MINIREVIEW

Prolactin in Autoimmune Diseases (44251)

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Abstract. **The immune system is still regarded by many as autonomous, and prolactin (Prl) has traditionally been considered as a lactogenic hormone. Over the last 10 years, the total number of publications considering Prl is decreasing, while the number of those investigating its role in immunity sustainly increased. In addition to the pituitary gland, Prl-like peptides can be produced by activated leukocytes and fibroblasts. Elevated serum levels of Prl in (rat) adjuvant arthritis, (murine) collagen type II-induced arthritis, (murine and human) systemic lupus erythematosus (SLE), and (murine and rat) autoimmune type I diabetes may influence the outcome of the disease. It is suggested that mild hyperprolactinemia is a risk factor for the development of autoimmunity. This can occur under certain circumstances, for example adrenocortical deficiency or postpartum. In human SLE, Prl appears to favor the production of anti-double stranded DNA. While glucocorticoids would damp the immune reactivity, Prl constitutes a stimulatory link between the neuroendocrine and immune systems. Future directions should include: 1) multicenter projects for evaluation of the therapy with Prl-inhibiting compounds in SLE, considering for example the HLA-DRB1 *0301 status; and 2) the regulation of extra-pituitary Prl-like cytokines ("proliferins") (e.g., in rheumatoid arthritis synovium) and their role in the production of catabolic enzymes.** [P.S.E.B.M. 1998, Vol 217]

Growth hormone (GH), prolactin (Prl) and placental lactogens presumably exert similar effects, because they have similarities in peptide sequence. The molecular evolution of this peptide family has been a complex process. Prl has a growth-promoting effect on mammary tissue in some circumstances, but will also stimulate growth of other tissues (e.g., liver, prostate, lymphoma cells). Both GH and Prl are trophic hormones for rat liver. It is possible that both hormones stimulate growth indirectly by eliciting the release of hepatic growth factors (somatomedins and "synlactin" (1)). The role of Prl during lactation is well known. However, in 1980, Nicoll (2) described more than 80 functions for this hormone and proposed the name "versatilin." In mammals, Prl influences reproductive functions, calcium metabolism, and immune reactivity. Over the last 10 years, Medline search revealed that the general interest for this hormone is decreasing, with the exception of its role during the immune reaction. First, a thymus-derived rat lymphoma cell line (Nb2) has been found to be Prl-dependent in growth. In 1983, Shiu *et al.* (3) demonstrated the expression of high-affinity receptors for Prl on Nb2 cells. One year later, Russell *et al.* (4, 5) showed that normal mammal leukocytes also express a low number of Prl receptors. At this time, the immune system was regarded by most investigators as autonomous. Nevertheless, the neuroendocrine system modulates the activity of the immune system *in vivo;* this has been suggested by many experimental and clinical evidences [e.g., the beneficial effect of glucocorticoid injections in patients with rheumatoid arthritis (RA)]. However, the existence of a stimulatory axis was more difficult to accept. The pioneer works of Berczi, Nagy *et al.* (6, 7) in the rat model of adjuvant arthritis (AA) decisively contributed to the breakthrough. The prolifera-

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tion of lymphocytes in primary and secondary lymphoid organs depends on both Prl and GH. Prl can act directly on the organs and cells of the immune system or modulate their activity *via* other factors (e.g., gonadal steroids). Prl could allow an optimal response to antigen and cytokines. It is now accepted that a dysfunctional communication between the neuroendocrine and immune systems can contribute to the development of autoimmune diseases in rodents, chickens, and humans. An increased serum level of Prl probably influences the outcome of at least four autoimmune diseases: AA in rats, collagen type II-induced arthritis in mice, type I diabetes in rats and mice, and systemic lupus erythematosus (SLE) in mice and humans (reviewed in Refs. 8–11).

Increased Prolactin and Ongoing Autoimmunity

In rats, within 10 days after inoculation at the base of the tail, Freund's complete adjuvant (FCA, heat-killed *Mycobacterium butyricum* in paraffin oil) induces an arthritic disease, which is preceded by multiple neuroendocrine alterations. Before the development of AA, this is reflected by the enhanced activity of ornithine decarboxylase (ODC, EC 4.1.1.17)—a marker for stimulation—in the hypothalamus, anterior pituitary gland, and the increased production of pituitary hormones [e.g., Prl, luteinizing hormone (LH), and adrenocorticotrophic hormone (ACTH)] (12). In rodents, serum Prl also increases within 1 hr following injection of lipopolysaccharide endotoxin (13) or *Corynebacterium parvum* which, like FCA, are potent activators of macrophage functions.

On Days 3–4 after FCA, Prl mRNA accumulates in the pituitary gland (14). The diurnal rhythm of Prl secretion shows that increased release mainly occurs during the night (dark phase) (15). In contrast, the secretion (and probably production) of GH, testosterone, and insulin are decreased (15, 16). In the later phase of the latency period (Days 4–9), the serum level of corticosterone (CS) decreases (14). On Day 7 after FCA, hypothalamic β-endorphin level diminuates, and met-enkephalin concentration increases. Thus, an extensive activation of the neuroendocrine network becomes obvious during the latency period.

