

REVIEW

The hypothalamic–pituitary–gonadal axis: immune function and autoimmunity

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Abstract

GnRH and sex steroids play an important role in immune system modulation and development. GnRH and the GnRH receptor are produced locally by immune cells, suggesting an autocrine role for GnRH. Experimental studies show a stimulatory action of exogenous GnRH on the immune response. The immune actions of GnRH *in vivo* are, however, less well established. Oestrogen and androgen receptors are expressed in primary lymphoid organs and peripheral immune cells. Experimental data have established that oestrogens enhance the humoral immune response and may have an activating role in autoimmune disorders. Testosterone enhances suppressor T cell activity. Although there are some clinical studies consistent with these findings, the impact of sex steroids in

autoimmune disease pathogenesis and the risk or benefits of their usage in normal and autoimmune-disordered patients remain to be elucidated. There are neither experimental nor clinical data evaluating functional GnRH–sex steroid interactions within the human immune system, and there is a paucity of data relating to GnRH analogues, hormone replacement therapy and oral contraceptive and androgen action in autoimmune diseases. However, a growing body of experimental evidence suggests that an extra-pituitary GnRH immune mechanism plays a role in the programming of the immune system. The implications of these findings in understanding immune function are discussed.

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Introduction

Although interactions between the nervous system, the immune system and the hypothalamic–pituitary–adrenal axis have been extensively investigated, there is recent evidence suggesting that the hypothalamic–pituitary–gonadal (HPG) axis may also modulate immune function.

Earlier observations established that compared with males, females display a strikingly increased incidence of autoimmune diseases, and despite intensive investigations, the mechanism of this sexual dimorphism remains elusive. There are other examples of sexual dimorphism in immune function, however. Thus, women have higher plasma immunoglobulin M levels, a difference most accentuated post-pubertally (Butterworth *et al.* 1967, Lichtman *et al.* 1967). Animal models also confirm the sexual dimorphism of both antibody- and cell-mediated immune responses, with females demonstrating greater responsiveness (Eidinger & Garrett 1972, Weinstein *et al.* 1984).

Gonadotrophin-releasing hormone (GnRH) and sex steroids are strongly implicated in the development and

modulation of the immune system (Schuurs & Verheul 1990, Mann *et al.* 1994). Both GnRH and the GnRH receptor (GnRH-R) are expressed in immune subsets (Marchetti *et al.* 1989a, Chen *et al.* 1999, Silveira *et al.* 2002), but hitherto, the physiological role of this extra-pituitary immunological axis has been poorly investigated. Sex steroids have binding sites in primary lymphoid organs and peripheral immune cells (Kawashima *et al.* 1992, Suenaga *et al.* 1998), implicating both GnRH and sex steroids in immune system modulation, acting through a local autocrine way and/or through HPG axis activation.

The interrelationship between GnRH and/or sex steroids and the immune system should be explored for several reasons. First, there are an increasing number of women using sex steroids, hormone replacement therapy (HRT) or oral contraceptives worldwide. Secondly, in recent years GnRH agonists have become widely used in a variety of disorders such as precocious puberty, endometriosis and prostatic carcinoma. Thirdly, the underlying mechanisms of sexual dimorphism in autoimmune conditions have not been established. Finally, the potential immune modulatory and programming actions of

currently used drugs (especially GnRH analogues) could be investigated in severe immune deficiency conditions such as HIV infection, bone marrow transplantation (BMT) and after high-dose chemotherapy in cancer patients when immune reconstitution has crucial importance.

This review focuses on the impact of GnRH and sex steroids in both immune system modulation and autoimmune disorders.

GnRH and the immune system: experimental studies

Expression and binding sites

Specific GnRH-binding sites have been reported in cultured porcine lymphocytes (Standaert *et al.* 1992), rat thymus (Marchetti *et al.* 1989a, Morale *et al.* 1991) and in rat spleen (Batticane *et al.* 1991). Maier and colleagues first reported the expression of thymic GnRH (identical to hypothalamic GnRH) in neonatal rat pups using PCR-based technology (Maier *et al.* 1992, Maier & Blalock 1994). Expression of GnRH mRNA was subsequently demonstrated in porcine immune cells (thymus, spleen, peripheral lymphocytes) (Weesner *et al.* 1997), in murine thymus and spleen (Jacobson *et al.* 1998) and in a rat immature T cell line (Wilson *et al.* 1995b).

