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COMMENTARY

Hormone Regulation of Immune Homeostasis
LOCAL OR LONG DISTANCE?

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ABSTRACT. The endocrine system and the immune system consist of dynamic biological processes involved on the one hand in the regulation of a complex array of metabolic and physiologic activities, and on the other hand in protection against infection and disease. Evidence for bidirectional functional involvement of immune–endocrine interactions can be seen at many levels, including codependence during critical stages of development, the complementary use of shared molecular mediators and receptors by both systems, and the integrated participation of the immune system and the endocrine system in resistance or susceptibility to disease. Moreover, recent findings—principally derived from studies of intestinal intraepithelial lymphocytes in mice—suggest that immune–endocrine interactions are essential for the proper development of intestinal T cells, and indicate that this most likely occurs via a local network of hormone synthesis and utilization. In the present article, these findings will be discussed in the context of immune–endocrine collaboration, with particular attention given to the involvement of the thymus in this process, and a hypothesis will be proposed which suggests that the homeostatic balance between health and disease is largely driven by local rather than systemic hormonal regulatory events. BIOCHEM PHARMACOL **56**;1:1–5, 1998. © 1998 Elsevier Science Inc.

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The likelihood that the immune system and the nervous system are inextricably linked through a series of physiological, biochemical, and functional activities has been acknowledged for many years. In fact, broadly speaking, the similarities between these two systems—in particular between the immune system and the neuroendocrine system—are striking. Each, for example, is, in part, a sensory organ that incorporates precise sets of receptors to interface with other tissues and/or with the external environment; each involves complex levels of communication for information transfer throughout the organism; and each employs intricate regulatory mechanisms, some of which consist of common mediators utilized by both systems. Therefore, it is not surprising that these two essential organs are dependent upon each other for the execution of their designated tasks. Yet, despite rapidly improving techniques for detailed studies into the molecular and cellular processes that drive the immune and endocrine systems, many basic questions have yet to be addressed regarding the means by which these systems cooperate in establishing and maintaining homeostasis within the organism.

Inasmuch as many excellent reviews and edited volumes are currently available which deal with the nature of immune–endocrine interactions [1–3], this paper will not attempt to provide a review of those findings, but rather will correlate the salient observations from that body of information with recent findings regarding immune–endocrine regulation of T cells, from which a general question can be posed concerning the potential combined workings of the immune and endocrine systems.

MAINTENANCE OF A CRITICAL BALANCE IN IMMUNITY AND METABOLISM BY BIDIRECTIONAL IMMUNE–ENDOCRINE INTERACTIONS

The intricate and elaborate connections between the immune system and the endocrine system—and the fundamental roles played by each—can be seen in a number of ways. Certainly, some of the most convincing examples of this are apparent in mice and humans during early periods of development, at a time when both the immune system and the endocrine system are in active stages of change or maturation. Within the immune system, the thymus, in particular, appears to play a major role in this regard. Thus, disruption of thymus function during fetal life is accompanied by concomitant endocrine-mediated alterations across a broad range of activities throughout both the HPA† and

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† *Abbreviations:* ACTH, adrenocorticotropic hormone; FSH, follicle stimulating hormone; GH, growth hormone; HPA, hypothalamus-pituitary-adrenal; HPT, hypothalamus-pituitary-thyroid; IEL, intraepithelial lymphocyte; IL, interleukin; LH, luteinizing hormone; NTX, neonatal thymectomy; PRL, prolactin; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; and T_4, thyroxine.

the HPT axes. For example, mice that are athymic from birth have impaired hypothalamic, pituitary, and adrenal hormone activities [4–7]. Conversely, disruption of endocrine development leads to alterations in many critical aspects of immunity, including but not limited to impairment of thymus-derived T cell development and function [8–11]. Restoration of the primary lesion, either by hormone replacement or by introduction of healthy thymus tissue, leads to at least partial functional restoration of the secondary defect. Such co-dependent interactions, however, are not restricted to developmental stages, but are evident throughout many periods of adult life as perhaps best seen by the dynamic relationship between thyroid function and immune activity of the thymus. Thus, for example, treatment of normal adult mice with thyroid hormones leads to an increase in the numbers of thymocytes [12, 13], and has marked immuno-augmenting effects on thymus function in aged mice [14, 15].