One of the first pieces of evidence suggesting the existence of a stimulatory axis was that hypophysectomy inhibits the development of AA, as well as other cell-mediated immune responses (e.g., delayed-type hypersensitivity, natural killer cells activity, and graft rejection (6, 7, 14, 17, 18)).

The Stimulatory Pituitary-Immune Axis

Hypophysectomy led to a rapid involution of the thymus and spleen, which is associated with a profound decrease in spontaneous DNA synthesis in these organs (19). Treatment with bovine GH, ovine Prl (but not with LH, follicle-stimulating hormone, or thyrotropin (TRH)) reverses the involution of the thymus and spleen. This treatment is capable of restoring immunocompetence, including AA (6, 7, 19). However, the effects of ovine Prl or bovine GH in rats may be due to their heterologous nature. Therefore, other models have been evaluated.

Genetically dwarf mice, which lack both Prl and GH, are also immunocompromised, and once again Prl and GH can correct the deficiencies. In dwarf mice, ectopically transplanted pituitary grafts produce enhancement of body weight gain, increase the low spleen and thymus weights, and restore "normal" blood leukocyte counts (particularly in males) (20).

Similarly, in hypophysectomized rats, pituitary implants under the kidney capsule restore AA (6, 7). Prl is probably the factor involved, since hypothalamic control of Prl secretion is predominantly inhibitory in nature, and mainly mediated by dopamine; thus, disruption of the hypothalamic-pituitary connection or ectopic pituitary transplantation can increase Prl release, whereas other pituitary hormones are not secreted under these conditions, due to lack of hypothalamic-releasing factors.

Hyperprolactinemia and Adaptive Changes

Surprisingly, Prl excess also may result in immunocompromise. Intact hyperprolactinemic rats (with pituitary implants) show a delay in the onset and a reduction in the severity of AA (14). Similarly, in intact rats, implantation of a GH- and Prl-secreting tumor produces immune suppression (21). In mice, destruction of the tuberoinfundibular region of the hypothalamus, which leads to an increased and uncontrolled secretion of Prl, also persistently suppresses immune functions (22).

In contrast, the mild hyperprolactinemia in hypophysectomized male rats (with pituitary implants) not only restores immunocompetence, but even results in an amplified autoimmune reaction (14). In intact female mice, the injection of homologous Prl makes collagen type II-induced arthritis worse (23, 24).

In humans, some data are available about the effect of chronic hyperprolactinemia (e.g., prolactinomas) on immune functions, but many clinical observations are contradictory. Similar to the observation in mice (22), the most consistent finding in human pathological hyperprolactinemia is the decreased number and/or activity of natural killer (NK) cells (25). Ten years ago, these results and other experiments using pharmacological agents (e.g., estrogen, haloperidol, metoclopramide, domperidone) to increase Prl secretion in intact rats—together with inconclusive *in vitro* experiments—seriously questioned the concept that Prl is an immunostimulatory hormone. The assumption has been made that long-lasting elevation of serum Prl concentration induces adaptive changes when the acute stimulatory effects on the immune system have subsided. However, the finding that mild hyperprolactinemia could be involved in the pathogenesis of SLE (26–33) was the beginning of a fascinating new field.

Elevated Prolactin in Systemic Lupus Erythematosus

NZB/NZW F1 mice (B/W) spontaneously develop an autoimmune disease similar to human SLE. As in murine collagen type II–induced arthritis (23, 24), the injection of homologous Prl accelerates autoimmunity in B/W lupus mice (34, 35). A modification in gonadal steroid metabolism seems not to be involved; it is postulated that Prl exerts a direct stimulatory effect on the immune system.

Fertile women are more susceptible to the onset of autoimmune diseases than men, but this increased susceptibility disappears after menopause. This is probably due to the hormonal changes. During pregnancy, in contrast to RA, SLE tends to flare more frequently. In SLE pregnancy, a longitudinal study has shown multiple associations between elevated Prl and clinical or laboratory markers of disease activity (36). Postpartum, in mice or humans, the situation seems clear: a variety of autoimmune diseases, including SLE (29) and RA (37), can be exacerbated. This period is accompanied by breast-feeding and increased Prl.

In SLE, recent studies found hyperprolactinemia only in very few patients (38–40) or reported a lack of correlation with disease activity (40–42). Many drugs, such as chloroquine and glucocorticoids, affect Prl secretion. In this type of investigation, the selection of the patients is primordial. Only patients without such drugs (even with low doses) can be evaluated; these drugs modulate both Prl release and production of autoantibodies. Because the therapy can affect the endocrine and immune parameters to a different extent, it alters the correlations.

