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Gonadal Steroids and Immunity*

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I. Introduction

THE remarkable female predominance of such diverse autoimmune diseases as systemic lupus erythematosus (SLE), Hashimoto's thyroiditis, rheumatoid arthritis, and primary biliary cirrhosis has suggested to many clinicians that hormonal differences between the sexes must confer some protective effect on males or enhance the susceptibility of females to these diseases. This review will examine the evidence in support of such an hypothesis. We will examine the effects of gonadal steroids on the function of the normal immune system as well as their impact on autoimmune processes. We will explore current knowledge of the cellular and molecular mechanisms by which gonadal steroids might modulate both normal and disordered function of the immune system.

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II. Physiology and Pathophysiology of Gonadal Steroid Effects on Immunity

A. Normal immune responses are sexually dimorphic

A significant body of data establishes that a number of aspects of immune responsiveness normally differ between males and females. These observations hold true in many species, including humans, and apply to both humoral and cellular responses. Women have higher plasma IgM levels than men (1); this difference becomes most significant at the time of puberty and is demonstrable in both African-American and white populations (Fig. 1) (2, 3). Levels of serum IgG have been found to be higher in black American women than in black American men, but no such gender difference in IgG levels are observed in the white population (2). In animal studies, females show more vigorous antibody responses to exogenous antigens (4). It has been suggested that this gender difference also holds in humans (5) although much of the data are derived from studies of infants, when gonadal hormonal effects seem less likely to be operative (6). Studies of several clinically useful vaccines, including those for hepatitis B, tetanus, and pneumococcus, have not revealed higher antibody responses in females compared with males (7–9). One possible explanation for the apparent discrepancy between animal and human data might be differences in the antibody classes that are produced. In general, vaccinations are designed to elevate levels of neutralizing or protective antibodies of the IgG class, while, as noted above, IgM levels show the most significant sexual dimorphism. Immunoglobulin class-specific responses were not measured in any of the vaccine studies cited; therefore, this possibility cannot be verified from the available data.

Cell-mediated immune responses are also sexually dimorphic. Thymocytes and lymphocytes from normal female mice respond more vigorously to exogenous and allogeneic antigens than do cells from male mice (10). Parallel studies of T cell function in humans are lacking; however, quantitative differences in relative numbers of functional T cells have been related to gender (11–14). Higher CD4:CD8 ratios are generally seen in females and hypogonadal males (15) due to relatively lower numbers of circulating CD8 T cells.

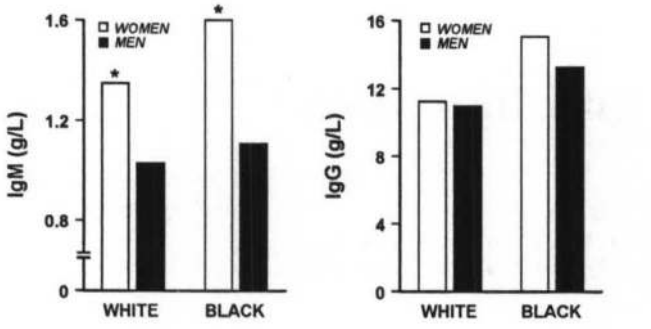

FIG. 1. Immunoglobulin levels are sexually dimorphic in humans, with higher levels observed in females. Serum levels of IgM (*left panel*) and IgG (*right panel*) in women (*open bars*) and men (*solid bars*) are shown for both white and black Americans. [Derived from (2).] Statistically significant differences between women and the corresponding group of men are noted by (*).

B. Autoimmune diseases are more common in females

A variety of disease processes believed to be autoimmune in origin are much more likely to occur in females than in males; this is true in both humans and experimental animal models. In humans the incidence of most autoimmune diseases is recognized to be greatly increased in women (Table 1)(16–19). SLE is up to 9 times more common in women than in men. Rheumatoid arthritis, Hashimoto's thyroiditis, and Graves' disease exhibit similar patterns of predominantly female incidence.

In mice, spontaneous autoimmune syndromes are also more prevalent and show greater severity in females compared with males. The best-studied example is the NZB/NZW F1 hybrid mouse, which develops a generalized autoimmune disorder similar to SLE in humans (20). Female NZB/W mice generally die within the first year of life due to glomerulonephritis while male mortality rates are 10% or less (20, 21). Other mouse strains that develop spontaneous lupus-like autoimmune syndromes also generally show a female predominance, including the MRL *lpr/lpr* (22) and the recently described SWRxSJL F1 hybrid (23). MRL *lpr/lpr* mice develop a lupus-like syndrome characterized by autoantibody production, lymphadenopathy, and proteinuria. The disease that develops in this strain is also sexually dimorphic, although not nearly so strikingly as in the NZB/W F_1 strain; at 7 months of age all females are dead of disease while 20–30% of males survive (22). Female SWRxSJL F_1 mice develop evidence of autoimmunity at a rate more than 7 times that of males (23).

The nonobese diabetic (NOD) mouse is an animal model of human type I diabetes. The diabetes in this mouse results from autoimmune destruction of the pancreatic islet β-cells. While pancreatic insulitis is observed in both sexes, progression to overt diabetes occurs predominantly in females. In most laboratories, including those establishing colonies under specific pathogen-free conditions, diabetes develops in 70–80% of females by 30 weeks of age (24). The male incidence has been noted to be more variable, from as low as 0% to as high as 39%, but is virtually always lower than the female incidence in the same colony.

Animal models of human rheumatoid arthritis also show a female predominance. These model syndromes generally do not develop spontaneously but are induced in susceptible animals by parenteral administration of bacterial or mycobacterial products. One such model is streptococcal cell wall-induced polyarthritis in Lewis rats, which develops with a greater incidence and severity in females than in males (25). It is clear that the Lewis genes conferring disease susceptibility are transmitted in autosomal fashion, excluding the possibility that the sexual dimorphism results from the transmission of X or Y chromosomal genes (26).

TABLE 1. Prevalence of various autoimmune diseases in females and males

Disease	Females:males
Rheumatoid arthritis	2:1
Systemic lupus erythematosus	9:1
Primary biliary cirrhosis	9:1
Grave's disease	5:1
Hashimoto's thyroiditis	40:1

C. Autoimmune diseases can be modulated by changes in levels of gonadal steroids

The ability of gonadal steroids to alter the course of autoimmune diseases has been suspected in the clinical setting for more than 100 yr, but the evidence for such hormonal modulation of autoimmune disease activity in humans is either circumstantial or anecdotal. Hypogonadal males may have a higher prevalence of autoimmune diseases including SLE (15). Women with systemic lupus have also been reported to have lower androgen levels than age-matched healthy female controls. In one study, 22 women with active SLE had mean serum testosterone concentrations of 18 ± 6.5 ng/dl compared with a control mean of 40 ± 22 ng/dl (27). This difference does not achieve statistical significance, but a trend was noted toward lower testosterone levels with more active disease (27). Similar results were obtained in another study of 13 women (28). Increased levels of active estrogen metabolites have also been reported in women with SLE (29–35). Administration of androgens has been reported to suppress the activity of SLE in individual cases of men with hypogonadism due to Klinefelter's syndrome (15, 36).

Animal models of autoimmune diseases have provided more durable evidence of the immunomodulatory effects of gonadal steroids. The first rigorous demonstration of such an immunomodulatory effect in an animal model of autoimmunity was accomplished more than 20 yr ago in the NZB/W F1 hybrid strain of mice (20, 21, 37). Female NZB/W mice develop anti-DNA antibodies by 4 months of age and by 10 months mortality approaches 100%. Males have less than 10% mortality over this same period of time. Prepubertal castration of NZB/W males results in an acceleration of the disease course, with 100% mortality by 11 months of age (21), a disease course indistinguishable from that of untreated females. Ovariectomy itself does not alter the disease course in females (20), but the administration of androgens to ovariectomized females largely prevents the appearance of the disease (20, 21, 37) (Fig. 2, *left panel*). Administration of estradiol to castrate females may even enhance disease progression compared with intact females (Fig. 2, *left panel*),

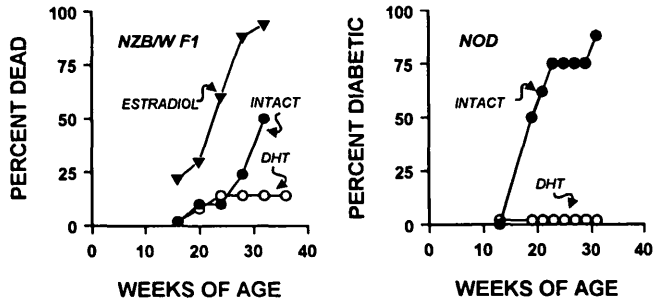

FIG. 2. Androgens suppress autoimmune disease activity. Hormonal effects on the course of two animal models of autoimmune diseases are shown. *Left panel*, Mortality in NZB/W F_1 mice. Female mice were sham operated (*closed circles*) or castrated prepubertally and treated with estradiol (*closed triangles*) or dihydrotestosterone (*open circles*). *Right panel*, Cumulative incidence of diabetes in intact NOD female mice treated with implanted dihydrotestosterone pellets (*open circles*) or carrier/binder pellets as control (*closed circles*). [Derived from (20) and (43).]

although the levels of estrogen achieved in these experiments were not reported.

The locus and mechanism of androgen action in the NZB/W lupus model have been explored in some detail. The disease-suppressing effect of androgens has been believed to be exerted at the level of the thymus, since thymectomy abrogates the protective effect of endogenous androgens in intact male NZB/W mice (38). Evidence for involvement of specific androgen receptors in the protective effect has come from experiments using the androgen receptor antagonist flutamide. This antagonist causes acceleration of disease appearance and progression in both males and females. The effect is, as expected, more pronounced in males but is also discernible in females (39). Less compelling, but also suggestive, is the failure of danazol, a weak androgen agonist, to alter the course of autoimmunity in NZB/W mice (40).

The MRL *lpr/lpr* mouse also develops a lupus-like syndrome that can be ameliorated by androgens, much like disease in the NZB/W (22). The lacrimal gland inflammation observed in these mice, which serves as a potentially useful model for Sjogren's syndrome, is significantly reduced by administration of testosterone (41).

Gonadal steroids modulate the autoimmune processes leading to diabetes in the nonobese diabetic (NOD) mouse. Castration accelerates the time of onset of diabetes in male mice. The prevalence of diabetes at 30 weeks of age was increased from 0% to 31% in one study (42). Administration of androgens to female NOD mice prevents development of overt diabetes (43) (Fig. 2, *right panel*). The findings in these animals are very similar to those previously described in the NZB/W model.

Disease transfer experiments have also yielded data supportive of a role for gonadal steroids in the modulation of islet-specific immunity in the NOD mouse. Neonatal NOD mice, in which no prior immunoablative procedures have been carried out, are susceptible to transfer of diabetes by splenocytes from overtly hyperglycemic animals (44). Diabetes develops in the males for the first 6 weeks at a rate similar to (or even faster than) that observed in female mice given the same donor cells neonatally. By 6 weeks of age more than 40% of males and 20% of females have overt diabetes. However, a remarkable arrest in the incidence of new cases of diabetes among the males occurs at the time of sexual maturation, while the appearance of new cases of diabetes among the females continues apace, up to a prevalence of 80% by 12 weeks of age. Occurrence of diabetes in the males after 5 weeks is rare, so that the overall prevalence in males after 20 weeks is still 40%. The protective effect of androgens against diabetes development in the NOD mouse appears to be exerted at the level of generation of the immune responsiveness, and not at the level of the pancreas, since androgen administration does not diminish susceptibility of irradiated (*i.e.* lymphoid-ablated) males to transfer of diabetes (43).