Observations in human cells yielded similar results. Immunoreactive and bioactive GnRH has been demonstrated in human peripheral T cells (CD4+, CD8+) and in a leukaemic cell line (Jurkat) similar to T lymphocytes (Azad *et al.* 1993, 1997); GnRH and GnRH-R mRNA transcripts are also demonstrable in human peripheral lymphocytes and in B lymphocytes (Chen *et al.* 1999, Silveira *et al.* 2002). GnRH-R expression is up-regulated by native GnRH, suggesting an autocrine function for this hormone in immune cells (Chen *et al.* 1999). Our laboratory has also confirmed not only the expression and presence of GnRH type-I but also of GnRH type-II transcript and peptides in human peripheral mononuclear cells and in B lymphocytes using RT-PCR, as well as by immunocytochemistry. Moreover, in an ongoing study, we have demonstrated the regulation of B lymphocyte proliferation by local and exogenous GnRH-I (F Tanriverdi & PMG Bouloux, unpublished observations). Although the clinical significance of GnRH mRNA expression and the presence of GnRH in immune cells is not clear yet, the activity of GnRH within the immune system appears distinct from its role within the pituitary. It has been established that GnRH concentrations are low in the portal system (Eskay *et al.* 1975) and further diluted (10–35 pg/ml) and metabolised in the general circulation; furthermore, no significant change in plasma GnRH concentration has been detected during the menstrual cycle (Aksel 1979). Hypothalamic GnRH is therefore unlikely to represent the endogenous ligand for immune cells. Rather, recent findings support a

probable physiological immune action of extra-pituitary GnRH (Chen *et al.* 1999, Jacobson *et al.* 1999a).

GnRH and immune system development

The direct involvement of GnRH in thymus maturation and development of cell-mediated and humoral immune responses in rats was first demonstrated by Morale *et al.* (1991). Treatment of neonatal female rats with a GnRH antagonist blocked the thymocyte proliferative response, decreased thymus weight, reduced CD4+ T lymphocyte subsets and decreased the antibody response to antigenic stimulus in adult age (3-month-old) animals. These findings clearly showed the important physiological role of GnRH in immune system development and the long-term effects of GnRH blockage on immune function (Morale *et al.* 1991). Recently, the potential role of GnRH in prenatal and postnatal programming of immune cells has been confirmed by Zakharova *et al.* (2000), within the rat embryo thymus. Intra-uterine injections of a GnRH antagonist and antibodies to 20-day-old rat fetuses were shown to suppress concanavalin-A-activated thymocyte proliferation, suggesting a prenatal action of GnRH in modulating T cell immune development and thymocyte proliferation.

GnRH antagonist injection into primates (*Callithrix jacchus*) within the first days after birth decreased the number of B and T lymphocytes in the thymus and spleen (Mann *et al.* 1998) and the number of circulating lymphocytes observed up to 5 years of age (Gould *et al.* 1998). By contrast, a single i.m. injection of a long-acting GnRH agonist in prepubertal mice produced a general suppression of leukocyte maturation in primary lymphoid organs (Rao *et al.* 1993). In a further study by the same authors, 50 µg of a GnRH analogue (Lupron depot) were administered to different strains of mice; a stimulatory effect of the GnRH agonist on stem cell maturation in lymphoid tissue was demonstrated in some strains (Rao *et al.* 1995). Taken together, these data suggest that GnRH has a modulatory effect in humoral and cellular immune system development, an effect mediated via a classical GnRH-R type-I.

Immune effects of GnRH

Studies in humans and rodents demonstrate that GnRH possesses potent stimulatory immune actions. In both the ageing male and female rat, the parallel decreases in both thymic GnRH-binding sites and thymus weight are reversed by chronic (45 day) potent GnRH analogue (GnRH-N-ethylamide) treatment (Marchetti *et al.* 1989b). Interleukin-2 (IL-2) is an important cytokine in proliferation and/or activation of T and B cells; its receptor (IL2R) expression was stimulated in rat thymocyte and splenocyte cultures incubated with native GnRH and its analogue in the absence of any other mitogenic stimulus,

Table 1 Summary of experimental data showing the effects of GnRH on the immune system

Expression of GnRH and GnRH-R	Both expressed in primary lymphoid organs and peripheral immune cells including human lymphocytes
Immune system development	Involved in immune system development No data in humans
Immune effects	Potent immune-stimulating action Increases the levels of IL2R, IFN- γ , T helper cells
Impact on autoimmunity	Potential to exacerbate autoimmune disease Effect independent from gonadal steroids
Sexually dimorphic immune response	Different expression of GnRH-R and/or G proteins in males and females?

an effect reversed by a GnRH antagonist (Batticane *et al.* 1991). In another study, native GnRH significantly enhanced *in vitro* interferon- γ (IFN- γ) production by human peripheral mononuclear cells (Grasso *et al.* 1998). In an animal model of immunodeficiency, total IgG levels and CD4+ lymphocytes increased after 7 weeks of native GnRH administration (Jacobson *et al.* 1999b), invoking a direct stimulatory effect of GnRH on immune function.

