention of microorganisms and their contact with immune cells. In rodents, this organ is deprived from lymphatic circulation and the control of blood flow is strictly based on the sympathetic

vascular tonus. As mentioned, we found that a decrease in sympathetic activity precedes the peak of a specific immune response. Paradoxically, it was shown that administration of IL-1 and LPS can increase SNS activity. This effect was not compatible with the increase in blood flow and accumulation that we have detected in lymphoid organs during immune process, which is likely based on a decrease in the sympathetic control of the vascular tonus. There is however an explanation for these seemingly contradictory findings. IL-1 produced in lymphoid organs selectively increases blood flow by interfering locally with the sympathetic tonus, causing a redistribution of blood flow. While stimulation of the splenic nerve *in vivo* decreases splenic blood flow, this effect is completely abrogated in animals treated with LPS or IL-1 as consequence of a postjunctional inhibition of NA release. Studies *in vitro* showed that IL-6 and TNF exert a comparable effect on NA release by sympathetic nerve fibers. These data reinforce the previously discussed evidence that activated immune cells are less exposed to the effects of noradrenergic nerves and suggest a mechanism by which pro-inflammatory cytokines could deviate blood supply to the sites where immune processes take place (Rogausch et al., 1997). This redistribution of blood supply would favor the contact between immune cells and antigens, a process that is essential for an efficient immune response.

Glucose is the main source of energy for the brain and for most peripheral tissues, including immune cells. In addition, essential immune processes such as endocytosis, phagocytosis, increased cell turnover, clonal expansion, production of numerous mediators and generation of effector cells and molecules are very expensive in terms of energy. We have shown that low, sub-pyrogenic doses of IL-1 induce a profound, long lasting, insulin-independent hypoglycemia in mice (del Rey and Besedovsky, 1989; del Rey and Besedovsky, 1992). This effect, which is also observed in insulin-resistant animals, develops in mice against increased levels of counter regulatory hormones such as catecholamines, glucocorticoids, and glucagon. There is also evidence that the hypoglycemic effect of IL-1 can be triggered at central levels since intracerebroventricular administration of the cytokine induces a reduction in glucose blood levels (del Rey et al., 1998). However, the most surprising effect is observed when mice and rats are challenged with a glucose load several hours after a single intraperitoneal injection of IL-1 (for review Besedovsky and del Rey, 1996). In this situation, it is clearly seen that, following a transient elevation of glucose levels in blood, its concentration returns to the previously reduced levels and IL-1-injected animals remain hypoglycemic for several hours more. These findings strongly indicate that IL-1 changes the rigid set point that characterizes glucose homeostasis. These changes are linked to the capacity of IL-1 to induce glucose transport and oxidation, e.g. in adipose cells and fibroblasts, and also to an effect on glucoregulatory mechanism under brain control (del Rey and Besedovsky, 1992). The combination of local and central effects of IL-1 would serve to deviate glucose to lymphoid organs and inflamed/infected tissues to satisfy the high cost of energy of immune responses (Fig. 1, branching arrows in brown).

4. 1997–2006

From 1997 on, the research in this field was mainly oriented to search for the physiological and pathological relevance of immune–neuro–endocrine interactions and to better understand the molecular basis underlying these interactions.

The importance of the immune–HPA axis regulatory circuit in controlling inflammatory and autoimmune processes was firmly established (Besedovsky and del Rey, 2006; Sternberg, 2006). Considerable experimental and clinical evidence underscores the relevance of the cytokine–HPA axis feedback circuit during autoimmune and infectious diseases. For example, adrenalectomy or blockade of glucocorticoid receptors aggravates disease and increases mortality in animal models of rheumatoid arthritis, multiple sclerosis, Hashimoto's thyroiditis and septic shock. Decreased response of the HPA axis or reduced sensitivity to glucocorticoids is also implicated in human pathologies such as rheumatoid diseases, multiple sclerosis, Sjogren's syndrome, allergic asthma, athopic skin disease, inflammatory bowel disease and fibromyalgia (for review Besedovsky and del Rey, 2006; Sternberg, 2006). The defects, detected in so many pathologies, are now explained by a better knowledge of glucocorticoid actions that can affect a plethora of inflammatory mediators. It has to be added that many effects of glucocorticoids are modulated by the adrenal androgen DHEA. In some cases, particularly during chronic diseases, the cortisol/DHEA ratio reflects the efficacy of the cytokine–HPA axis circuit. This circuit and its relevance in pathophysiology are schematically represented in Fig. 2 as an example because, at present, this is probably the best established neuro–endocrine immunoregulatory circuit.