Mechanisms of communication between the immune system and the endocrine system undoubtedly involve the reciprocal utilization of soluble hormone and cytokine mediators, coupled with the expression of appropriate receptors that are widely yet selectively distributed across both systems. These include receptors for hormones derived from nearly all branches of the neuroendocrine system (reviewed in Refs. 1 and 2). Conversely, it is now clear that cytokines classically associated with the immune system, most notably IL-1, IL-6, and cytokines of the tumor necrosis factor and interferon families, can profoundly influence endocrine responses, as well as other important activities of the central nervous system, such as sleep and behavior [16, 17]. Moreover, the thymus is itself vested with a high degree of endocrine-like activity mediated through a unique set of thymic peptides and hormones (e.g. thymulin, thymosin α1 and β4, and thymopoietin [1]), which are particularly sensitive to fluctuations in thyroid hormone activity [12, 13]. At a minimum, these shared molecular mediators, and the diverse activities regulated by them, point to a high level of information transfer and crosstalk between the immune system and the endocrine system.

Functionally, there is longstanding evidence establishing a direct correlation between endocrine function and susceptibility or resistance to disease. Such endocrine-associated events have been most closely linked to hormones of the HPA axis, in particular to glucocorticoids, and have been shown to significantly influence host immune responses to essentially all major types of infectious diseases, susceptibility to neoplasia, and/or expression of autoimmune disease [18]. Although many aspects of HPA–immune interactions remain to be elucidated, the capacity of endocrines to directly influence hemopoiesis [19], and the use of hormones and hormone receptors by the immune system, provide a potential clinical basis from which to view the involvement of hormones in the development of, or protection against, disease within the organism.

T CELL LYMPHOPOIESIS IN THE SMALL INTESTINE—DEVELOPMENTAL CORRELATIONS WITH THYMUS FUNCTION

The potential for the intestine to serve as a site of T cell lymphopoiesis has been a topic of controversy for many years [20]. Recently, however, studies from a number of laboratories have provided evidence that strongly favors this possibility. The results of these studies have been generally consistent, although interpretations have been confounded by variations in findings depending upon the type of experimental animal model used. Essentially all studies have relied to one degree or another on athymic mice drawn from one of three basic models, each of which differs according to the time at which mice became or were rendered athymic. Although in all three systems T cells and classical T cell functions, such as antigen-specific cytotoxicity, T helper cell activity, responsiveness to mitogens, and the ability of the animal to reject transplanted allografts, are virtually eliminated in peripheral T cell compartments, functionally-mature T cells (i.e. IELs) are present within the intestinal epithelium. However, the distribution, as well as the phenotypic composition and numbers of IELs, varies considerably, depending upon the experimental athymic animal model used. At the one extreme is the adult athymic bone marrow radiation chimera (athymic chimeras) in which adult mice are thymectomized, exposed to lethal total-body irradiation, and immunologically reconstituted with syngeneic bone marrow stem cells. IELs in athymic chimeras closely approximate, by all criteria, the types of IELs found in unmanipulated euthymic mice [21–23]. At the opposite end of the spectrum is the congenitally athymic nude mouse, which has notably reduced numbers of TCRαβ IELs (the T cell type commonly found throughout most extraintestinal peripheral lymphoid tissues), despite relatively normal numbers of TCRγδ IELs [24, 25]. The third athymic animal system used for IEL studies is the NTX mouse, in which the thymus is surgically removed within 24–72 hr of birth. IELs in adult NTX mice generally resemble in composition and numbers those of nude mice, although there may be some increases in TCRαβ IELs in NTX mice relative to the latter animals [26–28].

Despite the obvious differences in findings between these systems, several interpretations can nonetheless be drawn. Clearly, the results from studies of athymic chimeras strongly suggest that the thymus does not *directly* participate in the generation of intestinal T cells, given that functionally mature T cells of donor bone marrow origin are present within the intestinal epithelium but not elsewhere. However, when viewed across a developmental spectrum, the thymus must be involved in some manner in the maturation of intestinal T cells, since the absence of the thymus during fetal/neonatal life results in the incomplete development of that T cell compartment. Taken together, this implies that the IELs are thymus-dependent but not thymus-derived T cells.