III. Cell Biology of Gonadal/Immune System Interactions

A. The T cell compartment—gonadal steroids exert effects on thymus (Fig. 3A)

1. Androgens. It has been recognized for more than 100 yr that sex steroid hormones modulate thymus size in adult animals. In 1904, Henderson reported that castrate cattle (oxen) had significantly larger thymus glands than their intact male counterparts (45). These early studies have been confirmed in rats (46) and mice (47), and the reversibility of castration-induced thymic enlargement in rodents by androgen replacement has been documented in a number of studies (47, 48). The testicular feminization (Tfm) mouse, which expresses a mutant androgen receptor, rendering the animal insensitive to the effects of this hormone, also shows significant thymus enlargement (49).

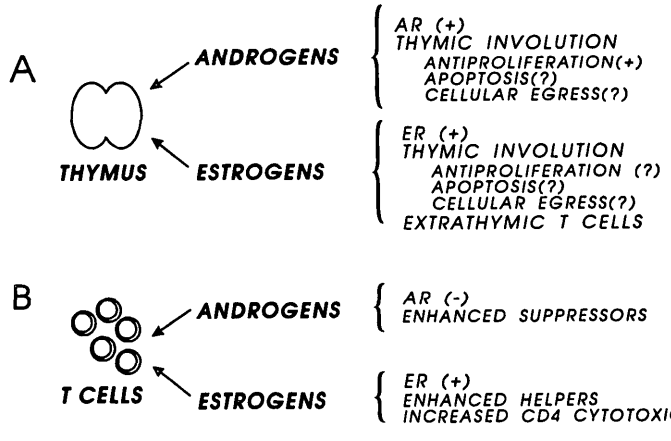

FIG. 3. Summary of known gonadal steroid interactions in the development and expression of cell-mediated immunity. A, Androgens and estrogens both appear to act at the level of the thymus via specific receptors (AR^+; ER^+). Both classes of hormones induce thymic involution, but mechanisms are largely unexplored; only an antiproliferative effect of androgens has been demonstrated. Estrogens have been found to induce the development of T cells in the liver, a known site of extrathymic T cell development under some conditions. B, Androgens and estrogens may also exert direct effects on peripheral T cells. No clear documentation of AR expression in peripheral T cells has been published, but the net effect of androgen action (direct or indirect) appears to be enhanced "suppressor" tone. Estrogens more likely exert direct effects on peripheral T cells to enhance helper activity. Under experimental conditions estrogens have been shown to increase the activity of an unusual class of cytotoxic $CD4^+$ cells.

The enlarged thymus in the castrate male mouse appears to have normal architecture with intact cortical and medullary areas. However, the relative distribution of thymocyte phenotypes is altered when androgens are removed by castration. The CD4 and CD8 markers define four major subpopulations of thymocytes (Fig. 4). "Double negative" $CD4^-CD8^-$ cells are the most immature thymocytes. The vast majority of thymocytes are $CD4^+CD8^+$ and are destined to undergo programmed cell death during the process of intrathymic selection. The $CD4^+CD8^-$ phenotype defines the mature helper cell, while the $CD4^-CD8^+$ phenotype comprises mature suppressor/cytotoxic cells as well as an immature population that includes precursors to $CD4^+CD8^+$ cells. The most consistent change after androgen deprivation is in the $CD4^-CD8^+$ subpopulation, which shows a small but significant decrease in castrate mice of several strains compared with their age-matched intact controls (Table 2) (47, 50). The $CD4^-CD8^+$ subset of thymocytes is heterogeneous (at least in the C57 Bl/6 mouse) in that it includes immature cells that have not yet acquired expression of $CD4^+$ to make them become double positive, as well as mature single positive $CD8^+$ cells. For this reason, it is difficult to draw inferences regarding functional or maturational differences effected by castration in these thymuses. The picture is somewhat clearer when the more mature cells are identified by T cell receptor expression. For example, cells expressing the CD3 T cell receptor component represented 17% of thymocytes from intact thymuses, but only 11% of cells from castrates ($P = 0.045$) (51). Androgen replacement reverses the balance toward more mature cell types, due, at least in part, to depletion of the double-positive cortical thymocytes (Table 2) (47, 52).

Mechanisms responsible for thymus enlargement after castration and thymic involution during androgen replacement are not completely understood but might involve one or more of three possible pathways: increased cell proliferation, decreased cell death, or diminished trafficking of thymocytes to the periphery. Recent studies have suggested that at least the first of these three possibilities does occur after castration in normal male mice (51). Under normal conditions in intact adult males, androgens apparently restrain active cell cycling of immature thymocytes. Androgen withdrawal triggers a wave of cellular proliferation. During the first week after castration, before significant thymus enlargement occurs, a wave of thymocyte proliferation is observed, peaking at day 4 after surgery (Fig. 5). As this wave subsides to baseline rates of proliferation, thymus enlargement becomes apparent by day 7, reaching a plateau level by day 10. Immunohistochemical examination of these thymuses indicates that the thymocyte proliferation occurs in the outer cortex and in areas of the medulla, locations where thymocytes normally proliferate. Phenotypic examination of the cell types undergoing proliferation indicates that the largest increase occurs in relatively immature cell types. For example, only thymocytes with low or absent levels of the CD3 component of the T cell receptor proliferate at significantly increased rates in the castrate animals; relatively mature thymocytes expressing high levels of CD3 show very low proliferation indices. Intrathymic proliferation of thymocytes therefore contributes, in large part, to the development of thymus enlargement after castration. Once the thymus has reached 150–200% of precastration size about 10 days after castration, thymocyte proliferation slows to baseline rates while thymic size is maintained.

If androgens exert direct effects on the thymus, then spe-

TABLE 2. Thymocyte phenotypes in normal male mice examining effects of castration and testosterone replacement

Phenotype	Experiment series 1		Experiment series 2	
	Control	Castrate	Oil	Test
$CD4^-CD8^+$	3.6 ± 0.6	2.7 ± 0.3[a]	3.2 ± 0.5[b]	12.4 ± 2.8[b]
$CD4^+CD8^-$	9.2 ± 1.0	9.2 ± 1.2	7.1 ± 0.6	11.6 ± 2.9
$CD4^+CD8^+$	81.0 ± 1.7	82.5 ± 1.5	85.4 ± 1.0[b]	59.3 ± 2.0[b]
$CD4^-CD8^-$	5.7 ± 0.7	5.3 ± 0.6	4.4 ± 0.8[b]	16.7 ± 3.4[b]

Values represent mean percent of total thymocytes. In Experiment series 1, castrate animals were compared to intact controls. In Experiment series 2, oil-treated castrates were compared to castrates undergoing testosterone replacement. [Derived from (47).]

[a] $P = 0.005$ comparing control and castrate groups.

[b] $P \leq 0.005$ comparing oil- and testosterone-treated groups.

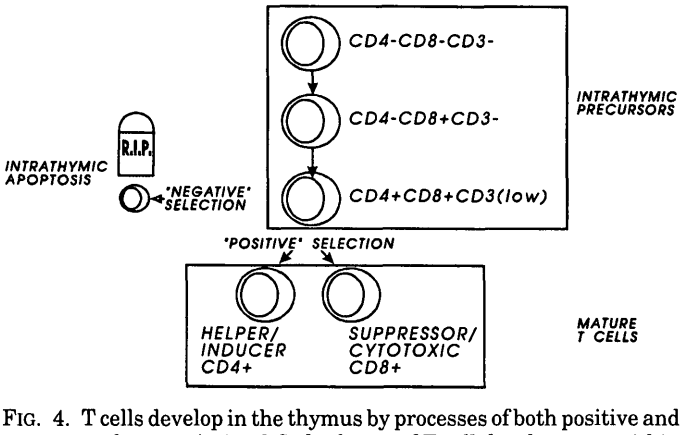

FIG. 4. T cells develop in the thymus by processes of both positive and negative selection. A simplified schema of T cell development within the thymus is shown. "Double-negative" precursors give rise to single positive $CD8^+$ and double-positive intermediates, the bulk of which are destined to die intrathymically. Positive selection processes give rise to clones of $CD4^+$ helper/inducer and $CD8^+$ suppressor/cytotoxic cells that express T cell receptors and exit to the periphery.

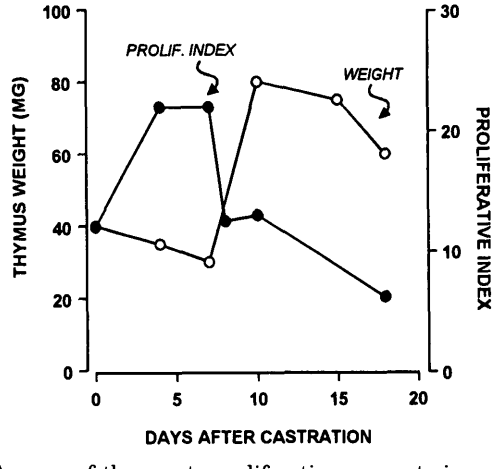

FIG. 5. A wave of thymocyte proliferation accounts in part for the subsequent thymic enlargement seen after androgen deprivation. Thymic weight (*open circles*) and thymocyte Proliferative Index (*solid circles*, the percentage of cells in S or G_2/M phase of the cell cycle as determined by flow cytometric determination of DNA content) are shown as functions of time after castration. [Derived from (51).]

cific receptors for these hormones would be expected to be expressed in the thymic target cells. Reports from diverse laboratories over more than a decade have indicated that such androgen receptors (AR) are detectable by radioligand-binding assays performed on homogenates of whole thymus tissue or by steroid autoradiography (53–62). Quantification of AR content of murine thymocytes by radioligand-binding assay shows that AR content (100 fmol/mg protein) is quite comparable to that of AR target tissues in the reproductive tract or in cultured AR-positive cell lines (63). The androgen binding activity detected satisfies the usual criteria of high affinity, saturability, and specificity that define the AR.

The specific cellular localization of thymic ARs has been addressed by a number of laboratories. Using thymocytes isolated from human and murine thymus (and documented to be pure populations on the basis of surface marker staining), AR expression in thymocytes has been demonstrated by radioligand-binding assay (55, 63), as well as western blot and multicolor flow cytometry (63). In the latter technique cells are permeabilized with lysolecithin to permit anti-AR antibody access to the interior. Surface markers are stained with antibodies directed against CD4, CD8, and the CD3 component of the T cell receptor. While all of the thymocytes defined by the markers CD4 and CD8 express AR, the level of expression of the receptor appears to be about 2-fold higher in the more immature $CD4^-CD8^-$ and $CD4^-CD8^+$cells (63).

Expression of AR in thymic stromal or epithelial cells has long been suspected but less rigorously proven. Thymocyte selection processes central to the development of the immune repertoire are dependent on thymic epithelial cells, so that these cells are important potential targets of gonadal steroid action. The earliest descriptions of "reticulo-epithelial" localization of AR were based on fractionation procedures involving only mincing of the tissue with or without straining through cheesecloth (54, 60). The widely requoted interpretation of these data as defining AR restriction to thymic epithelial cells has been justifiably questioned (55–58) since the actual cellular composition of the "reticulo-epithelial" tissue fragments was not examined, and almost certainly was comprised of mostly thymocytes. The availability of primary cultures of human thymic epithelium, as well as cell lines established from rat and murine thymic epithelium and stromal cells has permitted reexamination of the question. Several cell lines with fibroblastic morphology established from normal human thymus have been found to express AR at low levels (~1/10 those seen in genital fibroblasts). The IT45R1 rat thymic epithelial cell line (64) does not express detectable AR by ligand-binding assays. However, the thymic microenvironment is extremely complex, and the limited experimental evidence available cannot be taken to exclude the possibility that subpopulations of thymic stromal or epithelial cells in specific areas of the thymus express high levels of AR.