A novel aspect linked to immunoregulation by noradrenergic nerves derives from the finding that NA induces apoptosis in lymphoid cells via stimulation of β-adrenergic receptors. This finding led us to study whether pro-apoptotic effects of NA can affect immune responses that result in apoptotic-mediated specific T cell deletion (e.g. the immune response to superantigens) or during immune pathologies in which Fas-Fas ligand-mediated apoptosis is defective.

Superantigens, like staphylococcal enterotoxin B (SEB), induce a strong proliferative response followed by clonal deletion of a substantial portion of defined Vβ T cells. The remaining cells display *in vitro* anergy. We found that the immune response to SEB was paralleled by biphasic changes in the activity of the SNS. Furthermore, sympathetic denervation resulted in decreased SEB-induced cell proliferation and IL-2 production, and impeded the specific deletion of splenic CD4Vβ4 cells observed in intact animals without affecting anergy. These studies indicated that

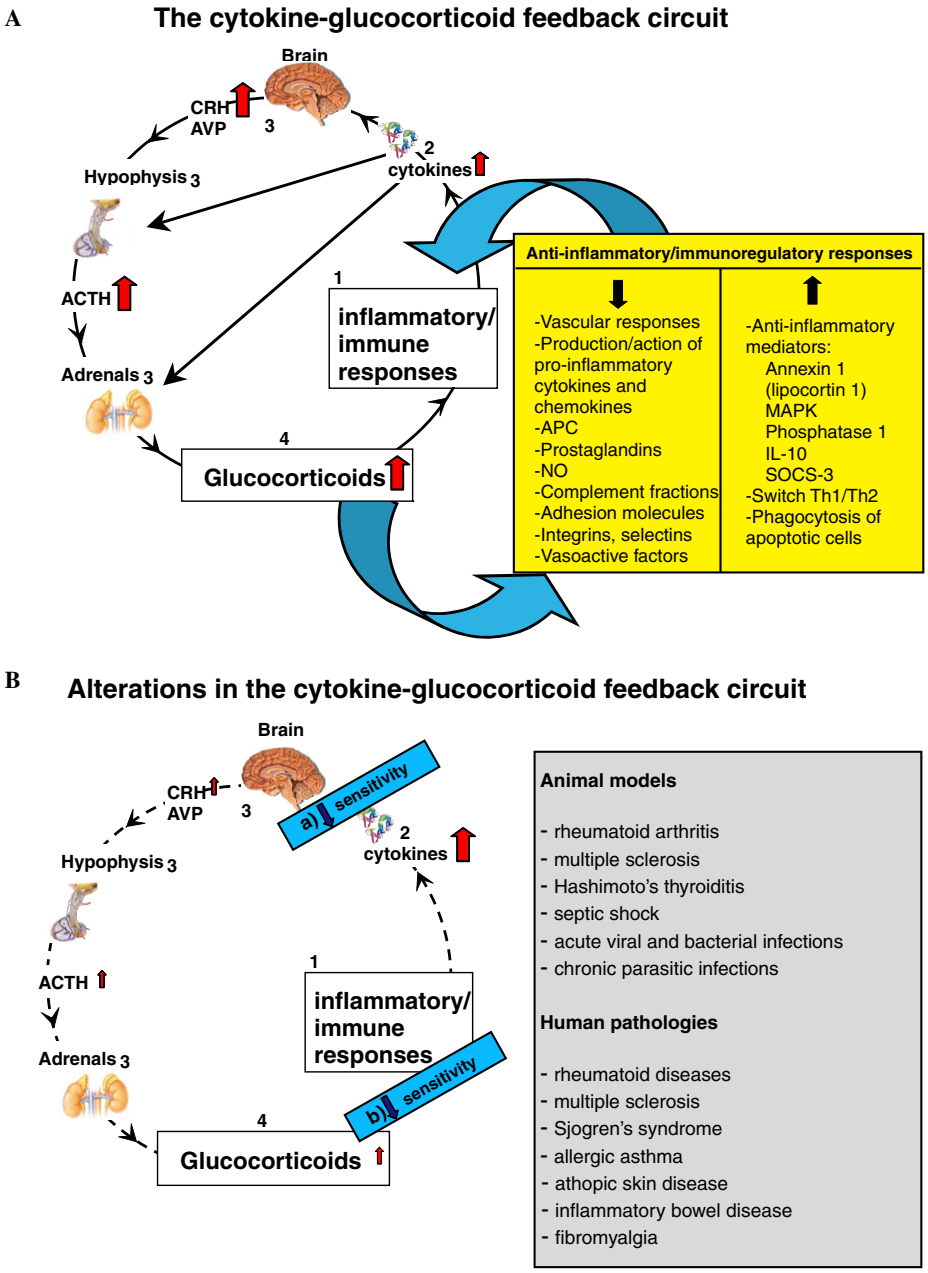

Fig. 2. The cytokine–HPA axis feed back circuit. This circuit is given as an example of immunoregulatory neuro–endocrine mechanisms. (A) Following certain inflammatory and immune responses, cytokines, particularly pro-inflammatory cytokines, can stimulate the HPA axis at different levels. As consequence, increased levels of glucocorticoids affect inflammatory and immune processes by down or up regulating several mechanisms or the production of mediators. (B) Disruption of the cytokine–HPA axis feed back circuit can aggravate the course of certain diseases. Experimental and clinical evidence indicates that the cytokine–HPA axis feed back circuit is altered in human pathologies and in animal models of certain diseases either because there is a reduced response of the HPA axis to cytokines (a) or because of the development of glucocorticoid resistance (b). APC: antigen-presenting cells; NO: nitric oxide; MAPK: mitogen-activated protein kinases; SOCS: suppressors of cytokine signaling.

the proapoptotic effect of NA can be expressed *in vivo* and is relevant during the immune response to superantigens.