An early study showed that, in mice, most IgA excreted by the mammary gland is regulated to a large extent by Prl (43). Later, it became apparent that hyperprolactinemia induced by suckling has an important stimulatory influence on IgM and IgA serum levels (44). Therefore, the influence of Prl on humoral immunity has been investigated in various conditions (i.e., in patients with connective tissue diseases (CTD) (30, 32, 45, 46)) and in other patients with chronic hyperprolactinemia (47, 48).

Mild Hyperprolactinemia and Autoantibodies

In young women (<50 years of age), hyperprolactinemia is associated with higher incidence of autoantibodies, including anti-double stranded DNA (ds DNA) and SSA/Ro (45, 47). Similarly, in hyperprolactinemic men, a high incidence of positive fluorescent antinuclear antibody tests has been reported (48). Thus, in SLE, it is not surprising to find a correlation between elevated serum Prl and antinuclear antibodies (30). In patients with relapsing SLE, increased serum Prl is associated with enhanced synthesis of IgG anti-ds DNA antibodies, and IgG antithyroid microsomal antibodies (TMA), as well as lymphocytopenia and erythrocytopenia (32). These correlations suggest a relation with disease activity. Anti-ds DNA autoantibodies are believed to be involved in the pathogenesis of SLE, and their presence in serum is of considerable diagnostic value. *In vitro,* Prl appears to induce the production of antibody by normal and SLE B-lymphocytes, particularly anti-ds DNA autoantibodies (49). In addition, elevated Prl can be associated with endometrial autoantibodies (46).

In humans, mild hyperprolactinemia also has been associated with autoimmune thyroiditis (50), multiple sclerosis (51), RA (52, 53), reactive arthritis (53), scleroderma (32, 54), polymyositis/dermatomyositis (32), as well as in blood donors with antibodies to human immunodeficiency (AIDS) (55) or hepatitis C-viruses (56). An increased Prl response to TRH occurs in women with RA and patients with reactive arthritis (53). In RA, a correlation has been reported between rising Prl levels and disease activity as measured by joint swelling (52). In other CTD than SLE, increased levels of Prl correlate with (IgG and IgM) anticardiolipin antibodies (32). These autoantibodies are associated with the "antiphospholipid syndrome" possibly implicating central nervous system (CNS) manifestations. In juvenile SLE, hyperprolactinemia is associated with psychosis or cognitive brain dysfunction, as well as an increased erythrocyte sedimentation rate, leukocytopenia, and lymphocytopenia (33).

Mild Hyperprolactinemia as a Risk Factor

In rats, a Prl peak precedes the onset of AA (12, 14–16); in mice, the injection of Prl exacerbates lupus (34, 35) and makes collagen type II-induced arthritis worse if treatment is performed during the induction stage of the disease, but not later (23, 24); in NOD mice, a chronic stimulation of Prl secretion with metoclopramide slightly aggravates the development of autoimmune diabetes (57); and in humans, increased Prl release predicts cardiac allograft rejection (58). In light of these observations, it can be hypothesized that certain events that provoke an acute hyperprolactinemia can increase immune reactivity, and therefore favor the subsequent development of an autoimmune disease.

Impressive epidemiological data support the concept that parity and breast-feeding (inducing pulses of Prl secretion) increase the risk of subsequent autoimmune diseases. The risk to develop RA is about six times higher in women with one child, than in the rest of the female population (including women with no or more than one child). In this group of patients, disease onset is 1–3 months postpartum (37) and breast-feeding is the rule (59). A more recent study confirmed that parity, and to a lesser extent breast-feeding, before RA onset worsened RA prognosis, whereas oral contraceptive pill use has a protective effect (60). Lactation raises the number of large granular lymphocytes in peripheral circulation (61). Prl and estrogens probably have a role in this effect. Similarly, the elevated incidence of hyperprolactinemia in women with breast implants is associated with higher risk of developing CTD (62). Recently, two patients with either autoimmune thyroiditis or dermatomyositis who presented with hyperprolactinemia preceding onset of the disease have been reported (63). Thus, experimental, clini-

cal and epidemiological data suggest that hyperprolactinemia constitutes a risk for the development of autoimmunity in certain situations.

Reduced Steroidogenesis as a Permissive Factor

Hypophysectomized rats show deficiencies in pituitary hormones and gonadal and adrenocortical dysfunctions. In such rats, the restoring effect of GH or Prl on immunocompetence (including AA) is antagonized by simultaneous administration of ACTH, which stimulates the production of CS by the adrenal cortex (6). Furthermore, the severity of AA is reduced in intact hyperprolactinemic rats (with pituitary implants), possibly due to increased plasma levels of CS that could contribute to a chronic suppression of immune reactivity; the catecholamine turnover and LH release also are increased. On the one hand, in FCA-treated rats (without pituitary implants), the Prl peak is followed by a period of decreased adrenocortical activity before the onset of AA (14, 64). On the other hand, after FCA, intact hyperprolactinemic rats show an increased baseline of production of proopiomelanocortin mRNA (POMC, the precursor of ACTH and endogenous opioid peptides), enhanced adrenocortical ODC activity, and increased serum CS levels (14).