2. *Estrogens.* Estrogens also induce thymic atrophy. A single 1 mg injection of estrogen induces profound thymic atrophy 10 days later (65). During pregnancy, when both estrogen and progesterone levels are high, thymic involution is accelerated (66, 67) and, at least in mice, thymic size gradually returns to normal in the first month postpartum (67). The thymic atrophy induced by estrogens in female mice is associated with a decrease in cortical double-positive thymocytes and an increase in more mature phenotypes (65, 68). Treatment of male animals with 17β-estradiol (E_2) also results in relative increases in mature thymocytes with a range of T cell receptor specificities identified by subtypes of the β-chain (Vβ's) (68). However, after estrogen administration, it is the $CD4^+CD8^-$ subset that is increased relative to the $CD4^-CD8^+$ subset (69), while the opposite occurs after androgen administration (47). Thus, it appears that while male and female gonadal hormones have similar effects on gross thymic size and on total thymocyte numbers, differential effects on specific thymocyte subsets might be responsible for shifting the balance of functional mature T cell subsets. Estrogen treatment also activates the process of extrathymic T cell maturation, especially in the liver (65). Cells that mature at this site express specificities that are normally forbidden, due to the lack of normal mechanisms of negative selection. Circulation of such potentially autoreactive cells might be postulated to accelerate the tendency toward autoreactivity (65).

Expression of the estrogen receptor (ER) in the thymus has been demonstrated by ligand-binding assays (70–85), immunohistochemistry (86), and northern blot (87). Not all studies agree on the cellular localization of ER within the thymus, but evidence has been reported for ER expression in both thymocytes (73, 75, 81, 87–89) and thymic epithelial cells (52, 74, 83, 86, 90, 91). Many of these studies predate the availability of reagents and techniques that permit unequivocal identification of thymic cell populations, and virtually all of the reported work suffers from the lack of such definitive typing of the cells under study. Nevertheless, it seems most likely that both developing thymocytes and thymic epithelial cells express ER.

3. *Progestins.* Since pregnancy must be accompanied by some form of "immunosuppression" for the mother to tolerate what is, in effect, a fetal hemigraft, a number of investigators have proposed that progesterone might serve to transmit an immunosuppressive signal in the pregnant state (92). A remarkable degree of thymic involution occurs during pregnancy and reverses in the postpartum period (67). Whether this involution is mediated during pregnancy by progesterone, and whether this thymic involution is at all related to immunological tolerance of the fetus is unknown, since alternative explanations for maternal tolerance of the fetal graft have been proposed (93). The thymus is, however, known to express specific high affinity progestin-binding activity (86, 92, 94–99). The specific cellular localization of these receptors is largely unexplored by currently available techniques, but immunohistochemical studies using progesterone-horseradish peroxidase conjugate (97) and specific anti-progesterone receptor antibodies (86) have localized progesterone receptor expression to cells of nonlymphoid morphology that are presumed to be thymic epithelial cells.

B. The T cell compartment—gonadal steroid effects on peripheral T cells may be direct or indirect (Fig. 3B)

1. *Androgens.* Androgens generally enhance the net functional capacity of $CD8^+$ cytotoxic/suppressor T cells in the

periphery. One crude measurement of the activity of these cells is their ability to suppress mitogen-induced proliferation of syngeneic target splenocytes. Unseparated spleen cells from castrated male mice are not as effective at inhibiting proliferation of other spleen cells as are cells from intact males; when the castrated male animals are treated with testosterone, the inhibitory capacity of spleen cells is increased (100). Phenotypic analyses and quantification of various subsets within the spleen were not carried out in the initial experiments, and therefore it was not possible to determine whether the observed functional differences were in fact due to quantitative differences in T cell subpopulations. More recent studies have confirmed these findings (47) and have suggested that at least some of the observed effects may be due to the presence of fewer mature T cells in the spleen of castrate male mice (101).

The demonstration of effects of *in vivo* manipulation of androgen levels on cells of the peripheral immune system does not necessarily imply that androgens act directly on these terminally differentiated cells. A number of laboratories have failed to demonstrate AR expression in circulating peripheral T cells by radioligand-binding assays (55, 102), and the spleen has generally been considered to be AR-negative by both radioligand-binding assays and western blot (103, 104). Few observations have been reported of direct effects of androgens on T cells *in vitro*. In some studies, supraphysiological levels of androgens (*i.e.* $\geq 10^{-6}$ M) have been required to show effects (105). At the present, the weight of evidence seems to indicate that androgens alter peripheral immune system function by effects exerted during process of thymocyte maturation.

2. *Estrogens. In vivo* administration of estrogens alters total peripheral T cell activity in a number of model systems in ways that suggest either enhancement of helper/inducer or reduction in suppressor/cytotoxic cellular activity. Disease activity in male NZB/W F_1 and MRL *lpr/lpr* mice is accelerated after castration and estrogen administration (21, 106). In the MRL *lpr/lpr* mouse there is evidence that at least part of the estrogen effect is to depress antigen-specific as well as mitogen-induced T cell responses measured *in vitro* (106).

The best evidence to date for a direct effect of estrogens on a specific T cell subset comes from experiments using the β_2-microglobulin-deficient mouse (107). These mice exhibit a defect in class I MHC expression and, as a consequence, have very low levels of functional $CD8^+$ cytotoxic T lymphocytes (CTL). Viral infection in these deficient mice is associated with induction of an unusual set of CTL that are class II-restricted and within the $CD4^+$ T cell population. The immune response mediated by these cells increases mortality in response to infection with lymphocyte choriomeningitis virus (LCMV). While no sexual dimorphism is observed in LCMV infections of normal mice, mortality from this infection is significantly decreased in male β_2-microglobulin-deficient mice compared with corresponding females. Estrogen treatment of castrated males results in a mortality curve that is similar to that of females, suggesting an active role for estrogen in modulating the immune response to the virus. Studies *in vitro* confirm that $CD4^+$ CTL activity is enhanced by addition of estrogen to the cultures, consistent with the *in vivo* observations. Mechanisms responsible for the estrogen-induced changes remain undetermined. In human cells *in vitro*, physiological concentrations of estradiol block the negative regulatory effects of a subset of T lymphocytes that suppress immunoglobulin production by B lymphocytes (108).

Direct exertion of estrogenic effects on T cells would be expected to require expression of ER in these putative targets. In peripheral T cells ER expression has been documented by specific [^{3}H]estradiol binding to be restricted to the $CD8^+$ subset (102, 109, 110). In synovial tissues from patients with rheumatoid arthritis, ER expression is found in $CD8^+$ T cells with phenotypic characteristics of immunological memory as well as in macrophage-like synovial lining cells (110). These observations of restriction of ER expression to $CD8^+$ cells are discordant with the physiological observations in the β_2-microglobulin "knockout" mouse and with the finding that estrogen amelioration of collagen-induced arthritis in rats does not require $CD8^+$ cells (111) and are currently unexplained. Uncharacterized tonsillar T cells also have detectable ER mRNA, although no autoregulation of the message by estrogen is demonstrable as it is in whole tonsil mRNA preparations (112).

C. The B cell compartment—gonadal steroids exert effects on bursa and bone marrow (Fig. 6)

1. *Androgens.* Only a few studies have examined effects of androgens on B lymphocyte-producing organs. In birds the B lymphocytes develop in a lymphoid organ called the bursa of Fabricius. Androgens induce bursal involution. In Japanese quail the normal age-related bursal involution is slowed by castration and accelerated by testosterone implants (113). In mammals B lymphopoiesis is carried out in the bone marrow. Castration of male mice results in expansion of the pre-B cell population in the bone marrow (114); this pre-B cell population can be depleted by treatment with testosterone or

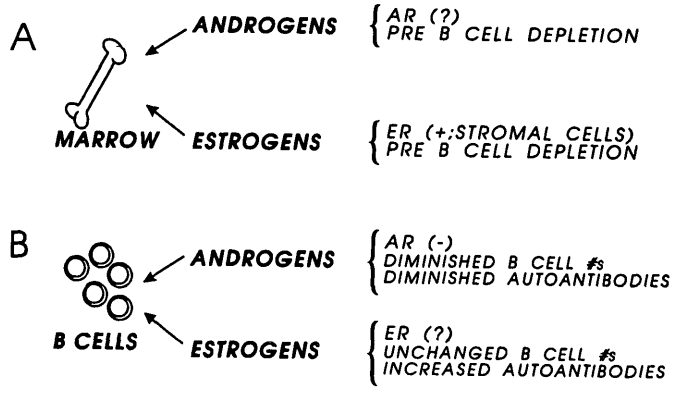

FIG. 6. Summary of known gonadal steroid interactions in the development and expression of humoral immunity. A, Androgens and estrogens both appear to act at the level of the bone marrow to cause pre-B cell depletion but to date only receptors for estrogens have been demonstrated in bone marrow (AR?; ER^+). B, Androgens and estrogens may also exert direct effects on peripheral B cells. No clear documentation of AR or ER expression in peripheral B cells has been published, but the net effect of androgen action (direct or indirect) appears to be a diminution of B cell numbers and autoantibody production. Estrogens do not affect peripheral B cell numbers, but they do enhance autoantibody production.

dihydrotestosterone (115, 116). Whether the effects are direct or indirect is unknown. Expression of AR in bone marrow B cell precursors has not been documented, but the Raji pre-B cell line does express AR (117).

2. *Estrogens.* The effects of estrogens on B lymphopoiesis have been examined more extensively and are best considered within the context of the recognized steps in B cell development (118). A schematic of the putative pathway of B cell maturation is shown in Fig. 7. The earliest committed lymphoid precursors in the bone marrow are characterized by the expression of terminal deoxynucleotidyl transferases. These cells subsequently acquire expression of B220 (CD45R) and leukosialin (CD43) defining the pro-B cell stage. With further progression, CD43 expression is lost and cytoplasmic μ-chains (which define pre-B cells), are acquired. Subsequently, surface IgM is expressed on virgin or immature B cells followed by coexpression with IgD on the mature B cell.

Pregnancy in normal mice does not alter numbers of early pro-B cells. However, all of the subpopulations developing after this stage, including cells responsive to IL-7, are reduced relative to other bone marrow cells (119). A number of observations support the notion that the alteration in B lymphopoiesis during pregnancy is the result of hormonal action. These include the finding that ovariectomy in nonpregnant female mice results in expansion of the numbers of bone marrow B cells; these changes are reversed by estrogen replacement (120). The hypogonadal (hpg) mouse (with partial deletion of the GnRH gene) also has greatly increased numbers of pre-B cells in bone marrow and exhibits a dose-dependent decrease in the numbers of these pre-B cells in response to estrogen replacement (121). A compelling argument for a role of estrogen in B cell development is derived from experiments in which nonpregnant female mice were implanted with pellets containing 17β-estradiol (E_2) sufficient to produce serum estrogen levels similar to those attained in pregnant mice. Changes in relative numbers of developing B cells in these estrogen-treated animals are generally parallel to those observed in pregnant mice (122). The diminution in numbers of bone marrow B lymphocyte precursors is seen within 48 h of a single intraperitoneal dose (1 mg) of E_2 (Fig. 8, top panel). Progesterone administered alone has no effect on B cell precursors but, when combined with E_2, synergism is observed so that lower doses of E_2 were effective in reducing pre-B cell numbers. These effects of E_2 on bone marrow B cells do not require the presence of the thymus. Bone marrow stromal cells, which have been shown to contain ER mRNA (123) as well as functional ER protein (124), appear to be required for estrogen to exert effects on developing B cells (Fig. 8, *bottom panel*) (120). Direct contact between the stromal cells and lymphocytes is not required for estrogen to suppress B lymphopoiesis, suggesting that the hormonal effects are mediated by soluble factors (123). Furthermore, estrogen-mediated effects occur in the presence of IL-7, suggesting the involvement of other stromal-derived molecules.

