Molecular Understanding of Cytokine–Steroid Hormone Dialogue

Implications for Human Diseases

JIMENA DRUKER,[a] ANA C. LIBERMAN,[a,b] MATÍAS ACUÑA,[a] DAMIANA GIACOMINI,[a,b] DAMIÁN REFOJO,[a,b] SUSANA SILBERSTEIN,[a,b] MARCELO PAEZ PEREDA,[c,d] GÜNTER K. STALLA,[d] FLORIAN HOLSBOER,[d] AND EDUARDO ARZT[a,b]

[a]*Laboratorio de Fisiología y Biología Molecular, Departamento de Fisiología y Biología Molecular y Celular, Facultad de Ciencias Exactas y Naturales (FCEN), Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II, (C1428EHA), Buenos Aires, Argentina*

[b]*Members of the IFYBINE-Argentine National Research Council (CONICET) Ciudad Universitaria, Pabellón II, Buenos Aires, Argentina*

[c]*Affectis Pharmaceuticals, Kraepelinstrasse 2, 80804 Munich, Germany*

[d]*Max-Planck Institute of Psychiatry, Kraepelinstrasse 2-10, 80804 Munich, Germany*

ABSTRACT: **Highly sophisticated mechanisms confer upon the immune system the capacity to respond with a certain degree of autonomy. However, the final outcome of an adaptative immune response depends on the interaction with other systems of the organism. The immune–neuroendocrine systems have an intimate cross-communication, making possible a satisfactory response to environmental changes. Part of this interaction occurs through cytokines and steroid hormones. The last step of this crosstalk is at the molecular level. In this article we will focus on the physical and functional interrelationship between cytokine signaling pathway–activated transcription factors (TFs) and steroid receptors in different cell models, where the signals triggered by cytokines and steroid hormones have major roles: (1) the ligand-dependent-activated glucocorticoid receptor (GR) influence the genetic program that specifies lineage commitment in T helper (Th) cell differentiation. How post-translational modifications of several TFs as well as nuclear hormone receptors could be implicated in the molecular crosstalk between the immune–neuroendocrine messengers is discussed. (2) glucocorticoid (GC) antagonism on the TCR-induced T cell apoptosis. (3) estrogen receptor/TGF-β family proteins molecular interaction implicated on**

Address for correspondence: Dr. Eduardo Arzt, Laboratorio de Fisiología y Biología Molecular, FCEN-Universidad de Buenos Aires, Ciudad Universitaria, Pabellón II (C1428EHA), Buenos Aires, Argentina. Voice: 54-11-4576-3368; fax: 54-11-4576-3321.
e-mail: earzt@fbmc.fcen.uba.ar.

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pituitary prolactinomas pathogenesis. The functional crosstalk at the molecular level between immune and steroids signals is essential to determine an integrative response to both mediators (which in the last instance results in a new gene activation/repression profile) and constitutes the ultimate integrative level of interaction between the immune and neuroendocrine systems.

KEY WORDS: **glucocorticoids; cAMP; TCR; apoptosis; GATA-3; T-bet; Th1-Th2 differentiation; BMP-4; TGF-β; prolactinoma; estrogens**

INTRODUCTION

The immune system is composed of a large variety of cells and molecules. It operates by a complex network of regulatory mechanisms that are able to confer on it a certain degree of autonomy. However, animal homeostasis is sustained by a group of systems that do not operate in isolation. Mutual influences between them are fundamental to make possible a satisfactory response to environmental changes. The existence of physiological interactions between the neuroendocrine and immune systems is reflected by the fact that several neuroendocrine responses occur during immune cell activation. Some of the main communicators between the immune and neuroendocrine systems are hormones and cytokines. Through these molecules cells receive information which, after processing, produces a biological response. Sustained activation of the immune system leads to an increase in both cytokine and steroid hormone blood levels. Immune responses elicit the production of soluble factors, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1 and IL-6, which stimulate the hypothalamus-pituitary-adrenal axis (HPA). One of the results of this activation is the elevation of circulating glucocorticoids (GCs).[1,2] Thus, a new metabolic hormonal state facilitates a homeostatic balance that limits some features of the immune reaction. The steroid receptors act both as transcription factors (TFs) themselves and as modulators of other TFs. The biological effect of cytokines and steroids is achieved by the activation of signaling cascades that elicit a specific gene expression program. Highly regulated go–stop signals between cytokines and steroid hormones are required to prevent cytokine overreaction. The ultimate level of integration of cytokine–steroid crosstalk is the molecular level.[3,4] It is widely known that the immunomodulatory actions of GCs are exerted by interfering with the proinflammatory signaling process.[4,5] Glucocorticoid receptor (GR) regulates gene transcription through DNA-dependent[6,7] and -independent mechanisms.[8–10] However, the most important anti-inflammatory effects of GCs occur independent of GR–DNA binding through direct inhibition of TF activity by a transrepression mechanism, which results in protein–protein interaction. The GR interacts with activator protein 1 (AP-1), nuclear factor-κB (NF-κB), and signal transducers and activators of transcription (STATs) that control proinflammatory gene