When studying the mechanisms by which NA induces apoptosis of lymphoid cells we found that this process is independent of a functional Fas. Thus, we used lpr/lpr mice, which lack functional Fas (CD95) expression and are, therefore, deficient in a critical mechanism for the maintenance of peripheral tolerance, to study whether noradrenergic nerves can affect the expression of the lymphoproliferative, autoimmune disease that they develop spontaneously. Early in ontogeny, the concentration of NA is significantly increased in the spleen of lpr/lpr mice but splenic sympathetic innervation gradually declines as the disease progresses. Furthermore, IgM blood levels and splenic NA concentration inversely correlate when the disease is overtly manifested. Neonatal sympathectomy, which experimentally advances the loss of sympathetic denervation that occurs spontaneously during adulthood,

results in a markedly increased concentration of IgM and IgG2a in blood, accelerates the appearance of lymphadenopathy and shortens significantly the survival time of lpr/lpr mice. These data show that, in addition to defects in the Fas pathway, an altered sympathetic innervation in lpr/lpr mice also contributes to the pathogenesis of the autoimmune disease, and strongly support the hypothesis that the SNS can modulate the expression of lymphoproliferative diseases (del Rey et al., 2002; del Rey et al., 2006).

An overall appraisal of today's view of sympathetic immunoregulation can be summarized as follows. The evidence available shows that almost all mechanisms involved in an immune response can be affected by noradrenergic neurotransmitters (for review Elenkov et al., 2000; Sanders, 2006). Indeed, NA can inhibit or stimulate an immune response depending on the dose of agonist applied and on the type of adrenergic receptor stimulated. The effect of NA also depends on the type of stimulus that triggers the immune response, the subset of cells affected, and, most importantly, at which step of the response, lymphoid and/or accessory cells are exposed to neurotransmitters. Among the processes directly or indirectly affected by sympathetic neurotransmitters are antigen presentation and the expression of costimulatory and adhesion molecules, lymphoid cell activation, cytokine production, clonal expansion and deletion, immunoglobulin production, and the generation of cytotoxic cells. The fact that the simultaneous stimulation of adrenergic receptors and the T cell receptor affects common intracellular signaling pathways may explain why adrenergic agonists can affect so many mechanisms involved in an immune response.