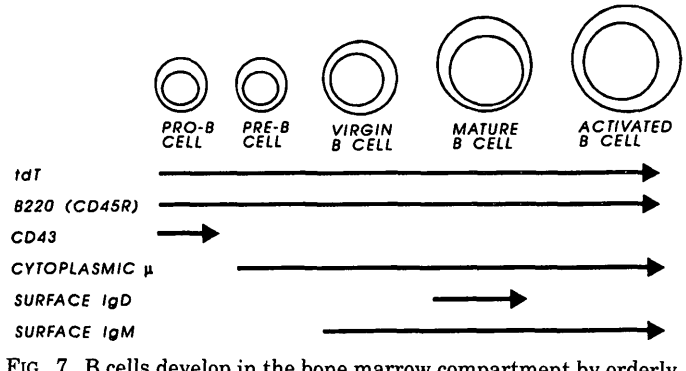

FIG. 7. B cells develop in the bone marrow compartment by orderly, cytokine-driven processes. The earliest committed lymphoid precursors are identifiable by expression of the enzyme terminal deoxynucleotidyl transferase (TdT). Pro-B cells are the first to express B220 (CD45R); subsequently pre-B cells express μ immunoglobulin heavy chains in cytoplasm, and eventually display whole IgM on the cell surface. Progression through latter stages of development is believed to be dependent on a number of factors, including interleukin 7 (IL-7) produced by marrow stromal cells and by T-cell derived interleukin 4 (IL-4). [Derived from (118).]

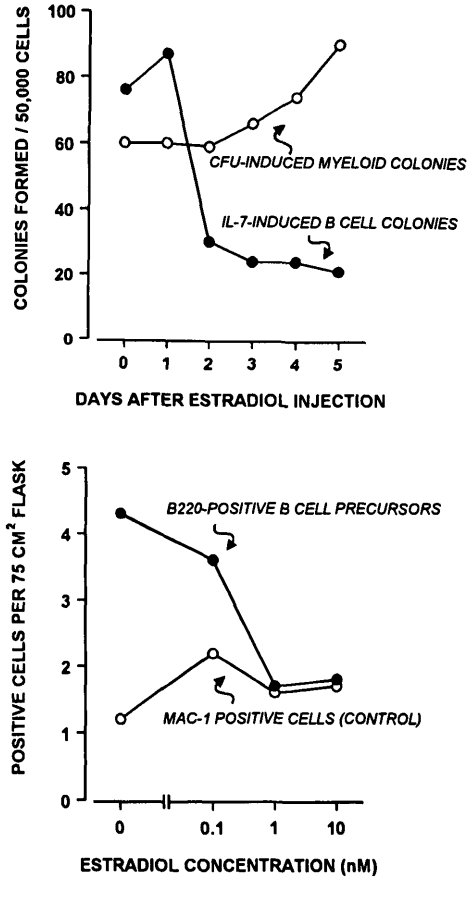

FIG. 8. Estrogens exert suppressive effects on B lymphopoiesis *in vivo* and *in vitro*. *Top panel*, Estradiol administered as a single 1 mg injection to adult female mice results in depletion of bone marrow B-lymphocyte precursors capable of forming colonies in agar under IL-7 stimulation. *Solid circles*, IL-7-dependent colony formation; *open circles*, control CFU-dependent colony formation. [Redrawn from Medina *et al.*: *J Exp Med* 178:1507–1515, 1993 (119).] *Bottom panel*, Estradiol prevents generation of B220-positive lymphocytes when bone marrow cells are cocultured with ST2 marrow-derived stromal cells. *Solid circles* show numbers of B220-positive cells; *open circles* show control Mac-1 positive cells. [Derived from (119) and (120).]

D. The B cell compartment—gonadal steroid effects on peripheral B cells (Fig. 6B)

Just as normal immunoglobulin production is quantitatively different between the sexes, the production of autoreactive antibodies by peripheral B cells (at least in mice) is sexually dimorphic. Female mice of several strains have been shown to produce higher levels of autoreactive antibodies directed against bromelain-treated erythrocytes, double-stranded DNA, and cardiolipin than age-matched males (125–127). Differences between males and females are most marked for the NZW strain, which has a normal phenotype but carries susceptibility genes that contribute to the development of autoimmune disease in the NZB/W F_1 hybrid (125). The hormonal differences between the sexes appear to exert modulating effects on the genetic factors that predispose toward development of autoimmunity.

1. Androgens. The effects of androgen removal on peripheral B cells have been examined in normal male mice (101). A 40% increase in spleen weight is observed after castration (101, 128). This splenic enlargement is accounted for, at least in part, by a relative expansion of the B cell population. In intact males, B cells constitute 45% of splenic lymphocytes compared with 56% for castrated animals; the major subpopulation of B cells, which do not express CD5, represents only 37% of control spleens but 51% of castrate spleens ($P = 0.015$) (101). In NOD mice, castration also results in an increase in spleen cell yield, which is largely accounted for by a relative expansion of the B cell population (42).

The functional significance of the expansion of peripheral B cell populations under androgen-deficient conditions is not clear, particularly with respect to the appearance of autoimmunity. The $CD5^+$ B cell subset, which has been implicated as an important source of autoantibodies (125), is not expanded in spleens of castrate males (101, 125). Nevertheless, spleen cells from castrate animals show a small, but statistically significant, increase in autoantibody production *in vitro* that cannot be attributed to polyclonal stimulation (101). These increases are observed only with the addition of pokeweed mitogen, but not lipopolysaccharide (LPS), to the cultures, suggesting T cell dependence of the enhanced response. However, alterations of androgen status in C57Bl/6 mice do not affect the spontaneous appearance in serum of autoantibodies directed against cardiolipin or DNA (126, 127), and spontaneous antibody production as measured by counts of plaque-forming cells is not increased by androgen deprivation in the NOD mouse (42), possibly due to defective T cell-mediated helper function.

There is little evidence to support direct actions of androgens on peripheral B cells. Examination of immortalized peripheral human B cells (blood or tonsillar B cells) does not reveal any lines expressing AR (117). Neoplastic B cells are generally AR-negative; one exception is the Raji pre-B cell line (117).

2. Estrogens. Estrogen treatment of normal male or female mice results in a large increase in numbers of autoantibody-producing cells without changes in total numbers of B cells (127). Autoantibodies to cardiolipin (126) and to double-stranded DNA (127) are increased in the estrogen-treated animals. These results suggest that estrogen enhances production of antibodies by B cells. Total antibody production was not reported in either of these studies, however, and therefore the relative specificity of the estrogen effect for autoreactive species could not be determined.

Overall, addition of estrogens or removal of androgens enhances B cell responses with a tendency toward production of autoreactive antibodies, even in normal animals. Mechanisms responsible for these hormonal effects on B cells have not been defined, but other data suggest that T cells are an important component of the response to hormonal effects (108, 129).

IV. Molecular Biology of Gonadal Steroid/Immune System Interactions: Cytokines as Mediators of the Effects of Gonadal Steroids in the Immune System

Soluble mediators produced by cells of the immune system contribute to both general and specific, as well as local and systemic, expressions of immune function. These mediators, generally termed cytokines, control innate, nonspecific "natural" immune responses that do not depend on prior foreign molecule exposure as well as highly specific, antigen-dependent responses of "acquired" immunity (Table 3). Autoimmune responses are known to be mediated by some of these cytokines. Understanding how gonadal steroids modulate immune responsiveness will obviously involve defining whether these hormones influence the development of cells that express specific cytokines, and whether gonadal steroids directly regulate the expression of specific cytokine genes in the differentiated cells of the immune system. Such explorations have only begun.

A. Mononuclear cell products that drive "natural" immunity (Table 3)

1. Interleukin-1 (IL-1). This polypeptide molecule produced predominantly by cells of the monocyte/macrophage

TABLE 3. Some cytokines experimentally implicated in hormonal effects on immune function: cytokines that drive "natural" (not antigen-specific) immunity

Cytokine	Cell source	Known targets	Known actions	Hormonal effect
IL-1	Monocytes	Thymocytes, T cells, endothelial cells, hypothalamus	Costimulation, induction of fever and acute phase proteins	Increased by estrogens (126–127)
IL-6	T cells	Thymocytes, B cells, hepatocytes	Activation, proliferation, acute phase response	Increased in PBMC's by estrogens (130)
IL-6	Bone marrow stromal cells	Thymocytes, B cells, hepatocytes	Activation, proliferation, acute phase response	Inhibited by estrogen and by androgens (132–135)

lineage was originally defined by its costimulatory influence on T lymphocytes, increasing their proliferation in response to polyclonal activators such as Concanavalin A (Con A) or phytohemagglutinin (130). Its major function is, however, now believed to be as a mediator of "natural" immunity, *i.e.* inflammatory responses not based on specific recognition of antigens by differentiated T cells.

Sexually dimorphic levels of expression of IL-1 have been demonstrated in normal humans and in normal mouse strains. In mice, plasma levels of IL-1 measured 4 h after injection of LPS are 4-fold greater in female mice than in males (131). Similarly, peritoneal adherent cells from adult female rats show greater spontaneous IL-1 secretion than cells from males. Ovariectomy leads to decreased levels of IL-1 synthesis, and this effect is reversed by estrogen replacement (132). In contrast, levels of TNFα, a related cytokine that is also produced by monocytes and has similar biological functions, does not show sexually dimorphic levels of expression (131, 132). Mononuclear cells isolated from peripheral blood from normal women and cultured under nonstimulating conditions secrete more IL-1 than cells from men (133). The highest levels of IL-1 secretion from women's cells are observed during the follicular phase of the menstrual cycle (133). The differences in cellular secretion of IL-1 correlate with differences in the urinary excretion of IL-1, suggesting that the *in vitro* measurements reflect *in vivo* levels of production. Under conditions of stimulation with LPS (bacterial endotoxin) *in vitro*, mononuclear cells from men and women produce similar amounts of IL-1, suggesting that while the capacity to produce IL-1 is not different in males and females, *in vivo* stimulators that are present in females cause higher levels of IL-1 to be actively produced.

The potential importance of gender differences in IL-1 levels as applied to arthritis is shown by observations made in an *in vivo* animal model of cartilage damage (134). Subcutaneous implantation of cartilage in normal mice results in formation of granulomatous tissue and degradation of the implant as measured by loss of glycosoaminoglycans. Female mice show more marked cartilage loss than males, and castration of males results in degradation levels similar to those seen in intact females. These changes in cartilage content parallel the measured IL-1 levels in the granulomatous tissues surrounding the implant with higher IL-1 levels in castrated males than in their intact counterparts. Androgen replacement in castrates reverses these changes.

Gender-specific differences in IL-1 secretion may relate to the function of this cytokine in the reproductive system, where it exerts effects on development of the ovarian follicle. Thus, although the primary reason for elevated IL-1 production in females may lie outside of the immune system, a secondary result may be that female "natural" immunity is in a higher state of activation, at least during reproductive years. IL-1 has not been implicated to date as a mediator in sexually dimorphic models of autoimmunity. However, cytokines induced by IL-1, such as interleukin-2 (IL-2) (see below), are important factors in inflammatory and immune responses that relate directly to autoimmunity. Whether gender-related differences in IL-1 expression exert effects leading to autoimmune responses remains unknown.

2. *Interleukin-6 (IL-6).* This monocyte-derived cytokine can also be derived from vascular endothelial cells or fibroblasts in response to IL-1 and has a range of activities that overlap with those attributed to IL-1. All of the elements of the acute phase response can be induced by IL-6, which transcriptionally activates acute phase protein production by hepatocytes. Important actions of IL-6 also include induction of lymphocyte proliferation and B cell differentiation with IgG secretion. Elevated levels of IL-6 have been linked to human autoimmune diseases including rheumatoid arthritis and SLE (135, 136).

Estradiol (E_2) stimulates IL-6 secretion by LPS-activated human peripheral blood mononuclear cells, but supraphysiological concentrations of hormone ($\geq 10^{-6}$ M) are required to achieve the effect (137). Progesterone and testosterone have no effect on IL-6 production in peripheral blood mononuclear cells, even at high concentrations (137). Studies of the effects of hormones on skeletal homeostasis have revealed that bone marrow stromal cells produce IL-6, which promotes osteoclastogenesis. Estrogens, at physiological concentrations, inhibit this stromal cell production of IL-6 (138, 139). Testosterone and progesterone are also generally suppressive in this system, but 100-fold higher concentrations are required (139). The findings in marrow stromal cells suggest that gonadal steroids could influence IL-6 expression in the immune system since both androgens and estrogens exert negative regulatory effects on the IL-6 promoter through specific, receptor-mediated mechanisms (140, 141). The ER-mediated inhibition at the IL-6 promoter involves direct interaction of ER with NfκB and C/EBP-β (142).