expression.[11] GCs also play a pivotal role in the regulation of the balance between Th1/Th2 subset during the immune response.[3,12,13] This occurs by interaction with transcriptional activity of T helper (Th) 1/Th2 TFs, T box expressed in T cells (T-bet) and GATA-3. Since the transcriptional activity of steroid receptors and TFs may be regulated by posttranslational modifications,[14] how such modifications could modulate the molecular crosstalk between the immune–neuroendocrine systems will also be discussed. GCs are able to induce apoptosis in T cells but also can counteract the signals of death, leading to cell survival.[3,15] T cell apoptosis is a physiological process that facilitates immune response regulation. T cell receptor (TCR) stimulation induces apoptosis in T cells and GCs exert an antiapoptotic effect on T cell activation–induced cell death.[16–18] These molecular mechanisms will also be discussed in this article. Similar to GCs, estrogens exert several roles not only in the female reproductive system, but also in other systems. Transforming growth factor-β (TGF-β) and bone morphogenetic proteins (BMPs) transduce signals through Smad-4, a signal cotransducer. This pathway regulates the expression of proto-oncogenes that control cell proliferation. The physical and functional interaction between Smad proteins and estrogen receptor that takes place in pituitary cells[19] will be also discussed in detail.

GC INTERACTION WITH Th FACTORS

Naive Th cells differentiate into two different functional subsets known as Th1 and Th2 cells, respectively.[20,21] There are several factors that determine the fate of Th cells, the most important being cytokine environment and the presence of specific TFs, T-bet and GATA-3, which are selectively expressed in Th1 and Th2 cells, respectively.[22,23] Th1 cells secrete interferon-gamma (IFN-γ) and IL-2 and Th2 lymphocytes produce IL-4, IL-5, IL-10, and IL-13. When T-bet and GATA-3 are ectopically expressed, they increase cytokine production of their own subset[24,25] (Th1 and Th2, respectively) and at the same time inhibit the opposite subset differentiation and cytokine production.[23,25,26] Some reports have shown that GCs favor Th2 differentiation,[27,28] whereas others have shown that GCs inhibit Th2 polarization measured as Th1/2 cytokine synthesis and expression.[29,30] Above all, there is little evidence about the molecular mechanisms implicated in Th cell polarization by GCs.[31,32] Therefore, we studied the interaction between GCs, T-bet, and GATA-3. Experiments in undifferentiated splenocytes showed a strong direct inhibition of T-bet and GATA-3 expression by GCs. In addition, GCs inhibited T-bet and to a lesser degree GATA-3 transcriptional activity. We also analyzed the molecular mechanisms involved in this differential inhibition and found that transrepression was the mechanism by which GCs inhibited T-bet activity, but not GATA-3. Therefore, GCs inhibitory effect is stronger on Th1 cells, supporting the notion that a differential inhibition of T-bet and GATA-3 may be the way by which

GCs induce Th2 differentiation. As shown in the example of T-bet, NF-κB, and AP-1, GRs interact with each other by protein–protein interaction. This may be further modified by posttranslational modifications. Transcriptional activity of steroid receptors as well as several TFs may be up- or downregulated by posttranslational modifications. Proteins can be modified by phosphorylation, acetylation, prenylation and even by covalent attachment of polypeptides, such as ubiquitin and small ubiquitin-related modifier (SUMO).[14,33,34] The most widely known function of the ubiquitin system is the selective degradation of targeted proteins by proteasome machinery.[35] On the other hand, SUMO modification regulates different cellular processes including subcellular localization, transactivation activity, protein–protein interactions,[36] and in particular situations SUMO covalently modified proteins that are able to avoid ubiquitin-mediated degradation, such is the case of the inhibitor of κB (I-κB).[37,38] One of the most important mechanisms by which cytokines transduce signals that elicit specific responses in target cells involves enzymes called Janus kinases (JAKs) and TFs called signal transducers and activators of transcription, STATs.[39] Protein inhibitor of activated STAT (PIAS) proteins (SUMO E3 ligases) were originally discovered as transcriptional coregulators of JAK–STAT pathway and subsequent studies have shown that PIAS proteins are implicated in the regulation of the activity of several TFs[39–41] and steroid receptors.[42,43] GR is modified by SUMO and this covalent modification regulates the stability of the GR and potentiates its transactivation activity.[44] However, sumoylation of androgen receptor (AR) represses AR-dependent transcription.[45] Nuclear targets of many cytokine signaling pathways (STATs, AP1, NF-κB) are sumoylated as are steroid receptors,[33,39,46,47] suggesting that SUMO modification of these proteins could play a key role in steroid hormone–cytokine interaction.