In humans, one-third of patients with SLE showed decreased adrenocortical activity, reflected by the reduced serum levels of cortisol (am and pm) (32). The HPA-deficiency in SLE, as in FCA-treated rats (14, 64), could be due to immune modulators and/or to a genetic predisposition. Meanwhile, in most cases with other CTD, the activity of the HPA axis seems to be normal. However, in such patients, an elevated Prl/cortisol ratio is associated with increased serum levels of tumor necrosis-α (TNF-α) (32).

A decreased glucocorticoid tone may have a permissive role for appearance of an autoimmune disease. Similarly to the hypophysectomized male rats with a pituitary implant, castrated male rats show an increased severity of AA. Thus, together with the observations postpartum, it can be hypothesized that increased Prl plays an important role under certain circumstances (e.g., during periods of reduced production of steroids—glucocorticoids or gonadal steroids). The relation between Prl and steroids is more complex than expected. On the one side, glucocorticoids antagonize the stimulatory effect of Prl, whereas gonadal steroids modulate the immune reactivity differently in males and females. On the other side, Prl influences steroidogenesis and can antagonize certain inhibitory actions of glucocorticoids, but probably has little effect on the modulating actions of gonadal steroids.

Prolactin in a Bidirectional System of Communication

A system of bidirectional communication exists between the neuroendocrine system and the cells of the immune system. Certain regions of the central nervous system (pineal gland, neocortex, hypothalamus) receive information from the immune system. They probably react by emitting neuroendocrine signals, which in turn modulate immune reactivity.

In rats, muramyl dipeptide, an important component of FCA, directly stimulates midbrain and hypothalamic serotoninergic pathways, which are involved in the production of fever. During immune stimulation, serotonin (together with the noradrenergic pathway) might enhance Prl secretion through stimulation of the release of hypothalamic vasoactive intestinal peptide (VIP), which in turn act as a Prl-releasing hormone (12).

In human SLE, of course, the mechanism is different. Altered estrogen metabolism has been claimed to be involved in the enhanced Prl secretion (33). Alternatively, in both rats and/or humans, the following cytokines could augment pituitary Prl secretion: interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-6 (IL-6) and TNF-α (65, 66). In rats, IL-1 can act in minute doses on the CNS, presumably on structures near the third ventricle, to stimulate GH and Prl release (67). In healthy humans, the serum levels of Prl and interleukin-1 receptor antagonist (which inhibits the action of IL-1) correlate with each other (32), suggesting a counterregulatory mechanism. At least in rats, IL-1 and IL-6 increase the release of ACTH at the pituitary level, while their effect on Prl secretion remains controversial. The direct effect of TNF-α on anterior pituitary hormone release is dependent on the time of exposure and age of the animal (68). Furthermore, interferon-γ could inhibit stimulated Prl, GH, and ACTH release from rat anterior pituitary cells *in vitro* (69). Thus, Prl release could be positively and negatively influenced by multiple cytokines, probably at both hypothalamic and pituitary levels.

Prolactin-Like Cytokines

A fascinating development is the finding that the neuroendocrine and immune systems have the ability to influence each other through common or related peptides (e.g., oxytocin, endorphins, ACTH, TRH). One of the first findings from *in vitro* studies was that Prl antisera block a number of immune reactions. This led to the discovery that normal lymphoid cells produce "Prl-like" peptides, in mice (70–73), rats (74), and humans (75–79).

Mitogen-activated murine splenocytes were thought to release a large 43–44-kDa "Prl-like" molecule (70, 71). Recently, it has been reported that the Prl-like activity produced by mouse splenocytes is not similar to pituitary Prl (73). Specifically, the 43–44-kDa "Prl-like" peptide has now been identified as aldolase A—unrelated to Prl (80).

In female rats, chronic administration of cocaine decreases the *ex vivo* production of a Prl-like peptide, whereas the secretion of IL-2 is unaffected (74), demonstrating different regulation mechanisms.

In humans, Prl mRNA has been detected in thymus, spleen, tonsil, lymph node, and lymphoid tumors (77). Human thymocytes produce a 23-kDa protein that is identical to pituitary Prl (78). Similarly, activated human peripheral

blood mononuclear cells (PBMC) produce a big (60-kDa) Prl-like molecule. PBMC from patients with SLE show an increased "spontaneous" production of Prl-like peptides (79). These Prl-like molecules are released *in vitro* as two different molecular weight forms (24–27 and 11 kDa), and appear to be derived from B lymphocytes rather than T lymphocytes.