One corollary of this is that GnRH may constitute one of the factors in thymic immune reconstitution. Thymopoiesis is an essential process for establishing the peripheral T cell pool in early life. The capacity of the thymus to export new T cells to the periphery begins to decrease at age 1 year (Steinmann *et al.* 1985), and although there is an age-dependent decline in thymic function (thymic ageing), T lymphopoiesis remains active in the human throughout life (Flores *et al.* 1999, Jamieson *et al.* 1999, Poulin *et al.* 1999). Thymic function gains additional importance in immune deficiency conditions such as HIV infection, BMT and the leukopenic period after high-dose chemotherapy, when immune reconstitution is required. Sempowski *et al.* (2000) identified a group of cytokines such as IL-2, IL-10 and IL-14 whose expression levels decreased during thymic ageing. GnRH administration has been shown to increase rat thymic expression of IL2R in rat thymus and circulating CD4+ levels in an animal model of immunodeficiency (Batticane *et al.* 1991, Jacobson *et al.* 1999b). Even though data in humans are sparse, GnRH and GnRH analogues increase IL2R expression in lymphocytes after 24 h exposure, in a dose-dependent manner (Chen *et al.* 1999). While the clinical significance of this short-term effect on lymphocyte function is not clear yet, these experimental data underline the potential implication of GnRH and GnRH analogues in immune reconstitution. Could GnRH analogues be used as adjunctive therapy in such indications as HIV or BMT?

Impact of GnRH on autoimmunity and gender-specific immune response

The pathogenesis of autoimmune diseases is complex, and hormonal, genetic, psychological and environmental

factors contribute to the development and activation of these conditions. Jacobson *et al.* (1994) suggested that GnRH might play a role in the exacerbation of autoimmune disorders. They assessed disease severity in intact and castrated, male and female, systemic lupus erythematosus (SLE)-prone (SWR \times NZB) F1 hybrid mice during treatment with a GnRH agonist (native decapeptide) and antagonist (Nal-Glu) (100 μ g s.c. injections with GnRH agonist or antagonist, six times weekly). After 12 weeks of GnRH antagonist administration, total serum levels of IgG and anti-DNA antibodies were reduced, severity of renal disease decreased and survival improved significantly. Interestingly these effects were noted in intact and castrated mice in both sexes, suggesting that the protection conferred by GnRH antagonists was independent of gonadal steroids (Jacobson *et al.* 1994). On the other hand, they also demonstrated sexually dimorphic actions (disease severity was higher in females) of GnRH agonists even in gonadectomised mice. Based on their data, it was hypothesised that differences in responsiveness to GnRH might be due to gender differences in expression of GnRH-R or due to gender differences in expression of G proteins (Jacobson *et al.* 1999a, Jacobson 2000, Walker & Jacobson 2000). These explanations might partially explain gender differences in immune function. However, could these observations explain the different expression of GnRH and/or G proteins between sexes? The relationship between GnRH and sex steroids in immunomodulation will be addressed later.

In summary, GnRH seems to be directly involved in cellular and humoral immune development. However, the role of GnRH in immune modulation and autoimmune responses during adulthood is still under investigation; current known actions of GnRH in the immune system are summarised in Table 1.

Sex steroids and the immune system: experimental studies

Several experimental studies suggest that sex steroids influence immune cell development in primary lymphoid tissues (bone marrow and thymus) and, in addition, have

immunomodulatory effects on both peripheral T cell and B cell subsets in adult life.

Receptor expression in primary lymphoid organs

Early studies showed that in rats and mice, castration induced thymic enlargement, an effect reversed by androgen replacement, suggesting the presence of specific androgen-binding sites in thymic cells (Fitzpatrick *et al.* 1985, Olsen *et al.* 1991). Androgen receptors (ARs) have been demonstrated in thymocytes by Western blot and flow cytometry in mice (Viselli *et al.* 1995) and radioligand binding assays in humans (Kovacs & Olsen 1987). Several studies have also demonstrated oestrogen receptor (ER) expression in thymocytes and thymic epithelial cells in both mice and humans (Nilsson *et al.* 1986, Kawashima *et al.* 1992). Oestrogen interacts with two distinct nuclear receptors, ER- α and ER- β . The human (Mosselman *et al.* 1996) and mouse (Tremblay *et al.* 1997) ER- β homologues have recently been cloned. In the male ER- α knockout mice, the role of ER- α in thymic development and oestrogen-induced thymocyte phenotypic shift has been clearly demonstrated (Staples *et al.* 1999), and the requirement for ER- α in thymic development in both sexes during the postnatal period established (Yellayi *et al.* 2000). A recent study in ER- α - and/or ER- β -disrupted mice established that ER subtypes have different roles in females and males. ER- α was shown to be essential for thymic and splenic development in males, whereas expression of ER- β was required for oestrogen-mediated thymic cortex atrophy and thymocyte phenotypic shift in females (Erlandsson *et al.* 2001).