GCs AND T CELL APOPTOSIS

In T cells, stimulation with either TCR or GCs induces apoptosis, and simultaneous addition of both results in cell survival.[16,17] Interestingly, cAMP, which is regulated by several neurotransmitters, such as vasoactive intestinal polypeptide (VIP), interacts with this system. cAMP inhibits apoptosis induced by TCR activation and also potentiates the apoptosis induced by GCs in the same cells, suggesting that cAMP might play an important role in adjusting T cells to the pro- and antiapoptotic stimulus.[18] cAMP also potentiates GC-induced glucocorticoid response element (GRE) activity cloned upstream of the luciferase gene. A dominant negative form of the protein kinase A (PKA) is able to block the effect of cAMP on GRE, suggesting that the effect of cAMP on GC-induced apoptosis is dependent on PKA activation. Gel shift experiments have shown that addition of cAMP enhances GR binding to GRE, a mechanism that is independent of GR concentration, as shown by the Western blot analysis of GR expression. Therefore, cAMP induces PKA, which

potentiates the GR-dependent transcriptional activity and apoptosis by regulating GR binding to the DNA. Adding H89 (a pharmacological inhibitor of PKA), which reverts TCR-induced apoptosis, and CRE, oligo decoy targeting strategies indicate that cAMP inhibits TCR-induced cell death through PKA and at least partially through CREB-like TFs. TCR-induced apoptosis involves Fas ligand (FasL) induction.[48] RT-PCR analysis has also shown that cAMP inhibition of TCR-induced FasL expression is blocked by H89 and is therefore mediated by PKA, whereas GC inhibition of TCR-induced FasL expression is blocked by adding RU38486 and is therefore dependent on the GR. The inhibition exerted by GCs and cAMP on TCR-induced cell death is associated with the inhibition of NF-κB and Erk1/2 activation, and also to the blockage of the transcriptional induction of FasL expression (D. Refojo and E. Arzt, unpublished results). During stress conditions, several neurotransmitters are released. These molecules are able to regulate the levels of cAMP, so during such events a strong influence of the modulatory action of cAMP in the crosstalk at the transcriptional level between GCs and the TCR signaling pathway may take place.

CROSSTALK BETWEEN TGF-β SUPERFAMILY CYTOKINES AND STEROID HORMONES

Prolactinomas are the most frequent pituitary functional tumors in humans. Growth factors and estrogens are also known to be involved in the control of lactotroph cell proliferation. The tumorigenic action of estrogen in prolactinoma development has been shown *in vitro* as well as by clinical evidence. Members of the TGF-β superfamily also exert inhibitory effects on prolactinomas;[49,50] in addition, TGF-β inhibits c-Myc expression.[51,52] Recently, we have demonstrated that BMP-4 (a member of the TGF-β superfamily) is overexpressed in different prolactinoma models including not only dopamine 2 receptor knockout (D2R-/-) mice, but also estradiol-induced rat and human prolactinomas, as compared to normal tissue and other pituitary adenoma types.[19] BMP-4 intracellular signaling is mediated by Smad-4 and in order to study the role of BMP-4 in tumor formation in nude mice we produced GH3 clones stably transfected with a Smad-4-dominant negative construct (GH3-Smad-4dn). GH3-Smad-4dn cells formed small, lows-growing tumors that did not express c-Myc as compared to control cells, demonstrating the involvement of BMP-4 in promoting prolactinoma development. Furthermore, we have also shown that cell proliferation is upregulated by an overlapping intracellular signaling mechanism between BMP-4 and estrogens, and that their action was partially inhibited by blocking either pathways with the reciprocal antagonist. Indeed, we demonstrated by coimmunoprecipitation studies that Smad proteins physically interact with the ER.[19] When GH3 cells were treated with BMP-4, TGF-β, or 17-β-estradiol, coimmunoprecipitation of ER-α/ER-β, and Smad-4 was