B. T cell products that regulate lymphocyte activation and differentiation (Table 4)

1. *Interleukin-2 (IL-2).* IL-2 is a small polypeptide mediator produced by the Th_1 subset of helper T cells; it acts in an autocrine and paracrine fashion to stimulate other T cells to proliferate, thus amplifying local immune responses (143). Receptors for IL-2 are up-regulated by IL-2 itself and are located on many cells within the immune system, including

TABLE 4. Some cytokines experimentally implicated in hormonal effects on immune function: cytokines that regulate lymphocytes

Cytokine	Cell source	Known targets	Known actions	Hormonal effect
IL-2	Th_1 cells	T cells, B cells, NK cells	Costimulation, IL-2 and IL-2R induction	Decreased by androgens (99)
IL-4	Th_2 cells	T cells, B cells	Costimulation, induction of differentiation, IgE production	Increased by estrogens (137, 139)
TGFβ	Thymocytes	T cells, monocytes	Inhibition of growth and activation, angiogenesis	Increased by androgens (142)

NK, Natural killer.

T cells, B cells, and macrophages. Processes that depend on T cell mediation can therefore be accelerated by IL-2.

The influence of hormonal status on IL-2 expression by T cells has been examined in only a few studies, but little evidence has been adduced for any physiologically significant effect. Studies in normal male C57 Bl/6 mice show that IL-2 production by Con A-stimulated mixed populations of spleen cells is roughly 50% higher for cells from castrates than for intact males (101). The result does not appear to be due to increased number of T cells, since the castrate spleens actually contain fewer T cells than spleens from intact animals but must be attributed to increased rates of cellular production. However, no sexual dimorphism of IL-2 production can be demonstrated in anti-CD3-activated spleen cells from males and females of the normal C3H mouse strain (144). These two studies differ in that anti-CD3 is a more generalized activator than Con A, which targets only a subset of T cells, and dimorphic responses of discrete subsets could be masked. Available evidence does not indicate that androgens directly regulate IL-2 production in T cells. Dihydrotestosterone, added *in vitro*, has no effect on production of bioactive IL-2 from T cell hybridomas (144). Whether these cells express ARs is unknown.

2. *Interleukin-4 (IL-4).* Originally defined as a growth factor for B lymphocytes, IL-4 has been shown to have a wide range of functions (145). In addition to stimulating B and T cell growth, this cytokine induces class II MHC expression on a variety of cells and acts as a switch factor for turning on B cell production of the IgG and IgE classes of immunoglobulins. IL-4 is a defining cytokine for the Th_2 subset of helper T cells and acts on target cells over short distances, which suggests an important local role directed at sites of inflammation (145).

IL-4 production by activated spleen cells has been reported to be higher in normal female mice than in males of the same strain [C3H/HeN] (144). The difference is small (332 U/ml in females *vs.* 189 U/ml in males), but statistically significant ($P = 0.012$). Furthermore, treatment of male mice with a 5α-reductase inhibitor to prevent conversion of testosterone to dihydrotestosterone results in a cytokine profile that approximates that of females. Hormone levels after treatment with the inhibitor were not measured, so effects on the relative levels of estrogens and androgens could only be inferred. Levels of IL-4 produced by splenocytes are increased during pregnancy in normal mice, while IL-2 levels do not change (146). These findings are consistent with enhanced antibody production and decreased cell-mediated cytotoxic responses that occur during pregnancy. Spleen cells from normal mice of the C57Bl/6 strain do not produce detectable levels of IL-4; effects of male castration on this cytokine could not be demonstrated (101). High levels of IL-4 production are present in the androgen-resistant *Tfm* (testicular feminization) mouse, but are not due to the androgen-receptor defect in these animals, since normal Tabby male littermates of these mice also produce high levels of IL-4 (147). Transcriptional regulation of IL-4 by steroid hormones has not been reported.

3. *Transforming growth factor-β (TGFβ).* This homodimeric molecule was originally described as a regulator of neoplastic cell growth, as implied by its name. In the past decade, interest in TGFβ has resulted in generation of a long list of actions and effects within the immune system (148). Most, but not all, of these effects are negative or down-regulatory. TGFβ has antiproliferative effects on T cells and thymocytes, interferes with the activities of other cytokines such as IL-2, and interferes with induction of the IL-2 receptor.

Levels of $TGF\beta_1$ mRNA and the corresponding bioactive protein are increased in the thymus after androgen replacement in castrate male mice (149). At least one source of this thymic TGFβ is the thymocytes themselves (149). This up-regulation of TGFβ within the thymus gland could contribute to androgen-induced thymic involution. Whether thymic production of TGFβ contributes to or mediates the apparent effects of androgens on systemic immune function is more speculative. Both androgens and TGFβ have ameliorating effects in at least one model of immune activation, streptococcal cell wall-induced arthritis in rats (26, 150).

C. T cell products that regulate immune-mediated inflammation (Table 5)

1. *Interleukin-5 (IL-5).* Originally identified as an eosinophil-differentiating factor, IL-5 was subsequently found to be a relatively large 45-kDa molecule that was distinct from other known stem cell-stimulatory factors. This product of activated Th_2 cells is now known to have B cell growth factor activity as well as the ability to induce the B cell switch to IgA synthesis. Receptors for IL-5 have been detected on B cells and eosinophils but not on T cells.

Reports of the effects of gonadal steroids on IL-5 gene expression appear to be contradictory. Some activated murine T cell hybridomas have been found to down-regulate bioactive IL-5 production in the presence of 10^{-7} M dihydrotestosterone (144). In the murine T cell hybridoma NIMP-TH1 and the T cell lymphoma EL-4, androgens induce IL-5 mRNA expression, apparently by a transcriptional effect (151). Progesterone treatment of antigen-specific $CD4^+$ T cell clones has been shown to stimulate production of IL-5 (152), although the effect required supraphysiological hormone concentrations (10^{-5} M). Expression of specific gonadal ste-

TABLE 5. Some cytokines experimentally implicated in hormonal effects on immune function: cytokines that modulate immune-mediated inflammation

Cytokine	Cell source	Known targets	Known actions	Hormonal effect
IL-5	T cell hybridoma, T cell lymphoma	Eosinophils, B cells	Activation, proliferation, IgA production	Increased (144) or decreased by androgens (137)
IFNγ	Th_1 cells	Monocytes, endothelial cells, other	Activation, MHC I and II induction	Increased by estrogens (150), decreased by androgens (99, 137)

roid receptors in the respective cell lines was not demonstrated in these reports.

2. *Interferon-γ (IFNγ).* IFNγ is a defining cytokine for the Th_1 subset of helper T cells but is also produced by $CD8^+$ cells. This cytokine has pleiotropic effects, generally augmenting cell-mediated responses by up-regulating expression of class I and class II MHC molecules in target cells both within and outside of the immune system (153). Up-regulation of these MHC molecules enhances antigen presentation to differentiated effector lymphocytes. In addition, IFNγ activates mononuclear and polymorphonuclear phagocytic cells and promotes the differentiation of Th_1 cells and the maturation of $CD8^+$ cells while inhibiting the proliferation of Th_2 cells.

Female mice produce higher levels of IFNγ than male mice in response to stimulation by infectious agents such as mycobacteria and viruses (154–156). The sexual dimorphism appears to be hormonally mediated since estrogen treatment of Con A-stimulated spleen cells increases steady state levels of IFNγ mRNA (157), castration of normal male mice leads to elevated production of IFNγ by activated spleen cells (101), and dihydrotestosterone inhibits IFNγ production by anti-CD3-activated murine T cells *in vitro* (144). Moreover, estrogens directly regulate the transcriptional activity of the IFNγ promoter, through a *cis*-acting element (or elements) in the DNA between 0.5 and 3.2 kb upstream of the transcriptional start site (Fig. 9) (157). Transcriptional enhancement through the putative estrogen response elements in this promoter is dependent on ER expression (157).

V. Therapeutic Implications for Autoimmune Diseases

It is not surprising that studies of hormonal modulation in animal models have triggered interest in the use of such agents in the treatment of human autoimmune disease (158). Testosterone treatment in hypogonadal males with rheumatoid arthritis results in significant decreases in joint tenderness scores and in levels of the rheumatoid factor autoantibody (159). Relative numbers of peripheral $CD8^+$ T cells also show increases after 3 to 6 months of testosterone. No significant side effects were associated with this treatment. On the other hand, treatment of women with androgens involves the obviously undesirable side effect of masculinization and, therefore, initial trials were performed using weaker androgens such as danazol or 19-nortestosterone (160, 161). In one series of seven female patients with lupus, treatment with danazol was reported to show some improvement in disease parameters with few side effects (160). However, this study was small and the patient population was too heterogeneous to form major conclusions. Data interpretation was complicated by the fact that some of the women were postmenopausal, and relatively high concomitant doses of prednisone (20 mg/day) were allowed. In studies with 19-nortestosterone, female patients showed some clinical improvement, but serological parameters were not significantly changed (161). Male patients in this study actually had worsening of disease, possibly due to *in vivo* conversion of the administered androgen to compounds with estrogenic activity. A total of three cases have been reported of hypogonadal males with Klinefelter's syndrome in whom normalization of the serum testosterone concentration was associated with correction of serological abnormalities (including elevated anti-double-stranded DNA titers in one case) and low complement levels (15, 36). These findings suggest that androgens can modulate disease status in patients with autoimmune disease, but that compounds with significant androgenic activity are required.

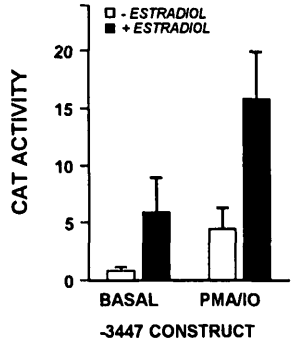

FIG. 9. Estrogens regulate the IFNγ promoter. The human T cell line Jurkatt was transfected with human ER expression vector and with reporter plasmids expressing chloramphenicol acetyl transferase (CAT) under control of the proximal −3447 bases of the murine IFNγ promoter. Cells were cultured in the absence (*open bars*) or presence (*solid bars*) of estrogen under basal conditions or under simultaneous stimulation with the T cell activators phorbol ester and calcium ionophore. Relative CAT activity compared with untreated cells is shown. [Derived from (157).]

Recent interest in the potential therapeutic effects of the adrenal androgen dehydroepiandrosterone (DHEA) was raised by a study of women with SLE (162). Clinical improvement was observed in this small, open-label trial, although many of the changes did not attain statistical significance. Serum levels of testosterone in the treated patients were elevated above the normal range for females, and some individuals may have had levels approaching the normal male range. Mechanisms underlying the observed clinical improvement were not determined, and the issue of whether this therapeutic effect was actually due to the elevated levels of testosterone or to an independent and direct effect of DHEA on cells of the immune system remains unresolved.

A therapeutically effective androgen that would produce acceptably low levels of masculinizing side effects would be a desirable goal for drug development. Such an agent would be possible if ARs or post-receptor signaling pathways in cells of the immune system differed from those in other target tissues. However, to date, no structurally or biologically different AR molecules have been described in nonmalignant tissues, and postreceptor signaling pathways have not been characterized fully in any cell. Identification of gene products that mediate androgen actions in the immune system would have obvious therapeutic implications for autoimmunity.

VI. Future Directions

It is difficult to propose any detailed model for the cellular and molecular mechanisms by which gonadal steroids mod-

ulate immune function. The observations on which such a model would be based are simply too fragmentary. However, it is clear that the era of phenomenology is about to close and that tools are now available for mechanistic studies of the nature of gonadal steroid/immune system interactions.