In mammals, B cells are produced in the bone marrow. Studies in mice have confirmed effects of androgens on developing B cells mediated through ARs, expressed in bone marrow stromal cells (Bellido *et al.* 1995, Olsen *et al.* 2001). These cells have also been shown to contain functional ER (Bellido *et al.* 1993, Smithson *et al.* 1995). Recent studies have demonstrated that B cell lymphopoiesis is normal in female ER- α -disrupted mice (Smithson *et al.* 1998), suggesting that ER- β might be responsible for regulation of B cell formation in bone marrow (Kincade *et al.* 2000).

Receptor expression in peripheral B and T cells

As in primary lymphoid organs, ER transcripts were identified in mature peripheral B and T lymphocytes both in healthy subjects and in patients with SLE (Suenaga *et al.* 1998). By contrast, ARs have not been documented in mature peripheral T and B lymphocytes (Olsen & Kovacs 2001), rendering genomic actions of androgens unlikely. Nevertheless it was demonstrated that testosterone induces calcium influx, presumably via non-genomic surface receptors, in activated murine splenic mature T cells

(Benten *et al.* 1997, 1999); the functional repercussions of this have not been established.

In summary, oestrogen and ARs are expressed in primary lymphoid organs and ERs are expressed in mature peripheral B and T cells, supporting the action of sex steroids in immune system development and modulation.

Effects of androgens on cellular and humoral immunity

Several studies in both animals and humans have been performed in an attempt to understand the influence of sex steroids on the immune system. Androgens exert considerable effects on the size and composition of the thymus. Removal of androgens by castration resulted in thymic enlargement even in old rats and androgen replacement reversed this effect (Greenstein *et al.* 1986). In one study, testosterone replacement in castrated male mice caused thymic regression, with a shift towards expression of mature thymocytes and predominance of the suppressor/cytotoxic CD4-CD8+ phenotype over the helper CD4+CD8- phenotype (Olsen *et al.* 1991). Mechanisms of androgen-induced thymus involution are incompletely understood, but decreased cell proliferation, changes in cell trafficking and increased apoptosis are some of the possible mechanisms involved. The role of apoptosis was suggested by one study from Olsen *et al.* (1998), in which a single dose of testosterone markedly decreased thymic size within hours in castrated mice; increased DNA fragmentation was shown, suggesting thymocyte apoptosis. Recently, it has been shown that testosterone specifically targets double positive (CD8+CD4+) thymocytes for apoptosis via upregulating tumour necrosis factor- α production (Guevara Patino *et al.* 2000). On the other hand, documentation of classical AR expression in peripheral T cells has not been reported but the net effect of androgen action (direct or indirect) seems to be an enhanced suppressor effect (Olsen & Kovacs 1996).

The number of pre-B cells in bone marrow and mature peripheral B cells increases in male mice after castration (Wilson *et al.* 1995a). It has been reported that treatment of mice with dihydrotestosterone (a non-aromatisable androgen) suppressed the expansion of lineage precursors (IL-7 responsive precursors) when added to short-term co-cultures of lymphocytes and stromal cells (Medina & Kincade 1994, Smithson *et al.* 1998).

There are conflicting data concerning the effects of castration and androgens on peripheral B cells. CD5+ cell subsets have been implicated as an important source of autoantibodies. An early study showed that this B cell subset did not expand in spleens of castrated male mice, whereas oestrogen treatment in female mice augmented the CD5+ cell subset activity (Ansar *et al.* 1989), suggesting the greater importance of oestrogen (rather than absence of androgen) in activation of CD5+ cell subsets. In a recent study, castration in male mice was shown to

selectively increase splenic cellularity and the number of peripheral blood lymphocytes due to newly emigrated immature B cells. It was concluded that the numbers of B cells in male mice were controlled by physiological levels of androgen (Ellis *et al.* 2001).

Pregnancy and the immune system

Pregnancy is a good physiological model for evaluating the effects of oestrogen and progesterone on the thymus and T cells. Thymic involution in mice is accelerated during pregnancy and the size of the thymus gradually returns to normal in the first month postpartum (Phuc *et al.* 1981). Activation of the humoral immune response, shift to the T helper 2 (TH2) pathway and a decreased cellular immune response have been determined during pregnancy. The most striking clinical example of this immune profile change is SLE, in which a humoral immune response is an important pathogenetic factor, exacerbated by pregnancy. By contrast, rheumatoid arthritis and multiple sclerosis (disorders related to cellular immune response TH1 pathways) improve during pregnancy (Lahita *et al.* 1986, Holmdahl 1989). Although the exact mechanism is unclear, high levels of oestrogen, progesterone and other possible hormones such as prolactin may be responsible for these immune profile changes (Walker *et al.* 1998, Whitacre 2001). Even though there is a reduction in circulating levels of immunoreactive GnRH during pregnancy (Petraglia *et al.* 1996), recently it has been reported that human placenta expresses GnRH and GnRH-R (Wolfahrt *et al.* 1998). In addition to being a potential autocrine/paracrine regulator of human chorionic gonadotrophin biosynthesis (Barnea & Kaplan 1989, Currie *et al.* 1993), placental GnRH may have a contribution in immune system changes during pregnancy via a paracrine route or indirectly through regulation of placental hormones, and given the aforementioned, this may be a fertile area of future investigation.