The interest in understanding the relevance of the other branch of the autonomic nervous system, the parasympathetic system, increased in the last years. There are indications that vagal nerve efferents exert a protective role during endotoxic shock. The evidence is based on the fact that bilateral cervical vagotomy aggravates the decrease in blood pressure caused by a lethal dose of LPS and that stimulation of the efferent vagal fibers moderates this effect. The conclusion that the vagus protects the host from the endotoxic shock was also indicated by the fact that the increase in cytokines such as TNFα occurs in vagotomized animals while stimulation of the efferent vagus reverses this effect. Furthermore, using a model of carrageenan-mediated inflammation, it was shown that activation of nicotinic receptors either by vagus nerve stimulation or by cholinergic agonists significantly inhibits the release of pro-inflammatory cytokines and blocks leukocyte migration. *In vitro* experiments using human macrophages confirmed that acetylcholine and cholinergic agonists inhibit the release of TNF, IL-1 and IL-18 in response to endotoxin and that this effect is exerted at post-transcriptional levels. The pharmacologic studies clearly indicate that stimulation of nicotinic cholinergic receptors can be involved in the control of excessive inflammatory responses (for review Tracey, 2002). This and previous studies constitute an important aspect of the research in immune–neuro–endocrine interactions that will most likely show the relevance of the parasympathetic nervous system for immunoregulation also during conditions less extreme than lethal endotoxic shock.

The relevance of neuropeptide release during inflammatory autoimmune processes has become more evident and this topic has been subject of several reviews. Some neuropeptides, particularly SP, are clearly pro-inflammatory while others, such as VIP and PACAP, are anti-inflammatory. These effects have been found in different models of disease, such as septic shock, rheumatoid arthritis and MS (Delgado et al., 2004; Jessop, 2002). It still needs to be clarified to what extent the stimulation of the HPA axis that results from the administration of these neuropeptides contributes to such effects (Nussdorfer and Malendowicz, 1998).

There is now considerable evidence that brain-born cytokines can affect CNS mechanisms. Cytokines have been shown to be "sleep factors" and to affect both non-rapid-eye-movement and REM sleep. Brain levels of IL-1 and TNF correlate with sleep propensity; for example, their levels increase after sleep deprivation. Furthermore, immune neutralization of IL-1 or blockade of its receptors in the brain affects slow wave sleep (Obal and Krueger, 2003), indicating that endogenous IL-1 and TNF are part of a complex biochemical cascade regulating sleep. It was also shown that IL-1 expression can be induced in the brain during stress (for review Besedovsky and del Rey, 1996) and that some of the symptoms of "sickness behavior" are integrated by cytokines produced in the brain. Sickness behavior refers to a coordinated set of subjective, behavioral, and physiological changes that develop in sick individuals during the course of an acute infection. These changes are caused by effects of IL-1 and other pro-inflammatory cytokines on brain cellular targets. Indeed, interference with the effects of these cytokines in the brain abolishes the expression of certain symptoms of sickness behavior. This evidence indicates the role of pro-inflammatory cytokines in orchestrating sickness behavior during acute diseases (Dantzer, 2004).

The possible role of cytokines produced in the CNS on brain physiology and their contribution to the integration of immune–brain interactions at central levels was approached in the last years. An essential question was whether an increase in neuronal activity in a "healthy" brain could affect the local production of cytokines. Direct evidence derives from the demonstration that pre-synaptic stimulation of defined neurons, as it happens during physiologic conditions, can control the local production of cytokines by glial cells and neurons. Long-term potentiation (LTP) of synaptic activity in the hippocampus has served as model to approach this issue. A clear increase in IL-1β gene expression, triggered by glutaminergic neurons via NMDA receptors, was observed in hippocampal slices and in freely moving rats during the course of LTP (Schneider et al., 1998). More recently, we have observed that the IL-6 gene is also over expressed during *in vivo*

and *in vitro* LTP (Balschun et al., 2004). These data constitute the first evidence that cytokine gene expression in the brain can be triggered by a pre-synapticaly induced increase in the activity of a discrete population of neurons.