Existing data do provide some general outline that could guide consideration of how gonadal steroids interact with the immune system (Fig. 10). First, it is a reasonable assumption that gonadal steroid effects in the immune system are exerted through specific receptors for the respective hormones. These receptor systems have been well characterized, and their expression in specific target cells is likely necessary. The target cell types need not be restricted to lymphoid cells but may include any cell type that interacts with immune effector cells, such as thymic epithelial cells or bone marrow stromal cells. Second, the majority of well characterized receptor-mediated effects of gonadal steroids are exerted by modulating specific gene expression. Steroid hormone receptors function as ligand-activated transcription factors, and mechanisms of immune system modulation are likely to involve effects on gene transcription. Third, it is of importance to remember that gonadal steroids function as regulators of development, as well as regulators of gene expression in differentiated cells. For example, testosterone initiates a program of differentiation on components of the urogenital tract that would otherwise follow a default pathway of involution (163). These developmental effects may involve processes and mediators distinct from those involved in the action of gonadal steroids on differentiated cells. Finally, consideration must be given to the possibility that some hormonally regulated cytokines have important local actions within the reproductive system with "spill-over" effects on the immune system.

What experimental approaches are likely to yield mechanisms of potential importance for understanding the female predominance of many autoimmune diseases? Two broad areas of inquiry suggest themselves. First, developmental studies in animals with specific defects in hormonal signaling targeted to the immune system will be of considerable interest. These studies may use antisense techniques, dominant negative receptor forms, or homologous recombination techniques to target disruption of gonadal steroid receptor expression or function in immune target cells. Naturally occurring human and animal mutations in AR or ER may also permit analysis of immune system development and function in affected subjects. Second, the design and implementation of *in vitro* systems for study of T and B cell maturation will permit the analysis of cellular interactions involved in hormonal effects. The identification of relevant cell-cell interactions that may mediate gonadal steroid effects on immunity will likely be achieved by use of such *in vitro* systems.

It is extremely unlikely that hormone-mediated differences in immune reactivity are of etiological importance in the development of autoimmune diseases. However, the weight of clinical, epidemiological, and experimental evidence implicates gonadal steroids as powerful modulators of immune system function and autoimmune disease activity. The exploration of these modulatory effects holds promise not only for the explanation of the observed phenomena but also for the application of new therapies to help those afflicted by these diverse diseases.

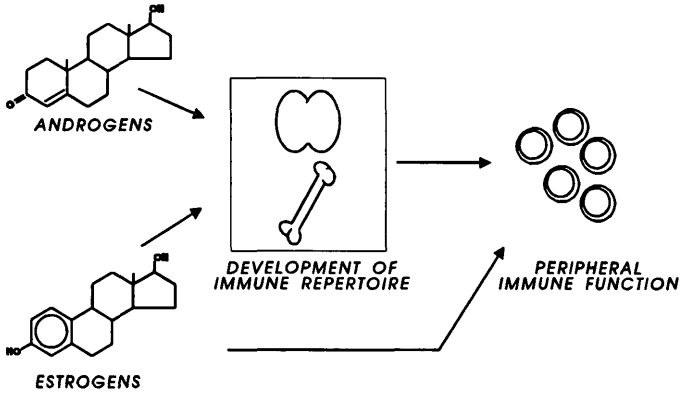

FIG. 10. Gonadal steroids likely modulate immune function by effects on developmental processes as well as effects on differentiated cells. A rudimentary working model of the sites of action of gonadal steroids on the immune system incorporates hormone actions exerted at the level of immune repertoire development (in thymus and bone marrow) as well as effects on mature peripheral T and B cell function. Available data indicate that both androgens and estrogens exert effects "centrally" on thymus and bone marrow. To date, estrogens, but not androgens, have been found to exert effects on mature peripheral immune cells.

References

1. **Butterworth M, McClellan B, Allansmith M** 1967 Influence of sex in immunoglobulin levels. Nature 214:1224–1225
2. **Lichtman MA, Vaughan JH, Hames CG** 1967 The distribution of serum immunoglobulins, anti-gamma-G globulins ("rheumatoid factors") and antinuclear antibodies in White and Negro subjects in Evans County, Georgia. Arthritis Rheum 10:204–215
3. **Grundbacher FJ** 1972 Human × chromosome carries quantitative genes for immunoglobulin. Science 176:311–312
4. **Eidinger D, Garrett TJ** 1972 Studies of the regulatory effects of the sex hormones on antibody formation and stem cell differentiation. J Exp Med 136:1098–1116
5. **Inman RD** 1978 Immunologic sex differences and the female predominance in systemic lupus erythematosus. Arthritis Rheum 21: 849–852
6. **Michaels RH, Rogers KD** 1971 A sex difference in immunologic responsiveness. Pediatrics 47:120–123
7. **Leen CL, Barclay GR, McClelland DB** 1986 Selection of plasma donors suitable for tetanus boosting. Vox Sang 51:197–201
8. **Vranckx R, Muylle L, Cole J, Moldenhaser R, Peetermans ME** 1986 HBV vaccinations in medical and paramedical staff: the impact of age on immunization results. Vox Sang 50:220–222
9. **Nurmi T, Leinonen M, Haiva VM, Tiilikainen A, Kouvalainen K** 1982 Antibody response to pneumococcal vaccine in patients with trisomy-21 (Down's syndrome). Clin Exp Immunol 48:485–490
10. **Weinstein Y, Ran S, Segal S** 1984 Sex-associated differences in the regulation of immune responses controlled by the MHC of the mouse. J Immunol 132:656–661
11. **Hallgren HM, Jackola DR, O'Leary JJ** 1983 Unusual pattern of surface marker expression on peripheral lymphocytes from aged humans suggestive of a population of less differentiated cells. J Immunol 131:191–194
12. **Nagel JE, Chrest FJ, Adler WH** 1981 Enumeration of T lymphocyte subsets by monoclonal antibodies in young and aged humans. J Immunol 127:2086–2088
13. **Mylvaganam R, Ahn YS, Harrington WJ, Kim CI, Gratzner HG** 1985 Differences in T cell subsets between men and women with idiopathic thrombocytopenic purpura. Blood 66:967–972
14. **Amadori A, Zamarchi R, DeSilvestro G, Forza G, Cavatton G, Danieli GA, Clementi M, Chieco-Bianchi L** 1995 Genetic control of the CD4/CD8 T cell ratio in humans. Nature Med 1:1279–1283

15. **Bizzarro A, Valentini G, Di Martino G, Daponte A, De Bellis A, Iacono G** 1987 Influence of testosterone therapy on clinical and immunological features of autoimmune diseases associated with Klinefelter's syndrome. J Clin Endocrinol Metab 64:32–36
16. **Hochberg MC, Spector TD** 1990 Epidemiology of rheumatoid arthritis: update. Epidemiol Rev 12:247–252
17. **Mikkelsen WM, Dodge HJ, Duff IF, Kato H** 1967 Estimates of the prevalence of rheumatic diseases in the population of Tecumseh, Michigan, 1959–60. J Chron Dis 20:351–369
18. **Furszyfer J, Kurland LT, McConahey WM, Woolner LB, Elveback LR** 1972 Epidemiologic aspects of Hashimoto's thyroiditis and Graves' disease in Rochester, Minnesota (1935–1967), with special reference to temporal trends. Metab Clin Exp 21:197–204
19. **Sherlock S, Scheuer P J** 1973 The presentation and diagnosis of 100 patients with primary biliary cirrhosis. N Engl J Med 289:674–678
20. **Roubinian JR, Papoian R, Talal N** 1977 Androgenic hormones modulate autoantibody responses and improve survival in murine lupus. J Clin Invest 59:1066–1070
21. **Roubinian JR, Talal N, Greenspan JS, Goodman JR, Siiteri PK** 1978 Effect of castration and sex hormone treatment on survival, anti-nucleic acid antibodies, and glomerulonephritis in NZB/NZW F1 mice. J Exp Med 147:1568–1583
22. **Steinberg AD, Roths JB, Murphy ED, Steinberg RT, Raveche E S** 1980 Effects of thymectomy or androgen administration upon the autoimmune disease of MRL/Mp-lpr/lpr mice. J Immunol 125: 871–873
23. **Vidal S, Gelpi C, Rodriguez-Sanchez J L** 1994 (SWR × SJL)F1 mice: a new model of lupus-like disease. J Exp Med 179:1429–1435
24. **Pozzilli P, Signore A, Williams AJ, Beales PE** 1993 NOD mouse colonies around the world–recent facts and figures. Immunol Today 14:193–196
25. **Wilder RL, Calandra GB, Garvin AJ, Wright KD, Hansen CT** 1982 Strain and sex variation in the susceptibility to streptococcal cell wall-induced polyarthritis in the rat. Arthritis Rheum 25:1064–1072
26. **Allen JB, Blatter D, Calandra GB, Wilder RL** 1983 Sex hormonal effects on the severity of streptococcal cell wall-induced polyarthritis in the rat. Arthritis Rheum 26:560–563
27. **Lahita RG, Bradlow HL, Ginzler E, Pang S, New M** 1987 Low plasma androgens in women with systemic lupus erythematosus. Arthritis Rheum 30:241–248
28. **Jungers P, Nahoul K, Pelissier C, Dougados M, Tron F, Bach JF** 1982 Low plasma androgens in women with active or quiescent systemic lupus erythematosus. Arthritis Rheum 25:454–457
29. **Lahita RG, Bradlow HL, Fishman J, Kunkel HG** 1982 Abnormal estrogen and androgen metabolism in the human with systemic lupus erythematosus. Am J Kidney Dis 2:206–211
30. **Lahita RG, Bucala R, Bradlow HL, Fishman J** 1985 Determination of 16 alpha-hydroxyestrone by radioimmunoassay in systemic lupus erythematosus. Arthritis Rheum 28:1122–1127
31. **Bucala R, Lahita RG, Fishman J, Cerami A** 1985 Increased levels of 16 alpha-hydroxyestrone-modified proteins in pregnancy and in systemic lupus erythematosus. J Clin Endocrinol Metab 60:841–847
32. **Lahita RG, Kunkel HG, Bradlow HL** 1983 Increased oxidation of testosterone in systemic lupus erythematosus. Arthritis Rheum 26:1517–1521
33. **Lahita RG, Bradlow L, Fishman J, Kunkel HG** 1982 Estrogen metabolism in systemic lupus erythematosus: patients and family members. Arthritis Rheum 25:843–846
34. **Lahita RG, Bradlow HL, Kunkel HG, Fishman J** 1981 Increased 16 alpha-hydroxylation of estradiol in systemic lupus erythematosus. J Clin Endocrinol Metab 53:174–178
35. **Lahita RG, Bradlow HL, Kunkel HG, Fishman J** 1979 Alterations of estrogen metabolism in systemic lupus erythematosus. Arthritis Rheum 22:1195–1198
36. **Olsen NJ, Kovacs WJ** 1995 Case Report:testosterone treatment of systemic lupus erythematosus in a patient with Klinefelter's syndrome. Am J Med Sci 310:158–160
37. **Roubinian JR, Talal N, Greenspan JS, Goodman JR, Siiteri PK** 1979 Delayed androgen treatment prolongs survival in murine lupus. J Clin Invest 63:902–911
38. **Roubinian JR, Papoian R, Talal N** 1977 Effects of neonatal thymectomy and splenectomy on survival and regulation of autoantibody formation in NZB/NZW F1 mice. J Immunol 118:1524–1529
39. **Walker SE, Besch-Williford CL, Keisler DH** 1994 Accelerated deaths from systemic lupus erythematosus in NZB × NZW F1 mice treated with the testosterone-blocking drug flutamide. J Lab Clin Med 124:401–407
40. **Roubinian JR, Talal N, Greenspan JS, Goodman JR, Nussenzweig V** 1979 Danazol's failure to suppress autoimmunity in NZB/NZW F1 mice. Arthritis Rheum 22:1399–1402
41. **Ariga H, Edwards J, Sullivan DA** 1989 Androgen control of autoimmune expression in lacrimal glands of MRL/Mp-lpr/lpr mice. Clin Immunol Immunopathol 53:499–508
42. **Fitzpatrick F, Lepault F, Homo-Delarche F, Bach JF, Dardenne M** 1991 Influence of castration, alone or combined with thymectomy, on the development of diabetes in the nonobese diabetic mouse. Endocrinology 129:1382–1390
43. **Fox HS** 1992 Androgen treatment prevents diabetes in nonobese diabetic mice. J Exp Med 175:1409–1412
44. **Bendelac A, Carnaud C, Boitard C, Bach JF** 1987 Syngeneic transfer of autoimmune diabetes from diabetic NOD mice to healthy neonates. Requirement for both L3T4$^+$ and Lyt-2$^+$ T cells. J Exp Med 166:823–832
45. **Henderson J** 1904 On the relationship of the thymus to the sexual organs. I. The influence of castration on the thymus. J Physiol 31:222–229
46. **Fitzpatrick FT, Kendall MD, Wheeler MJ, Adcock IM, Greenstein BD** 1985 Reappearance of thymus of ageing rats after orchidectomy. J Endocrinol 106:R17–R19
47. **Olsen NJ, Watson MB, Henderson GS, Kovacs WJ** 1991 Androgen deprivation induces phenotypic and functional changes in the thymus of adult male mice. Endocrinology 129:2471–2476
48. **Greenstein BD, Fitzpatrick FT, Adcock IM, Kendall MD, Wheeler MJ** 1986 Reappearance of the thymus in old rats after orchidectomy: inhibition of regeneration by testosterone. J Endocrinol 110: 417–422
49. **Olsen NJ, Kovacs WJ** 1989 Increased thymic size and thymocyte interleukin 2 production in androgen-resistant mice. Scand J Immunol 29:733–738
50. **Aboudkhil S, Bureau JP, Garrelly L, Vago P** 1991 Effects of castration, Depo-testosterone and cyproterone acetate on lymphocyte T subsets in mouse thymus and spleen. Scand J Immunol 34:647–653
51. **Olsen NJ, Viselli SM, Shults K, Stelzer G, Kovacs WJ** 1994 Induction of immature thymocyte proliferation after castration of normal male mice. Endocrinology 134:107–113
52. **Barr IG, Khalid BA, Pearce P, Toh BH, Bartlett PF, Scollay RG, Funder JW** 1982 Dihydrotestosterone and estradiol deplete corticosensitive thymocytes lacking in receptors for these hormones. J Immunol 128:2825–2828
53. **Sato N, Kyakumoto S, Kurokawa R, Ota M** 1986 Characteristics of cytosol androgen receptor in rat thymus. Biochem Int 13:15–23
54. **McCruden AB, Stimson WH** 1984 Androgen receptor in the human thymus. Immunol Lett 8:49–53
55. **Kovacs WJ, Olsen NJ** 1987 Androgen receptors in human thymocytes. J Immunol 139:490–493
56. **Pearce P, Khalid BA, Funder JW** 1981 Androgens and the thymus. Endocrinology 109:1073–1077
57. **Vojtiskova M, Hilgertova J, Draber P** 1981 Localization of androgen receptors in mouse thymus. Folia Biol (Krakow) 27:203–208
58. **Raveche ES, Vigersky RA, Rice MK, Steinberg AD** 1980 Murine thymic androgen receptors. J Immunopharmacol 2:425–434
59. **Grossman CJ, Sholiton LJ, Helmsworth JA** 1983 Characteristics of the cytoplasmic and nuclear dihydrotestosterone receptors of human thymic tissue. Steroids 42:11–22
60. **Grossman CJ, Nathan P, Taylor BB, Sholiton LJ** 1979 Rat thymic dihydrotestosterone receptor: preparation, location and physiochemical properties. Steroids 34:539–553
61. **McCruden AB, Stimson WH** 1981 Androgen binding cytosol receptors in the rat thymus: physicochemical properties, specificity and localisation. Thymus 3:105–117
62. **Stites DP, Pavia CS, Clemens LE, Kuhn RW, Siiteri PK** 1979 Immunologic regulation in pregnancy. Arthritis Rheum 22:1300–1307
63. **Viselli SM, Olsen NJ, Shults K, Steizer G, Kovacs WJ** 1995 Im-