ENDOCRINE REGULATION OF INTESTINAL T CELLS

How, then, might the above experimental differences be reconciled? One possibility is that thymus participation in intestinal T cell development is tied to a signaling process—possibly involving neuroendocrine mediators—during early stages of animal development, and that the functional consequence of that event is to render the intestinal epithelium suitable for local T cell maturation. To test this hypothesis, intestinal T cell maturation was studied in NTX and normal euthymic mice treated at various age intervals with hormones of the HPT axis (TRH, TSH and T_4), based on previous studies demonstrating a role for HPT hormones in immune regulation during development, as discussed above. TRH or TSH administered to 6-week-old NTX mice resulted in the regeneration of intestinal T cell subsets that are normally lacking in NTX mice, but are routinely present in euthymic mice and athymic chimeras; T_4 treatment had no detectable effect on IELs in NTX mice [28, 29]. Conversely, TRH or TSH treatment of 6-week-old normal euthymic mice did not alter in either a positive or negative manner the overall composition of intestinal IELs; however, T_4 treatment of normal euthymic mice at either 3 or 6 weeks of age (a period of active IEL development in mice) suppressed the development of the same IEL subsets up-regulated by TRH and TSH in NTX mice [30]. These results are particularly striking given that they occurred in the absence of direct immune manipulation by either thymus introduction or removal, or by hematopoietic cell transfer, but rather occurred following exogenous hormone supplementation.

The functional involvement of HPT hormones in the regulation of gastrointestinal immunity was further demonstrated, using a murine model of experimental autoimmune gastritis. In this system, day 1–3 NTX mice, depending upon animal strain, develop organ-specific autoimmune disease that is histologically evident in adult animals with peak incidence from 9 to 12 weeks post-thymectomy [31]. Disease is mediated, in part, by autoantibodies to stomach parietal cell components, and appears to be linked to the disruption of a regulatory T cell population(s) [32]. Using a large panel of day 1–3 NTX mice treated with TRH, TSH, or T_4 administered to neonates beginning 24 hr post-thymectomy, or to young adult mice beginning at 6 weeks of age, it was found that mice treated with T_4 at 6 weeks of age, i.e. at about the time of disease onset, had significantly lower incidence and severity of autoimmune gastritis [33]. In contrast, TRH and TSH treatment had a minimal modulating effect on the outcome of disease, once again implying a dynamic process of immune regulation by HPT hormones. Findings from these sets of experiments are summarized in Table 1.

TABLE 1. Effects of HPT hormones on intestinal T cell development and differentiation and gastrointestinal inflammation

Type of hormone	Effect on T cell development	Effect on experimental autoimmune gastritis*
TRH	Up-regulate	Minimal
TSH	Up-regulate	Minimal
T_4	Down-regulate	Reduce or exacerbate†

*Autoimmune gastritis induced in neonatally thymectomized BALB/c mice [31, 32].

†Outcome of hormone treatment, reduction or exacerbation of disease, is a function of the time of hormone exposure and the onset of disease [34].

IDENTIFICATION OF A LOCAL PARACRINE HORMONE CIRCUIT IN THE SMALL INTESTINE

The results discussed above, although novel, are at the same time perplexing since they evoke the question as to why systemic administration of HPT hormones should have immunological effects that are targeted principally to the intestine. One potential explanation is that the hormone-mediated effects observed during periods of active T cell development reflect a normal ongoing process that occurs throughout the life of the animal, and that under natural conditions this process is not endocrine-driven but is regulated via local paracrine and/or autocrine hormone circuits. To explore this, murine small intestine IELs and epithelial cells were studied for TRH receptor expression, TSHβ production, and TSH receptor expression using molecular, biochemical, and immunochemical techniques. These studies revealed a potentially dynamic process of immune–endocrine crosstalk between hematopoietic and non-hematopoietic cells of the intestine in which intestinal epithelial cells produce TSH in a TRH-dependent manner, and intestinal IELs are the primary TSH-responsive cell population [34]. Within this system, the actual effector molecule appears to be TSH under regulation by both TRH and T_4 in a manner analogous to the TRH $\rightarrow$ TSH $\leftrightarrow$ T_4 pathway across the classical HPT axis. Thus, up-regulation of TSH by TRH has the effect of activating the intestinal immune response, whereas down-regulation of TSH by T_4 results in local immunological suppression.

Although the significance of this local hormone-mediated communication network to the overall biology of intestinal immunity is not yet fully evident, there are a number of ways in which it may be functionally important. Clearly, a distinct advantage for the host resides with its ability to efficiently adjust the immune response to environmental pathogens or antigens at the level of the gastrointestinal tract by altering the composition of lymphocytes at the point of antigenic challenge, rather than through large-scale remodeling of the intestinal immune system. Activation may occur via TRH, as reported [34], but under natural conditions may also involve other external stimuli, such as bacterial endotoxin [35] (Fig. 1).