Effects of oestrogens on cellular and humoral immunity

Oestrogen treatment has been shown to cause significant thymic atrophy and to decrease the number of thymocytes in mice (Okuyama *et al.* 1992). In a study with ovariectomised female rats, ovariectomy increased thymic size and had a profound effect on the thymocyte profile, leading to an increase in the CD4+CD8+ immature cells, with a decrease in the relative proportion of the mature cells, which was opposed by treatment with physiological doses of oestradiol-17 β (Leposavic *et al.* 2001). Oestrogen also stimulates CD4+CD8- cells and can activate an extra-thymic pathway of autoreactive T cell differentiation in the liver (Screpanti *et al.* 1989, Okuyama *et al.* 1992, Muller *et al.* 1995).

Several studies have established that oestrogen is a potent inhibitor of stromal cell-dependent B cell lym-

phopoiesis *in vitro*. In bone marrow, all the precursors beyond the early pro-B cell stage are affected by oestrogen (Medina *et al.* 1993, Medina & Kincade 1994, Smithson *et al.* 1995). In a recent study, the same authors observed a dramatic reduction in B cell lineage differentiation and expansion when oestrogen was added to stromal-free cultures, suggesting a direct effect of oestrogen on early pro-B cells (Kincade *et al.* 2000).

Oestrogen also affects peripheral B cells and humoral immunity. Oestrogen treatment in normal male or female mice increased the number of antibody-producing cells and the levels of circulating autoantibodies (against double-stranded (ds) DNA) without any increase in B cell count (Verthelyi & Ahmed 1994). In another study, using human peripheral blood mononuclear cells, oestrogen treatment was showed to enhance immunoglobulin production, partially by increasing IL-10 production (Kanda & Tamaki 1999).

In summary, androgens and oestrogen are potent immune modulators. Sex steroids act as negative regulators in both the thymus and bone marrow, but androgens and oestrogen tend to affect different subsets of immune cells. In general, androgens seem to inhibit immune activity, while oestrogen seems to have a more powerful effect on immune cells and to stimulate immune activity.

Impact of sex steroids on autoimmunity and gender-specific immune response

Autoimmune disorders are typically more common in women and, therefore, a role for sex steroids in the sexually dimorphic response of the immune system might be expected. (NZB \times NZW) F1 mice (B/W mice), an animal model of SLE, have been widely used to investigate the impact of sex steroids in autoimmunity.

In early studies, premature death and a disease course indistinguishable from untreated females were reported in orchietomised B/W mice. In females, ovariectomy did not alter the disease course, but further administration of dihydrotestosterone lowered anti-DNA antibody levels and improved the prognosis (Roubinian *et al.* 1977a), suggesting a protective role for androgens rather than a deleterious effect of oestrogen (Roubinian *et al.* 1978, Siiteri *et al.* 1980). On the other hand, thymectomy of intact male B/W mice decreased the protective effects of endogenous androgens (Roubinian *et al.* 1977b).

In contrast to androgen administration, oestrogen treatment of castrated females enhanced disease progression and death (Roubinian *et al.* 1978, Siiteri *et al.* 1980). It was reported that normal female mice (C57BL/6) have higher autoantibody levels than males. In addition, chronic administration of oestradiol increased the autoantibody (IgG anti-cardiolipin and anti-dsDNA) production in both normal female and male mice (Ahmed & Verthelyi 1993, Verthelyi & Ahmed 1994, Verthelyi & Ansar 1997).

Table 2 Summary of experimental data showing the effects of sex steroids on the immune system

Expression of receptors	ER and AR expressed in all immune subsets, except AR in mature B and T cells
Thymus and bone marrow effects	Testosterone: thymic atrophy, thymocyte apoptosis and B cell precursor depletion in bone marrow Oestrogen: same as testosterone, but on different immune subsets
Peripheral T and B cells	Testosterone: enhances suppressor T cells and diminishes B cell number Oestrogen: enhances helper T cells and increases autoantibody production
Impact on autoimmunity	Androgens: seem to have suppressor activity Oestrogens: seem to have stimulating activity
Sexually dimorphic immune response	Impact is more during development of immune response; mechanism is unclear

The relationship between sex steroids and autoimmunity was demonstrated not only in the SLE mouse model, but also in insulin-dependent diabetes mellitus and an animal model of autoimmune thyroiditis (Ahmed & Talal 1990, Fox 1992). In all these studies, oestradiol seemed to accelerate the progression of humoral immune response-dependent autoimmune diseases via enhancing the TH2 pathway, while androgen had a protective effect. Another hormonally modulated autoimmune disease model which has been extensively studied is experimental allergic encephalitis (EAE). EAE is a cellular immune response (TH1 pathway)-dependent autoimmune disease that has proved a useful model of multiple sclerosis. Several studies in EAE rat and mouse models have demonstrated the potent suppressive effects of oestrogens on disease activity (Jansson *et al.* 1994, Kim *et al.* 1999, Hoffman *et al.* 2001).