Prolactin and Primary Lymphoid Tissues

Hypophysectomized rats show normocytic anemia, as well as decreased DNA and RNA synthesis in the bone marrow (81, 82). These deficiencies are normalized after ectopical pituitary implantation, and the restoring effect can be blocked by additional treatment with anti-rat Prl antiserum. In fact, more than 90% of bone marrow cells express Prl receptors (83, 84). *In vitro,* Prl (as GH and placental lactogen) stimulates the proliferation of rat bone marrow cells (83) and can promote erythropoiesis and DNA synthesis in bone marrow progenitor cells (85, 86). Furthermore, in untreated rats, serum Prl correlates with ODC activity in a group of tissues showing peaks during the dark phase (i.e., liver, adrenal cortex, pancreas, and kidney) (16). After FCA, the increased serum level of Prl and enhanced bone marrow ODC activity are correlated during the dark phase. It is concluded that, in untreated control rats and FCA-treated rats, Prl plays different roles in bone marrow metabolism.

The thymus is a primary lymphoid organ in which bone marrow–derived T-cell precursors undergo a complex maturation process in the context of the thymic microenvironment, represented by nonlymphoid epithelial cells and extracellular matrix components. Hypophysectomized rats showed decreased thymus weight and decreased DNA and RNA synthesis in this organ (6, 19, 82). These defects can be restored after ectopical pituitary implantation. The relation between Prl and the thymus is bidirectional. The rat Prl receptor exists as two forms, short and long, and both are found in the rat thymus (86, 87). Thymic epithelial cells, the major component of the thymic microenvironment, bear Prl receptors. Prl stimulates their proliferation and increases their production of thymus hormones (thymulin and thymosin α_1) (88), which in turn influence the differentiation of thymocytes. Either ovine or rat Prl or GH induce a rapid expression of the proto-oncogene c-*myc* in rat thymus (19). The protein induced by the c-*myc* gene acts as an intracellular competence factor, which enables the cell to synthesize DNA and eventually undergo mitosis. The protein products of several proto-oncogenes (e.g., c-*fos,* c-*jun* and c-*myc*) are known to interact with the regulatory region of the ODC gene. After FCA, the greatest increase of ODC activity in lymphoid tissues occurs in the thymus (16); this enhancement is Prl-dependent.

In turn, thymic epithelial cells could secrete Prl-releasing factors (e.g., thymic neuroendocrine–releasing factor, thymosin fraction V/MB-35, thymomodulin) (89–93). They can influence LH and Prl release, through stimulation of LH-releasing hormone and interaction with opiate receptors.

Seventy-five to > 90% of rat thymocytes have Prl receptors (84, 94, 95). A minority of double negative ($CD4^-$, $CD8^-$) cells and single positive CD4 cells highly express the Prl receptors. In mice, Prl participates in two interrelated mechanisms: the regulation of peripheral single positive T lymphocytes ($CD4^+$ or $CD8^+$) and the maintenance of thymocyte viability during the double-positive stage ($CD4^+$, $CD8^+$) of intrathymic differentiation (96). Prl preferentially stimulates the development of $CD4^+$ T-helper lymphocytes. Thymocytes produce a Prl-like cytokine (78), which can influence their maturation directly in an autocrine fashion or indirectly through its effect on thymus epithelial cells and the production of thymus hormones.

Prolactin and Secondary Lymphoid Tissues

In rodents, all B lymphocytes and monocytes/macrophages, as well as the majority of T lymphocytes from lymph nodes express Prl receptors (97). After FCA, lymph node ODC activity reaches its maximum level more rapidly, compared to the activation in the spleen (16). The increase of ODC activity is Prl-dependent. It can be hypothesized that, as in footpad immunization (99), a rapid, but transient, enhancement of Prl receptor expression occurs in the draining lymph nodes.

Hypophysectomized rats show a decreased spleen weight, as well as reduced DNA and RNA synthesis (6, 19), that can be restored with ectopic pituitary implants. Twenty percent of the rat splenocytes express Prl receptors, including the majority of B lymphocytes and monocytes/macrophages (97), and minorities of $CD4^+$ and $CD8^+$ T lymphocytes (87, 94). The expression of Prl receptors is developmentally regulated in the spleen (100). Prl at a physiological dose stimulates the proliferation of rat splenocytes (101) and induces a rapid expression of c-*myc* in rat spleen (19). About 40% of the rat splenocytes respond to Prl, probably including more $CD8^+$ than $CD4^+$ T lymphocytes (102). As for other lymphoid tissues, the FCA-induced enhancement of spleen ODC activity is Prl-dependent (16).