munochemical and flow cytometric analysis of androgen receptor expression in thymocytes. Mol Cell Endocrinol 109:19–26
64. **Itoh T, Kasahara S, Mori T** 1982 A thymic epithelial cell line, IT-45R1, induces the differentiation of prethymic progenitor cells into postthymic cells through direct contact. Thymus 4:69–75
65. **Okuyama R, Abo T, Seki S, Ohteki T, Sugiura K, Kusumi A, Kumagai K** 1992 Estrogen administration activates extrathymic T cell differentiation in the liver. J Exp Med 175:661–669
66. **Phuc LH, Papiernik M, Dardenne M** 1981 Thymic involution in pregnant mice. II. Functional aspects of the remaining thymocytes. Clin Exp Immunol 44:253–261
67. **Phuc LH, Papiernik M, Berrih S, Duval D** 1981 Thymic involution in pregnant mice. I. Characterization of the remaining thymocyte subpopulations. Clin Exp Immunol 44:247–252
68. **Screpanti I, Meco D, Morrone S, Gulino A, Mathieson BJ, Frati L** 1991 In vivo modulation of the distribution of thymocyte subsets: effects of estrogen on the expression of different T cell receptor V beta gene families in CD4-, CD8- thymocytes. Cell Immunol 134: 414–426
69. **Screpanti I, Morrone S, Meco D, Santoni A, Gulino A, Paolini R, Crisanti A, Mathieson BJ, Frati L** 1989 Steroid sensitivity of thymocyte subpopulations during intrathymic differentiation. Effects of 17 beta-estradiol and dexamethasone on subsets expressing T cell antigen receptor or IL-2 receptor. J Immunol 142:3378–3383
70. **Nilsson B, Carlsson S, Damber MG, Lindblom D, Sodergard R, von Schoultz B** 1984 Specific binding of 17 beta-estradiol in the human thymus. Am J Obstet Gynecol 149:544–547
71. **Athreya BH, Moore WC, Wadsworth SA, Gupta C, Goldman A S** 1989 Estrogen receptor levels in a murine model of systemic lupus erythematosus. Clin Exp Rheumatol 7:589–593
72. **Morgan DD, Grossman CJ** 1984 Analysis and properties of the cytosolic estrogen receptor from rat thymus. Endocr Res 10:193–207
73. **Gulino A, Screpanti I, Torrisi MR, Frati L** 1985 Estrogen receptors and estrogen sensitivity of fetal thymocytes are restricted to blast lymphoid cells. Endocrinology 117:47–54
74. **Nilsson B, Bergqvist A, Lindblom D, Ljungberg O, Sodergard R, von Schoultz B** 1986 Characterization and localization of specific oestrogen binding in the human thymus. Gynecol Obstet Invest 21:150–157
75. **Carbone A, Piantelli M, Musiani P, Larocca LM, Aiello FB, Maggiano N, Scoppetta C, Crucitti F, Ranelletti FO** 1986 Estrogen binding sites in peripheral blood mononuclear cells and thymocytes from 2 myasthenia gravis patients. J Clin Lab Immunol 21: 87–91
76. **Morgan DD, Grossman CJ** 1985 Studies on the cytosolic estrogen receptor from rat thymus. Thymus 7:279–286
77. **Imanishi Y, Seiki K, Haruki Y** 1980 Cytoplasmic estrogen receptor in castrated rat thymus. Endocrinol Jpn 27:395–399
78. **Seiki K, Imanishi Y, Haruki Y, Enomoto T** 1979 Estrogen receptor in the thymus of the castrated mice. Endocrinol Jpn 26:159–165
79. **Screpanti I, Gulino A, Pasqualini JR** 1982 The fetal thymus of guinea pig as an estrogen target organ. Endocrinology 111:1552–1561
80. **Malacarne P, Piffanelli A, Indelli M, Fumero S, Mondino A, Gionchiglia E, Silvestri S** 1980 Estradiol binding in rat thymus cells. Horm Res 12:224–232
81. **Danel L, Souweine G, Monier JC, Saez S** 1983 Specific estrogen binding sites in human lymphoid cells and thymic cells. J Steroid Biochem 18:559–563
82. **Grossman CJ, Sholiton LJ, Blaha GC, Nathan P** 1979 Rat thymic estrogen receptor. II. Physiological properties. J Steroid Biochem 11:1241–1246
83. **Grossman CJ, Sholiton LJ, Nathan P** 1979 Rat thymic estrogen receptor. I. Preparation, location and physiochemical properties. J Steroid Biochem 11:1233–1240
84. **Reichman ME, Villee CA** 1978 Estradiol binding by rat thymus cytosol. J Steroid Biochem 9:637–641
85. **Imanishi Y, Haruki Y, Seiki K** 1980 Estrogen receptor in rat thymus cytosol. Tokai J Exp Clin Med 5:263–267
86. **Kawashima I, Sakabe K, Seiki K, Fujii-Hanamoto H, Akatsuka A, Tsukamoto H** 1991 Localization of sex steroid receptor cells, with special reference to thymulin (FTS)-producing cells in female rat thymus. Thymus 18:79–93
87. **Kawashima I, Seiki K, Sakabe K, Ihara S, Akatsuka A, Katsumata Y** 1992 Localization of estrogen receptors and estrogen receptor-mRNA in female mouse thymus. Thymus 20:115–121
88. **Gillette S, Gillette RW** 1979 Changes in thymic estrogen receptor expression following orchidectomy. Cell Immunol 42:194–196
89. **Gulino A, Screpanti I, Pasqualini JR** 1983 Estrogen and antiestrogen effects on different lymphoid cell populations in the developing fetal thymus of guinea pig. Endocrinology 113:1754–1762
90. **Thompson Jr EA** 1981 The effects of estradiol upon the thymus of the sexually immature female mouse. J Steroid Biochem 14:167–174
91. **Haruki Y, Seiki K, Enomoto T, Fujii H, Sakabe K** 1983 Estrogen receptor in the "non-lymphocytes" in the thymus of the ovariectomized rat. Tokai J Exp Clin Med 8:31–39
92. **Pearce P, Funder JW** 1986 Cytosol and nuclear levels of thymic progesterone receptors in pregnant, pseudopregnant and steroid-treated rats. J Steroid Biochem 25:65–69
93. **Nelson JL, Hughes KA, Smith AG, Nisperos BB, Branchaud AM, Hansen JA** 1993 Maternal-fetal disparity in HLA class II alloantigens and the pregnancy-induced amelioration of rheumatoid arthritis. N Engl J Med 329:466–471
94. **Nilsson B, Ferno M, von Schoultz B** 1990 Estrogen and progesterone receptors in the human thymus. Gynecol Obstet Invest 29: 289–291
95. **Fujii-Hanamoto H, Seiki K, Sakabe K, Ogawa H** 1985 Progestin receptor in the thymus of ovariectomized immature rats. J Endocrinol 107:223–229
96. **Fujii-Hanamoto H, Grossman CJ, Roselle GA, Mendenhall CL, Seiki K** 1990 Nuclear progestin receptors in rat thymic tissue. Thymus 15:31–45
97. **Sakabe K, Seiki K, Fujii-Hanamoto H** 1986 Histochemical localization of progestin receptor cells in the rat thymus. Thymus 8:97–107
98. **Naray A** 1981 Progesterone receptor in the chick thymus. Biochem Biophys Res Commun 98:866–874
99. **Pearce PT, Khalid BA, Funder JW** 1983 Progesterone receptors in rat thymus. Endocrinology 113:1287–1291
100. **Weinstein Y, Berkovich Z** 1981 Testosterone effect on bone marrow, thymus, and suppressor T cells in the (NZB × NZW)F1 mice: its relevance to autoimmunity. J Immunol 126:998–1002
101. **Viselli SM, Stanziale S, Shults K, Kovacs WJ, Olsen NJ** 1995 Castration alters peripheral immune function in normal male mice. Immunology 84:337–342
102. **Cohen JH, Danel L, Cordier G, Saez S, Revillard JP** 1983 Sex steroid receptors in peripheral T cells: absence of androgen receptors and restriction of estrogen receptors to OKT8-positive cells. J Immunol 131:2767–2771
103. **Kumar N, Shan L-X, Hardy MP, Bardin CW** 1995 Mechanism of androgen-induced thymolysis. Endocrinology 136:4887–4893
104. **Takeda H, Chodak G, Mutchnik S, Nakamoto T, Chang C** 1990 Immunohistochemical localization of androgen receptors with monoclonal and polyclonal antibodies to androgen receptor. J Endocrinol 126:17–23
105. **Grossman CJ** 1984 Regulation of the immune system by sex steroids. Endocr Rev 5:435–455
106. **Carlsten H, Tarkowski A, Holmdahl R, Nilsson LA** 1990 Oestrogen is a potent disease accelerator in SLE-prone MRL lpr/lpr mice. Clin Exp Immunol 80:467–473
107. **Muller D, Chen M, Vikingsson A, Hildeman D, Pederson K** 1995 Oestrogen influences $CD4^+$ T-lymphocyte activity *in vivo* and *in vitro* in β_2-microglobulin-deficient mice. Immunology 86:162–167
108. **Paavonen T, Andersson LC, Adlercreutz H** 1981 Sex hormone regulation of *in vitro* immune response. Estradiol enhances human B cell maturation via inhibition of suppressor T cells in pokeweed mitogen-stimulated cultures. J Exp Med 154:1935–1945
109. **Stimson WH** 1988 Oestrogen and human T lymphocytes: presence of specific receptors in the T-suppressor/cytotoxic subset. Scand J Immunol 28:345–350
110. **Cutolo M, Accardo S, Villaggio B, Clerico P, Bagnasco M, Coviello DA, Carruba G, lo Casto M, Castagnetta L** 1993 Presence of estrogen-binding sites on macrophage-like synoviocytes and $CD8^+$, $CD29^+$, $CD45RO^+$ T lymphocytes in normal and rheumatoid synovium. Arthritis Rheum 36:1087–1097
111. **Larsson P, Goldschmidt TJ, Klareskog L, Holmdahl R** 1989 Oes-