detected. On the contrary, coimmunoprecipitation of ER-α/ER-β and Smad-1 (a BMP-4-specific Smad-transducer protein) was detected only in the presence of BMP-4 or 17-β-estradiol, and coimmunoprecipitation of ER-α/ER-β and Smad-2 (TGF-β-specific Smad-transducer proteins) was detected only in the presence of TGF-β or 17-β-estradiol.[19] These results demonstrated that different proteins are involved in the ER/Smad complex upon stimulation. The nature of this complex may change not only the transcriptional activity of the proteins involved, but also the transcriptional regulation of the target promoters. Moreover, the crosstalk between estrogen and TGF-β/BMP-4 cytokines may be present not only in the prolactinoma cells but also in other cells, such as those of the breast and bone, in which both estrogens and the TGF-β superfamily

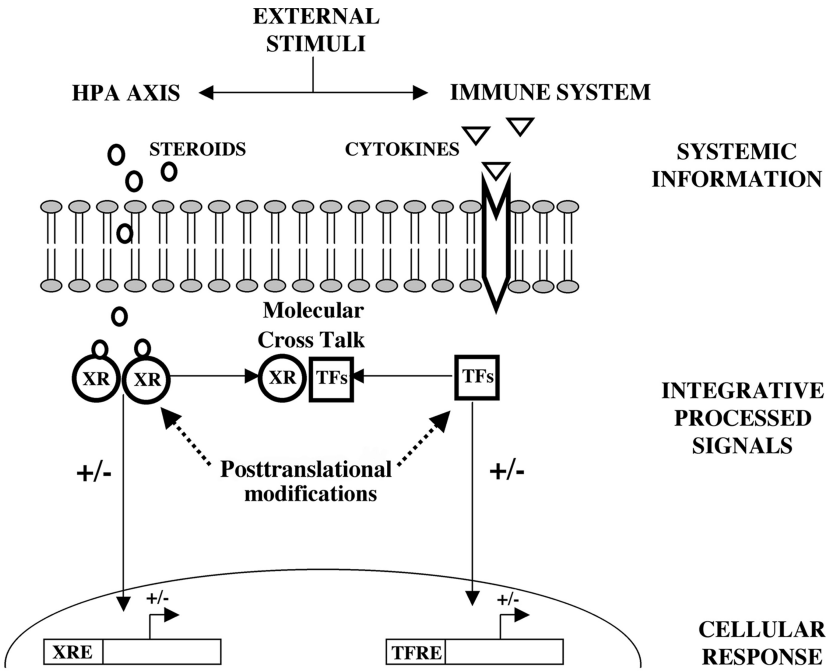

FIGURE 1. External stimuli may induce the immune system to activate the HPA axis. The result of this activation is an increase of systemic cytokines and steroid hormone levels, which are able to trigger multiple signaling cascade pathways in target cells. The cross-interaction of target proteins of these signaling pathways leads to an integrative cell response, which is reflected by a new gene activation/repression profile. Posttranslational modification of steroid receptors and TFs could be involved in the regulation of signaling crosstalk among the immune and neuroendocrine systems. XR = steroid receptors; RE = DNA response element; TFs = transcription factors.

play important roles,[53,54] implicating similar mechanisms in the progression of other diseases.

CONCLUSIONS

A coordinated functional interaction between the immune and neuroendocrine systems is essential to develop an adaptive response to environmental changes. The goal of this process is achieved by the molecular interaction of each system: cytokines and steroid hormones. As shown in FIGURE 1 the molecular crosstalk between immune and endocrine signals occurs at the cellular level, where the signaling pathways triggered by these messengers cross-interact and are mutually influenced, with functional consequences that will determine the final cell response. Inside this network, posttranslational modification of target proteins is important for signal-dependent regulation of protein activity and also for gene expression–repression profile. Increased understanding of how systemic information is integrated at cellular and molecular levels will help to elucidate the intimate cross-communication between signaling triggered by steroid hormones and cytokines in both immune and nonimmune cells. The elicited biological response achieved by each cell will result from the balance of overlapping signals released by the immune and neuroendocrine systems in response to external or internal environmental factors. Understanding how this information is integrated at the molecular level is a challenge to further dissect the complex interaction of these systems and its impact on human diseases, providing new molecular targets for pharmacological approaches.

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