Prolactin and Peripheral Blood Leukocytes

Low levels of Prl receptors are expressed on human and rodent lymphocytes (4, 5, 77, 83, 94, 95, 103), on pig and rodent monocytes/macrophages (83, 94, 95, 97, 104–108), as well as on human neutrophils (109). In rats, an average of 80% of peripheral blood mononuclear cells, comprising all B lymphocytes, all monocytes, and 60%–75% of T lymphocytes express the Prl receptors (83, 94, 95). Regarding T-cell subsets, similar percentages of Prl receptor positive cells are observed in the $CD4^+$ and $CD8^+$ compartments. The density of receptors is lower on T lymphocytes than on B cells and monocytes. Some imbalances of Prl/Prl-receptor interaction may exist in autoimmune situations (94). Thus, in contrast to the pattern observed in normal mice, the fre-

quencies of Prl receptor–bearing T lymphocytes in blood, as well as the density of Prl receptors per cell, increase with age in the autoimmune W/B lupus mice.

In mice, elevated Prl favors the development of $CD4^+$ T-helper lymphocytes (96) and appears to augment the number and function of antigen-specific T lymphocytes in the periphery (110). In healthy women, plasma Prl correlates with the numbers of peripheral blood B lymphocytes and $CD4^+$ T lymphocytes (111). Among peripheral blood $CD4^+$ T lymphocytes, elevated Prl is associated with more transient cells ($CD45RA^+$, $CD45RO^+$) in women and more naive cells ($CD45RA^+$, $CD45RO^-$) in men (112). After menopause, the decrease in Prl levels appears to be involved in the reduction of activated lymphocytes, thus lowering the risk for the onset of autoimmune diseases (111). In major depression, a correlation has been reported between plasma Prl and soluble IL-2 receptors, suggesting a relation with lymphocyte stimulation (113). Furthermore, Prl induces immunoglobulin synthesis by PBMC (49). Activated PBMC have the potential to produce Prl-like cytokines (76). In healthy men and cancer patients, depending on the concentration, Prl *in vitro* produces stimulation (low dose) or inhibition (high dose) of resting NK cells (112).

Prolactin and the Control of Proliferation

Prl stimulates ODC and protein kinase C activities, which are pivotal enzymes in the differentiation, proliferation, and function of cells of the immune system. As reported for the Nb2 T-lymphoma cells, Prl may regulate lymphocyte proliferation responses by stimulating the transcriptional induction of various proto-oncogenes, including c-*myc,* c-*Raf*-1, *pim*-1, p21*ras,* p59*fyn* and p95*vav* (c-*Raf*-1 and *pim*-1 encode for serine/threonine protein kinases, p59*fyn* for a tyrosine protein kinase, p21*ras* and p95*vav* for other signaling proteins) (19, 114–116). Furthermore, the expression of Prl receptors, and therefore the capacity to respond to Prl, depends on the activation state of the cells (86, 117). From *in vitro* experiments, it can be recognized that Prl: 1) acts as a co-factor during lymphocyte activation and proliferation (86, 103, 120–123); and 2) acts in synergy with cytokines (e.g., interferon-γ (INF-γ) (104, 122–126) and IL-2) (127–130). Purified or recombinant Prl employed *in vitro* must be free of contaminant (e.g., endotoxin) (131).

Prolactin and the Cytokine Cascade

Kupffer cells are an important source of pro-inflammatory cytokines and contribute to systemic inflammatory response following hemorrhagic shock. Prl is involved in blunting the inflammatory response associated with cell and organ depression. On the one side, Prl decreases the expression of IL-1β, IL-6 and TNF-α in Kupffer cells (132). Prl also reduces the release of IL-1α from murine splenocytes stimulated with staphylococcal enterotoxin A by 80%, but has no effect on INF-γ release (125). In contrast, Prl inhibits the release of INF-γ from murine splenocytes stimulated with porins of *Salmonella typhimurium* by 20%, without influence on the production of IL-1α (126). On the other hand, in mice with toxoplasmosis (*T. gondii*), treatment with Prl protects against death possibly through the stimulation of TNF-α production (133). Prl also enhances the production of INF-γ by PBMC (119). In humans, domperidone *in vivo* induces an elevation of plasma Prl and increases the *ex vivo* production of INF-γ by leukocytes treated with Newcastle disease virus (124). Thus, Prl differentially affects the release of cytokines *in vivo,* depending on the circumstances.

Prl increases proliferation in response to IL-2 and mitogens (120). Prl can induce IL-2 receptor (IL-2R) expression in murine splenocytes and lymph node T lymphocytes, as well as in peripheral blood T- and B lymphocytes (121, 127, 130, 134). Prl-treated T lymphocytes produce bioactive IL-2 in a dose- and time-dependent fashion (122). In turn, IL-2 induces the translocation of the Prl/receptor complex to the nuclear periphery (127). These observations suggest that Prl is involved in the IL-2 positive feedback loop, that it stimulates lymphoid organs, and under certain circumstances, can increase immune reactivity. Positive feedback systems are relatively rare; for example, oxytocin released after mechanical distention of the reproductive tract in turn stimulates uterine contraction. Both Prl and IL-2 activate the MGF-STAT5 transcription factor and specific gene expression in Nb2, YT, and C196 T lymphocytes (135, 136). MGF-STAT5 was originally identified as a mammary gland factor induced by Prl in lactating breast cells. The MGF-STAT5 DNA recognition site is the same as the INF-γ-activated site (GAS) in the interferon regulatory factor 1 gene. The GAS element is necessary and sufficient for transcriptional induction by both IL-2 and Prl in T lymphocytes.