It is proposed that specific responses of different immune subsets to androgens and oestrogens could be one of the contributing factors in sexual dimorphism in the immune system (Olsen *et al.* 1998). Previous studies have shown that the dimorphic immune response is fully established after puberty (Blazkovec & Orsini 1976, Aaron *et al.* 1985). However, in a recent study, it was clearly demonstrated that sexual dimorphism in the composition of thymocyte subsets persisted after the gonadectomy of adult rats, suggesting that gonadal steroids are more important for the development than for the maintenance of the gender difference in immune responsiveness (Leosavic *et al.* 1996).

The main relationships between sex steroids and immune system are summarised in Table 2. Although the full nature and underlying mechanisms are still poorly understood, future molecular studies are likely to elucidate the relationships between sex steroids and immune cells.

Sex steroids and GnRH interaction

Is there an interaction demonstrable between sex steroids and GnRH within the immune system? Oestrogen regu-

lates the number of GnRH-Rs in the pituitary, modulating the responsiveness of gonadotrophs to GnRH (McArdle *et al.* 1992). One suggested mechanism for oestrogen regulation of GnRH-R includes modulation of GnRH-R mRNA levels (Bauer-Dantoin *et al.* 1993, Funabashi *et al.* 1994, Quinones-Jenab *et al.* 1996). Direct regulation of human GnRH-R expression by progesterone at pituitary and placental levels was recently shown, and the presence of a putative progesterone response element in the 5'-flanking region of the human GnRH-R gene has been established (Cheng *et al.* 2001).

In an elegant study, Azad *et al.* (1998) demonstrated that gonadal steroids could also modulate the production of GnRH by immune cells. In this study, thymic GnRH concentrations were significantly increased in castrated rats, but testosterone replacement inhibited this increment. Other studies showed that the increase in oestrogen concentrations during the oestrous cycle in mice and rats was associated with increased expression of GnRH and GnRH-R mRNA in both spleen and thymus (Jacobson *et al.* 1998, Marchetti *et al.* 2000, Morale *et al.* 2001). Interactions between GnRH, sex steroids and immune cells are shown in Fig. 1.

The HPG axis and the immune system: clinical studies

GnRH agonist treatment

GnRH analogues are used in the treatment of several conditions, such as precocious puberty (Neely *et al.* 1992), endometriosis (Waller & Shaw 1993), leiomyomas (Coddington *et al.* 1992), prostatic carcinoma (Huben 1992) and other hormone-dependent cancers. Studies in women receiving GnRH analogue treatment for endometriosis showed an increase in natural killer (NK) cell numbers and up-regulation of T cell mitogenic activity after 1 year of follow-up (Garzetti *et al.* 1996, Hsu *et al.* 1997). No new case of autoimmune disorder was reported among the women participating in these studies. Ho *et al.*