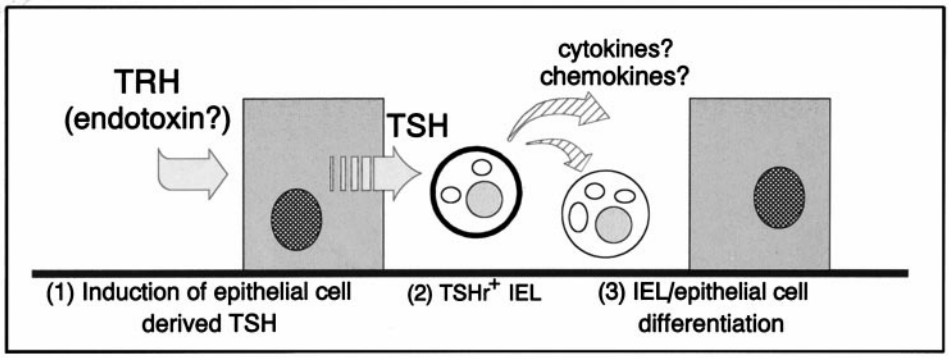

FIG. 1. Model for regulation of intestinal immune homeostasis by HPT hormones. Secretion of epithelial cell-derived TSH is induced by TRH, or by other stimuli such as endotoxin [35]. TSH binds to selected TSH receptor-positive ($TSHr^+$) IELs, which are induced to release cytokines and/or chemokines used to promote IEL and/or epithelial cell differentiation.

SYNTHESIS AND SPECULATION

Evidence establishing a functional link between the immune system and the endocrine system continues to accumulate from many fronts. Moreover, from an immunological perspective, there is a growing awareness that the immune system is not an autonomous organ, and that understanding immunity *en toto* cannot be achieved through purely reductionist approaches. Rather, immunity must be viewed as a dynamic biochemical and cellular balance, both within the organism overall and within the immune system itself. In terms of immune–endocrine interactions, an essential question continues to be how communication is mediated across long distances. In some cases, this may occur via direct neuronal connection through the autonomic nervous system. In this scenario, rapid long-range networking could be initiated by sentinel immune cells strategically located within appropriately innervated tissues such as the spleen, lymph nodes, or Peyer's patches [36], followed by autonomic nervous system-activated hormone release. The cycle would then be completed by endocrine-driven hormone regulation of the immune response within appropriate tissues. But is it possible for classical methods of endocrine hormone dissemination to operate throughout the immune system with sufficient specificity so as not to result in unwanted broad-spectrum immunological perturbations? Certainly, the differential expression of hormone receptors on immune cells, and the regulation of those receptors, would impart a significant degree of specificity to the system. Moreover, expression of hormone receptors on lymphocytes could be modulated as a consequence of signal transduction upon ligation of the T-cell receptor on T cells or immunoglobulin on B cells by appropriate antigens, or concomitant with the expression of co-receptors or other activation molecules. At present, however, there is little evidence to support that possibility.

Alternatively, therefore, hormone regulation of immunity may be controlled through the local production and utilization of hormones in a manner similar to that which occurs in the intestine. This, of course, would require the synthesis of immunologically relevant hormones to occur within the various lymphoid tissues themselves. At present, there is ample evidence to support this possibility, however. Note that the thymus, in particular, has the capacity to produce a remarkable array of classical neuroendocrine hormones (GH, PRL, ACTH, FSH, LH, and TSH), derived either from the thymocytes themselves or from thymic epithelial cells [1]. Moreover, nearly all murine lymphoid tissues studied to date by our laboratory express TSH receptors, but to varying degrees, with bone marrow stem cells expressing unusually high levels. TSH stimulation of those TSH receptor-bearing cells leads to rapid yet selective synthesis of immunologically relevant cytokines and/or chemokines (Whetsell M, Bagriacik U and Klein JR, unpublished observations), implying a very active and dynamic process of immune–endocrine regulation of lymphoid cell maturation and differentiation, which inherently would be most efficiently controlled through local hormone sources. Additional studies into this aspect of immunity may help to provide further insight into the process by which the endocrine system and the immune system cooperate in the overall scheme of disease protection.

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