The production of IL-2 and INF-γ are reduced in lupus-mice, and the expression of IL-2R on spleen $CD4^+$ T lymphocytes is decreased. Since the injection of Prl increases the expression of IL-2R, it can be hypothesized that the deleterious effect of the hormone (34, 35) is mediated, at least in part, by the restoration of "normal" IL-2R levels. The suggestion that Prl may mediate the expression of normal IL-2R levels in animals deficient in the expression of the receptor on $CD4^+$ T lymphocytes has been demonstrated using the Snell dwarf mouse (137).

In light of these observations, it is less surprising that elevated Prl can favor the development of an ongoing autoimmune reaction. With the exception of possible antagonistic effects on NK cell activity (22, 25, 114), Prl and IL-2 seem to act in synergy on cells of the immune system. These *in vivo* and *in vitro* experiments justified the use of Prl-blocking agents (e.g., bromocriptine) in the therapy of rodent (139–141) and human autoimmunity (122, 142).

Future Directions

In health, the neuroendocrine-immuno regulatory network is fundamental to the host's defense and to the transfer of immunity to offsprings; the network also plays important roles in intestinal physiology and in tissue regeneration,

healing, and reproduction. Defects in regulatory processes, which are involved in immune disorders and inflammatory diseases, thus may lie at least in part in the neuroendocrine system (8–11, 143, 144).

Cytokines are capable of inducing fever and altering neurotransmitter activity in the brain and hormone secretion by the pituitary gland (12, 143). During the latency period after FCA, the CNS-mechanisms leading to the increase of Prl secretion are unknown. Prl could be released by the pituitary after stimulation with neuropeptides (serotonin, TRH, substance P, or VIP), and could be modulated by several cytokines (66, 67, 145, 146). IL-1α and β are potent modulators of neuroendocrine processes, probably increasing the release of Prl (66, 146).

The spontaneous hypertensive rats (SHR) did not develop AA. The proliferative response of SHR splenocytes is decreased compared with other strains (Wistar-Kyoto and Fisher F-344) (147). Addition of Prl, but not IL-1 or IL-2, to the cultures increases proliferation. The injection of Prl or ectopically transplanted pituitary grafts in SHR rats could allow the development of AA. This should be tested; however, multiple other factors probably are involved in the resistance of SHR rats to AA.

The nature of the adaptive changes occurring during long-lasting elevation of serum Prl concentration (e.g., increased CS) has to be elucidated. In addition to the change in adrenocortical metabolism, the possible immunomodulatory effects *via* catecholamines and gonadal steroids should not be neglected. The distinction between the effects of Prl and sex steroids, and their interactions, on immune reactions *in vivo* is unclear. Prl is a natural immune enhancer, whereas progesterone and androgens are natural immune suppressors, and estrogens can act in both ways. For example, both bromocriptine and estradiol protect the mouse from postpartum flare of type-II collagen-induced arthritis (148, 149). A better understanding of the role of Prl and its interactions with other immunoregulatory hormones may lead to novel therapies.

In SLE, the disease exacerbation during oral contraceptive therapy, pregnancy, and the postpartum period has been attributed to altered serum concentration of gonadal steroids, especially estrogens. Often it has been overlooked that gonadal steroids and their metabolites stimulate Prl production and release. In this context, the comparison between the implications of elevated Prl levels in pregnant patients with SLE and in those patients with postpartum exacerbation are particularly interesting. Pregnancy is associated with elevated productions of both gonadal steroids and Prl. The postpartum period, however, is characterized by low plasma gonadal steroids and high Prl levels, necessary for the initiation of lactation.

The association between mild hyperprolactinemia and autoantibodies raises several questions. A triangular relationship could exist between increased Prl, anti-ds DNA, and the development of glomerulonephritis (32). Alternatively, an alteration in renal metabolism could be responsible for elevated serum Prl. Furthermore, increased levels of antithyroid autoantibodies also have been reported in patients with prolactinoma. The association of Prl and TMA raises the question of whether the hormonal manifestations are related to discrete autoimmune thyroid diseases.