We and others have studied to what extent cytokines produced in the brain during LTP can affect synaptic plasticity and performance. At this stage, it is necessary to distinguish between studies based on exogenous administration of cytokines and those that focus on the effects of cytokines endogenously produced by brain cells. There is a vast literature showing that exogenous *in vivo* and *in vitro* administration of cytokines can affect LTP induction and synaptic plasticity (for references Besedovsky and del Rey, 1996). These studies, although important from the pharmacological point of view, cannot reliably reveal the effect of cytokines produced in the brain under natural conditions. In fact, LTP is a complex phenomenon that involves a number of receptors and mediators that influence its inducibility, establishment, and maintenance in different ways. In particular, the maintenance of LTP is protein synthesis-dependent and involves the activation of genes in a given sequence and the release of their products in certain quantity. Thus, it is almost impossible to mimic the time-dependent effect of an endogenously produced cytokine by its exogenous administration. For example, as it is discussed below, lL-1, a cytokine that inhibits LTP when administered exogenously, contributes to the consolidation and maintenance of this process when it is produced endogenously.

Using the specific IL-1 receptor antagonist (IL-1ra), we found that blockade of IL-1 receptors, both *in vivo* and in hippocampal slices, results in the inhibition of LTP maintenance. This effect is reversible and occurs only when the antagonist is administered after LTP is triggered, that is at a time when, according to the studies mentioned above, increased IL-1 levels are expected. Studies in Type 1 IL-1 receptor knock out mice are in line with this finding (Avital et al., 2003). We have recently found that, in contrast to the supportive effect of IL-1, IL-6 contributes to the extinction of a well-consolidated LTP (Balschun et al., 2004). Collectively, these results strongly suggest that IL-1β and IL-6 can control the maintenance of LTP in the brain, a process that is assigned a role in memory formation and in certain types of learning. Furthermore, these studies provide evidence for a physiologic, neuromodulatory role of cytokines originally described as immune mediators.

As in the case of LTP, the effects of cytokine administration on learning, memory, and behavior in general, have been extensively investigated (for review Anisman et al., 2005). Again, these studies are undoubtfully of pharmacological relevance but may not reflect the physiological effect of cytokines, which is the main scope of this article. Thus, only possible physiologic effects of endogenous cytokines on memory and learning are discussed below. As mentioned above, a transient blockade of endogenous IL-1 in hippocampal slices and in the brain of freely moving rats results in inhibition of LTP maintenance. Considering that it is currently accepted that LTP underlies certain forms of memory, it was predicted that this process would be inhibited in animals in which IL-1 effects cannot be manifested. This is the case of IL-1 receptor type I knockout mice (Yirmiya et al., 2002). These mice display significantly longer latency to reach a hidden platform in the spatial version of the water maze test and exhibit diminished contextual fear conditioning, but behave similarly to control animals in hippocampal-independent memory tasks. Blockade of IL-1 receptors in the brain of normal animals following a learning task (Morris water maze) causes hippocampal-dependent memory impairment. These results suggest that IL-1 signaling within the hippocampus plays a critical role in learning and memory processes (Avital et al., 2003). It is worth noting that in the previously mentioned studies, the impediment of IL-1 signaling was induced after the training procedure. In contrast, there is evidence that blockade of IL-1 effects prior training by using an adenovirus vector expressing IL-1ra causes an improvement of both short-term and long-term memory retention scores (Depino et al., 2004). However, as mentioned, endogenously produced IL-1 during learning significantly contributes to memorize an established task.

The role of IL-6 endogenously produced in the brain has also been studied. As discussed above, IL-6 is produced during LTP. Blockade of endogenous IL-6 after hippocampus-dependent spatial alternation learning resulted in significant improvement of long-term memory (Balschun et al., 2004). Furthermore, IL-6 KO mice exhibited a facilitation of radial maze learning over 30 days, in terms of lower number of working memory errors (Braida et al., 2004).

Taken these results together, it appears clear that, although having an opposite role, endogenous IL-1 and IL-6 produced in the "healthy" brain, are important in the control of synaptic plasticity and in the hippocampal processing of memory. The mechanism underlying the role of IL-1 and IL-6 on these processes is still unknown but recent data indicate the involvement of NFk-B, a transcription factor that mediates the production and effects of multiple cytokines (for review Meffert and Baltimore, 2005).