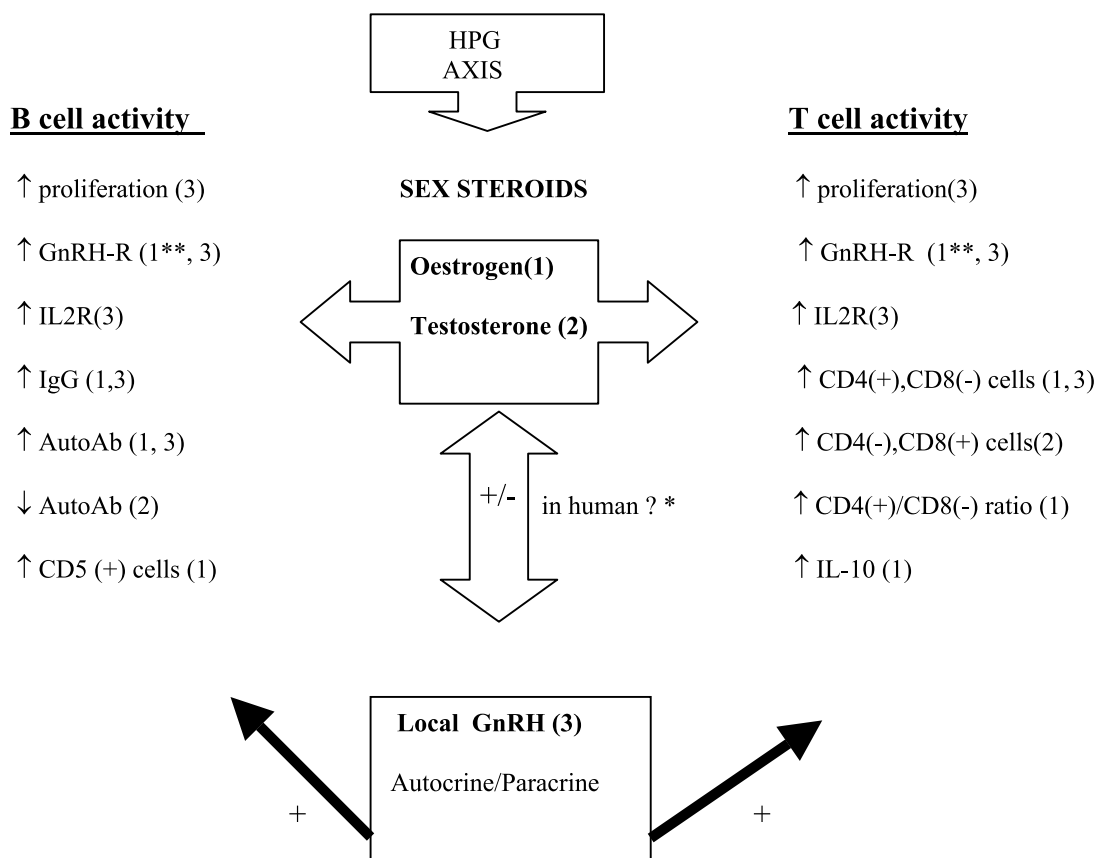


Figure 1 Diagrammatic representation of experimental data for sex steroid, GnRH and immune system interactions. The HPG axis affects B and T cell activity via sex steroids in both stimulatory or inhibitory ways. Local GnRH produced by immune cells or tissues has an immune stimulatory action in autocrine/paracrine ways. There are possible interactions between sex steroids and local GnRH at the immune system level. *In the human, sex steroid–GnRH interaction at the immune system level is unknown; **Oestrogens increase GnRH mRNA in immune cells in mice. (1) Oestrogen effect; (2) testosterone effect; (3) GnRH effect.

(1995) analysed the immune profile of patients receiving GnRH analogues during *in vitro* fertilisation programmes and, in contrast to data obtained in experimental studies, no changes were observed in peripheral B cells, NK cells, CD4+ and CD8+ T cells, and serum IL-2 and IL2R levels. On the other hand, exacerbation of SLE nephritis and thrombocytopenia after GnRH analogue administration has been reported in two different case reports (Miyagawa *et al.* 1994, Metcalfe & Boulton-Jones 1997). In a contradictory study, Catania *et al.* (1989) reported improvement in the disease course of six SLE patients due to down-regulation of gonadotrophin secretion after GnRH analogue administration. More clinical studies are warranted to establish the role of GnRH agonists in immune function.

Hypogonadism and autoimmunity

In primary hypogonadism, hypothalamic–pituitary production of GnRH and gonadotrophins is increased, but

there are no data about the effects of this condition on the GnRH and GnRH-R expressed in immune cells. Nevertheless, in either primary or secondary hypogonadism some relevant immune changes might be expected due to low sex steroid levels.

Increased incidence of autoimmune disorders in hypogonadal states have been reported in several studies. Examples of this include increased occurrence of autoimmune thyroid diseases in premature ovarian failure (Belvisi *et al.* 1993) and SLE in Klinefelter's syndrome (Stern *et al.* 1977, French & Hughes 1983). The latter is characterised by low androgen levels, often associated with elevated oestrogen levels. Increased CD4+ T cell count, CD4+/CD8+ ratio, immunoglobulin levels and IL-2 levels have been reported in untreated Klinefelter's syndrome patients with no autoimmune disorder, confirming experimental studies. Androgen replacement significantly reversed all these changes (Kocar *et al.* 2000). In some Klinefelter's syndrome patients with autoimmune disorders, androgen replacement decreases the activity of the disease and

depresses the humoral immune response via lowering CD4+/CD8+ ratios (Bizzarro *et al.* 1987, Olsen & Kovacs 1995).

Similar immune profile changes were demonstrated in male patients with idiopathic hypogonadotrophic hypogonadism (IHH) before and after gonadotrophin treatment; testosterone normalisation was associated with a decrease in CD4+/CD8+ ratio and immunoglobulin levels (Yesilova *et al.* 2000). However, in a previous study, although CD4+ cells were significantly higher in untreated IHH patients compared with normal adults and treated patients, the CD4+/CD8+ ratio did not change after normalisation of testosterone (Kiess *et al.* 1991).

The impact of HRT and oral contraceptives on immune function: risk or benefit?