Time courses of serum Prl levels in various autoimmune diseases would confirm that mild hyperprolactinemia is a risk factor. Possibly, the diurnal rhythms of cortisol, GH, and Prl are altered in patients with SLE and RA (as in rats with AA). Since Prl has a diurnal rhythm of secretion with a peak at about 02.00 hr, it may contribute to the nocturnal worsening of RA (150). In such patients (but not in patients with osteoarthritis), both Prl and estradiol correlate with interpersonal stress, depression, and clinician ratings of disease activity (151). In men with RA, increased Prl could be involved in the disturbance of sexual functions (152). In women, the risk of developing RA seems to be associated with reduced fecundity and with breast-feeding (37, 59, 60, 153); these apparently contradictory risk factors can be explained by their association with high Prl concentrations. The only consistent genetic association with RA is for genes encoded in the HLA complex, namely HLA-DR4, which enhances disease susceptibility, and HLA-DRB1 alleles *0401 and *0404, which modify disease severity in rheumatoid factor positive patients. The effects of breast-feeding and nulliparity are modified by HLA-DR4 status, suggesting an interaction between genetic and reproductive risk factors in the etiology of RA (154). As observed in reactive arthritis (55), the Prl response to TRH is increased in women with RA (155), particularly in HLA-DR4 cases (154). The Prl gene is in close proximity to the HLA region on the short arm of chromosome 6. The associations between HLA-DR4 (and possibly the DRB1 *0401 allele) and reproductive risk factors could be due to linkage disequilibrium between the HLA complex, an abnormally regulated Prl gene expression, and Prl protein polymorphism, which could be important in relation to pathological processes (156).

It has been claimed that leukocytes [and possibly synovial fibroblasts (157)] are capable of synthesizing and responding to Prl-like peptides in an autocrine, paracrine, and endocrine fashion (74–79). It has been shown that the Prl receptor is a member of the superfamily of cytokine receptors (158) and is expressed by various leukocytes and fibroblasts (94–101, 157). However, the concept of Prl-like cytokines (which collectively could be called "proliferins") needs to be explored in more detail. In patients with inflammatory arthritis, the synovial fluid levels of Prl-like immunoreactivity parallel the concentrations in the blood (159). The net biopentency of circulating Prl-like immunoreactivity could reflect the sum of activity of structurally and functionally diverse peptides. Both size and charge variants of pituitary Prl influence its biopotency; probably, the biological functions of the different "proliferins" also are heterogenous.

Prl-like mRNA and/or "proliferins" are detected in

human endometrium, myometrium, and connective tissues, as well as in epithelial cells and vascular endothelial cells. Murine 3T3 fibroblastic cells stimulated with growth factors, as well as human fibroblasts obtained from the rectus fascia, produce proliferins. Female gonadal steroids favor the decidualization of endometrial fibroblasts, induce cell aggregation and the expression of proliferins. Extrapituitary Prl-like peptides are part of a negative feedback loop; they inhibit the proliferation of endometrial fibroblasts and capillary endothelial cells (*via* antagonism of fibroblast growth factors). Dermal fibroblasts also produce a Prl-like peptide (157). These observations justify the question of whether or not rheumatoid synovial fibroblasts and/or T-lymphocyte infiltrates produce Prl-like peptides. Preliminary results revealed that Prl-like immunoreactivity is found in the area of human RA synovium where T lymphocytes are accumulating. Proliferins produced by infiltrating T lymphocytes could induce the proliferation of synovial fibroblasts and could stimulate the production of pro-inflammatory cytokines and enzymes involved in the destruction of articular cartilage. It is attractive to consider that proliferins may be involved in the local control of cell proliferation and antibody production, in both the T-cell-dependent pathway of inflammation and T-cell-independent pathway of joint destruction and, finally, in the regulation of angiogenesis.

In turn, cytokines could regulate the expression of proliferins. Exposure of primary decidual cell cultures from term pregnancies to TNF-α, IL-1 or IL-8 inhibit the production and release of a Prl-like peptide (160). These observations suggest that pro-inflammatory cytokines also may have a paracrine role in the regulation of proliferins.

Monokines could modulate Prl secretion; in turn, macrophages have Prl receptors, and the hormone influences their tumoricidal and bactericidal activities. The modulating effects of neuroendocrine factors, including Prl-like peptides, on (monocytic-derived) dendritic cells in autoimmunity could be an exciting field of future investigations (161).

Interestingly, mice receiving the Prl release-inhibiting compound, bromocriptine, exhibit a drastic reduction in the serum level of antibody induced by orally-given bovine rotavirus (162). Together with the increased serum levels of Prl in patients with AIDS and hepatitis C (55, 56), the link between Prl and induction of proto-oncogenes, and the possible association of SLE with retroviral sequences, it can be hypothesized that: 1) Prl and proliferins allow the optimal response to viral antigens; but 2) in SLE, they could exacerbate the ongoing autoimmune process. In some studies, hyperprolactinemia was detected in very few SLE patients, and a lack of association with clinical or laboratory markers of disease activity has been reported (38–42, 163). The therapy is certainly not the only cause of discrepancy, and it is not easy to explain the differences. High Prl levels have a role in autoimmunity, at least in a subset of patients. In women with SLE, there may be a linkage disequilibrium between the HLA-DRB1 allele *0301 and microsatellite marker alleles close to the Prl gene (156). Future studies should include a larger sample size and consider the differences between gender and HLA-DRB1 status. Additional multicenter projects would clarify the situation.

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