In conclusion, there are examples that cytokines produced in the healthy brain can contribute to brain physiology by controlling neuronal activity affecting in this way neuro–endocrine control systems, the set point for the regulation of essential homeostatic mechanisms and intrinsic functions of the CNS like memory and learning. There is also evidence that brain-born cytokines coordinate physiologic adjustments during disease and neuro–endocrine immunoregulatory responses (Fig. 1, red circle). Cytokines appear to play a dual role during brain pathology, on one hand they can control the local immune response by cytokine–neuronal interactions and on the other hand they can behave as effector mediators capable to contribute to the disease.

5. 2007: Waiting for the beginning

This subtitle is clearly optimistic since it pretends to stress the almost infinite work left to the new generations. For example, we often referred to neuro–endocrine immunoregulatory responses that are elicited following stimulation of the IS. This is an oversimplification that derives from the experimental models used initially to uncover immune–neuro–endocrine interactions. There is no ''one'' immune response: there are probably as many types of immune responses as pathological conditions. While immunologists make distinctions between different aspects of natural immunity and type of adaptive immune response, e.g. Th1/Th2-mediated responses, in real conditions immunity is based on interwoven mechanisms that in many cases are the product of a trade-off between immune cells and the causal agent of a disease, e.g. a microorganism, and, why not, the therapeutic intervention used. During each stage of a disease, pathogen-associated molecular patterns (PAMPs) mediated mechanisms are activated, particular combinations of immune cells and their specific products are detected and a cocktail of other mediators are found in the circulation and in inflamed/infected tissues. Thus, neuro–endocrine responses during disease and their immunoregulatory outcome are expected to be different and would require investigation at all levels. Furthermore, today we could state that local, short-loop immune–neuro–endocrine interactions are likely to occur probably in all organs or tissues during health and during the course of a disease.

An important breakthrough in the last decade derived from the exploration of the complex intracellular machinery involved in the response of a cell following stimulation of a given receptor. For example, the different protein-kinases, second messengers, transcription factors, post-transcriptional events and intracellular modulators can be points of encounter during the simultaneous or sequential activation of receptors by immune and neuro–endocrine derived ligands on immune and non-immune cells. This would result in an almost infinite potential synergism or antagonism between the effects of these ligands under normal and pathological conditions. However, from the physiological point of view, the investigation of immune–neuro–endocrine interactions at cellular level should not be isolated from the upstream homeostatic events that result in changes in the production and liberation of different ligands and in the expression of their receptors on the target cell.

Today it would not sound exaggerated to state that almost all pathologies have an inflammatory/immune component that interacts with neuro and endocrine mechanisms. In fact, immune cells, particularly antigen-presenting cells, are present in all tissues where they are exposed to hormones and neurotransmitters.

As stated in the introduction, it is difficult to find the limit between physiological or pathological situations when one refers to the IS. We wonder whether the different denominations given to our field are somehow self-imposed restrictions. For example, should a field of research devoted to study the psychoneuroimmunology of skin diseases be denominated psychoneuroimmunodermatology? This would apply to all medical specialties and also to Biology (psychoneuroimmunobiology?) and we could continue adding all possible acronyms to the denomination of our field. What are we really doing when we study immune–neuro–endocrine interactions in each organ? In our view, what we are doing is a serious attempt to integrate the present knowledge in biology and medicine based on a multidisciplinary approach. This attempt serves to unify data obtained using systemic approaches with data derived from the implementation of refined technologies that allow the analysis of a phenomenon at molecular levels. In our view, our work serves to reestablish the balance between deduction and induction, analysis and synthesis, which is, in fact, the essence of science. The need to reestablish such balance derives from the actual predominance of analytic approaches that sometimes degenerate in reductionist views. Such predominance probably derives from the immense technologic progress of the last decades that results in the temptation (sometimes favored by grant agencies and high impact factor journals) to invest resources in understanding molecular mechanisms isolated from their physiological and pathophysiological significance. Indeed, even if all products that serve as messengers for intra, extra, and intercellular communication would be identified, we would not be able to decipher all the messages they could convey. A messenger can be purified but not the message that it carries, which depends on a large variety of temporal and spatial conditions and on the present state of the target that receives the information. Thus, the question that comes to our mind is: has the time arrived to re-name our field by simply calling it ''Integrative Biology and Medicine''?

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