The associations between oestrogen treatment and the risk of development or exacerbation of autoimmune disorders have been evaluated in several studies. Consistent with experimental studies, increased levels of oestrogens and active oestrogen metabolites have been shown in SLE patients (Lahita *et al.* 1982, Folomeev *et al.* 1992). Moreover, it is known that exacerbations of SLE commonly occur during pregnancy, a high-oestrogen state, due to increased TH2 cytokines (IL-4, IL-10) and correspondingly enhanced humoral immunity (Ostensen 1999). In some clinical studies, it was suggested that long-term use of postmenopausal oestrogen therapy might be associated with increased SLE risk (Sanchez-Guerrero *et al.* 1995). Sanchez-Guerrero *et al.* (1995) investigated nearly 70 000 postmenopausal women in a prospective study (Nurses' Health Study), using health questionnaires, and found an approximately 2-fold increased age-adjusted relative risk for SLE in women receiving HRT (Meier *et al.* 1998). However, this study was not a randomised trial designed to test the association between HRT and SLE, and different HRT schedules were not assessed (Buyon *et al.* 1995). In a recent study, B cell subsets were evaluated in postmenopausal women. CD5⁻ cells were decreased in late postmenopausal women and increased after 6 months of HRT; however, HRT did not affect autoantibody-producing cell CD5⁺ count, and moreover, HRT did not appear to increase the risk of autoimmune disease (Kamada *et al.* 2001). In addition, it was suggested that HRT might protect women from an aberration of the immune system via improving the cellular and humoral immune response balance (Deguchi *et al.* 2001).

SLE and other autoimmune pathologies seem to be associated with impaired NK cell activity and B cell hyperactivity. In an elegant study, effects of sex steroids on immune cells' profile levels were assessed in 30 male-to-female and 30 female-to-male transsexuals. Oestrogen plus anti-androgen administration to males significantly decreased NK cell numbers and a slight increase was found in the number of B cells. In addition, a shift towards the

TH1 phenotype was found in females receiving testosterone, while the opposite was seen in males after oestrogen plus anti-androgen treatment (Giltay *et al.* 2000).

Effects of HRT and oestradiol treatment on autoimmune disease progression are uncertain. Although the number of patients analysed was small, retrospective (Arden *et al.* 1994) and prospective studies (Kreidstein *et al.* 1997, Mok *et al.* 1998) have shown that HRT does not increase flare rates and disease severity. Women with SLE have an elevated risk of osteoporosis and coronary artery disease due to ovarian dysfunction and prolonged use of corticosteroids (Petri *et al.* 1992, Kipen *et al.* 1998). In theory, these patients might have potential benefit from HRT treatment but there are insufficient data available at the moment to confirm this. The SELINA Trial (the Safety of Estrogens in Lupus Erythematosus – National Assessment) is currently in progress in the USA. This randomised, placebo-controlled, multicentre trial is assessing the use of oral contraceptive and HRT in female patients with SLE and may shed more light on this issue.

Conclusions and future directions

Although definitive mechanisms have not been elucidated yet, GnRH and sex steroids seem to be important components of immune system modulation and may play a role in the pathogenesis of autoimmune disorders. Effects of these hormones are not only limited to immune modulation. Actions of sex hormones during embryonic development appear to be important for immune system programming and gender differences in immune responsiveness.

GnRH and GnRH-R are produced locally by immune cells, suggesting an autocrine role for GnRH. Most experimental studies have shown that exogenous GnRH stimulates the immune response. However, there are conflicting results in clinical studies regarding the immune effects of currently used GnRH analogues.

ERs and ARs are expressed in primary lymphoid organs and peripheral immune cells, except for mature B and T cells in which ARs are not demonstrable. Experimental data have established that oestrogens enhance the humoral immune response and seem to have an activating role in autoimmune disorders, whereas testosterone enhances suppressor T cell activity. Although there are some clinical studies consistent with these findings, the impact of sex steroids on autoimmune disease pathogenesis, and the risk or benefits of their usage in normal and autoimmune disordered patients, require further investigation.

There are neither experimental nor clinical data evaluating GnRH and sex steroid interaction at the immune system level in humans, and several questions remain to be answered in this area. For example, does GnRH-R have signalling capacity on human immune cells and can sex steroids modulate GnRH and GnRH-R expression in

human immune cells as they do in the HPG axis? Does GnRH have a direct effect on immune cells, via stimulation of local GnRH-Rs? Or does it only act indirectly via activating the HPG axis and increasing circulating levels of sex steroids? Answers to these questions are important if the immune-modulating mechanisms of GnRH and sex steroids are to be clarified.

The immune aspects of currently used medications such as GnRH analogues, oestrogens and androgens need to be further evaluated for risk of autoimmune disorder development and for potential application in new indications in decreased immune response conditions such as HIV infection and after BMT.

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