trogen-mediated suppression of collagen-induced arthritis in rats. Studies on the role of the thymus and of peripheral $CD8^+$ T lymphocytes. Scand J Immunol 30:741–747
112. **Evagelatou M, Farrant J** 1995 Effect of oestradiol-17 beta on the expression of oestrogen receptor mRNA in human tonsillar cells. J Mol Endocrinol 14:13–19
113. **Mase Y, Oishi T** 1991 Effects of castration and testosterone treatment on the development and involution of the bursa of fabricius and the thymus in the Japanese quail. Gen Comp Endocrinol 84: 426–433
114. **Wilson CA, Mrose SA, Thomas DW** 1995 Enhanced production of B lymphocytes after castration. Blood 85:1535–1539
115. **Medina KL, Kincade PW** 1994 Pregnancy-related steroids are potential negative regulators of B lymphopoiesis. Proc Natl Acad Sci USA 91:5382–5386
116. **Olsen N, Viselli S, Reese K, Fan J, Kovacs W** 1996 Castration of normal male mice results in B cell expansion which is reversible with androgen replacement. J Invest Med 44:38A (Abstract)
117. **Danel L, Vincent C, Rousset F, Klein B, Bataille R, Flacher M, Durie BG, Revillard JP** 1988 Estrogen and progesterone receptors in some human myeloma cell lines and murine hybridomas. J Steroid Biochem 30:363–367
118. **Tarlinton D** 1994 B-cell differentiation in the bone marrow and the periphery. Immunol Rev 137:203–229
119. **Medina KL, Smithson G, Kincade PW** 1993 Suppression of B lymphopoiesis during normal pregnancy. J Exp Med 178:1507–1515
120. **Masuzawa T, Miyaura C, Onoe Y, Kusano K, Ohta H, Nozawa S, Suda T** 1994 Estrogen deficiency stimulates B lymphopoiesis in mouse bone marrow. J Clin Invest 94:1090–1097
121. **Smithson G, Beamer WG, Shultz KL, Christianson SW, Shultz LD, Kincade PW** 1994 Increased B lymphopoiesis in genetically sex steroid-deficient hypogonadal (hpg) mice. J Exp Med 180:717–720
122. **Kincade PW, Medina KL, Smithson G** 1994 Sex hormones as negative regulators of lymphopoiesis. Immunol Rev 137:119–134
123. **Smithson G, Medina K, Ponting I, Kincade PW** 1995 Estrogen suppresses stromal cell-dependent lymphopoiesis in culture. J Immunol 155:3409–3417
124. **Bellido T, Girasole G, Passeri G, Yu XP, Mocharla H, Jilka RL, Notides A, Manolagas SC** 1993 Demonstration of estrogen and vitamin D receptors in bone marrow-derived stromal cells: up-regulation of the estrogen receptor by 1,25-dihydroxyvitamin-D3. Endocrinology 133:553–562
125. **Ansar Ahmed S, Dauphinee MJ, Montoya AI, Talal N** 1989 Estrogen induces normal murine $CD5^+$ B cells to produce autoantibodies. J Immunol 142:2647–2653
126. **Ahmed SA, Verthelyi D** 1993 Antibodies to cardiolipin in normal C57BL/6J mice: induction by estrogen but not dihydrotestosterone. J Autoimmun 6:265–279
127. **Verthelyi D, Ahmed SA** 1994 17 beta-estradiol, but not 5 alpha-dihydrotestosterone, augments antibodies to double-stranded deoxyribonucleic acid in nonautoimmune C57BL/6J mice. Endocrinology 135:2615–2622
128. **Castro JE** 1974 Orchidectomy and the immune response. I. Effect of orchidectomy on lymphoid tissues of mice. Proc R Soc Lond [Biol] 185:425–436
129. **Brick JE, Wilson DA, Walker SE** 1985 Hormonal modulation of responses to thymus-independent and thymus-dependent antigens in autoimmune NZB/W mice. J Immunol 134:3693–3698
130. **Dinarello CA** 1984 Interleukin-1 and the pathogenesis of the acute-phase response. N Engl J Med 311:1413–1418
131. **Li P, Allen H, Banerjee S, Franklin S, Herzog L, Johnston C, McDowell J, Paskind M, Rodman L, Salfeld J, Towne E, Tracey D, Wardwell S, Wei F-Y, Wong W, Kamen R, Seshadri T** 1995 Mice deficient in IL-1 beta-converting enzyme are defective in production of mature IL-1 beta and resistant to endotoxic shock. Cell 80:401–411
132. **Hu SK, Mitcho YL, Rath NC** 1988 Effect of estradiol on interleukin 1 synthesis by macrophages. Int J Immunopharmacol 10:247–252
133. **Lynch EA, Dinarello CA, Cannon JG** 1994 Gender differences in IL-1 alpha, IL-1 beta, and IL-1 receptor antagonist secretion from mononuclear cells and urinary excretion. J Immunol 153:300–306
134. **DaSilva JAP, Larbre J-P, Seed MP, Cutolo M, Villaggio B, Scott DL, Willoughby DA** 1994 Sex differences in inflammation induced cartilage damage in rodents. The influence of sex steroids. J Rheumatol 21:330–337
135. **Linker-Israeli M, Deans RJ, Wallace DJ, Prehn J, Ozeri-Chen T, Klinenberg JR** 1991 Elevated levels of endogenous IL-6 in systemic lupus erythematosus. A putative role in pathogenesis. J Immunol 147:117–123
136. **Dasgupta B, Corkill M, Kirkham B, Gibson T, Panayi G** 1992 Serial estimation of interleukin 6 as a measure of systemic disease in rheumatoid arthritis. J Rheumatol 19:22–25
137. **Li ZG, Danis VA, Brooks PM** 1993 Effect of gonadal steroids on the production of IL-1 and IL-6 by blood mononuclear cells *in vitro*. Clin Exp Rheumatol 11:157–162
138. **Jilka RL, Hangoc G, Girasole G, Passeri G, Williams DC, Abrams JS, Boyce B, Broxmeyer H, Manolagas SC** 1992 Increased osteoclast development after estrogen loss: mediation by interleukin-6. Science 257:88–91
139. **Girasole G, Jilka RL, Passeri G, Boswell S, Boder G, Williams DC, Manolagas SC** 1992 17 Beta-estradiol inhibits interleukin-6 production by bone marrow-derived stromal cells and osteoblasts *in vitro*: a potential mechanism for the antiosteoporotic effect of estrogens. J Clin Invest 89:883–891
140. **Bellido T, Jilka RL, Boyce BF, Girasole G, Broxmeyer H, Dalrymple SA, Murray R, Manolagas SC** 1995 Regulation of interleukin-6, osteoclastogenesis, and bone mass by androgens. The role of the androgen receptor. J Clin Invest 95:2886–2895
141. **Pottratz ST, Bellido T, Mocharta H, Crabb D, Manolagas SC** 1994 17β-Estradiol inhibits expression of human interleukin-6 promoter-reporter constructs by a receptor-dependent mechanism. J Clin Invest 93:944–950
142. **Stein B, Yang MX** 1995 Repression of the interleukin-6 promoter by estrogen receptor is mediated by NF-kappa B and C/EBP-β. Mol Cell Biol 15:4971–4979
143. **Smith KA** 1988 Interleukin-2: inception, impact, and implications. [Review]. Science 240:1169–1176
144. **Araneo BA, Dowell T, Diegel M, Daynes RA** 1991 Dihydrotestosterone exerts a depressive influence on the production of interleukin-4 (IL-4), IL-5, and gamma-interferon, but not IL-2 by activated murine T cells. Blood 78:688–699
145. **Paul WE** 1991 Interleukin-4: a prototypic immunoregulatory lymphokine. Blood 77:1859–1870
146. **Dudley DJ, Chen CL, Mitchell MD, Daynes RA, Araneo BA** 1993 Adaptive immune responses during murine pregnancy: pregnancy-induced regulation of lymphokine production by activated T lymphocytes. Am J Obstet Gynecol 168:1155–1163
147. **Olsen NJ, Watson MB, Kovacs WJ** 1991 Studies of immunological function in mice with defective androgen action. Distinction between alterations in immune function due to hormonal insensitivity and alterations due to other genetic factors. Immunology 73: 52–57
148. **Wahl SM** 1992 Transforming growth factor beta (TGF-beta) in inflammation: a cause and a cure. J Clin Immunol 12:61–74
149. **Olsen NJ, Zhou P, Ong H, Kovacs WJ** 1993 Testosterone induces expression of transforming growth factor-beta 1 in the murine thymus. J Steroid Biochem Mol Biol 45:327–332
150. **Brandes ME, Allen JB, Ogawa Y, Wahl SM** 1991 Transforming growth factor beta 1 suppresses acute and chronic arthritis in experimental animals. J Clin Invest 87:1108–1113
151. **Wang Y, Campbell HD, Young IG** 1993 Sex hormones and dexamethasone modulate interleukin-5 gene expression in T lymphocytes. J Steroid Biochem Mol Biol 44:203–210
152. **Piccinni MP, Giudizi MG, Biagiotti R, Beloni L, Giannarini L, Sampognaro S, Parronchi P, Manetti R, Annunziato F, Livi C, et al** 1995 Progesterone favors the development of human T helper cells producing Th2-type cytokines and promotes both IL-4 production and membrane CD30 expression in established Th1 cell clones. J Immunol 155:128–133
153. **Farrar MA, Schreiber RD** 1993 The molecular cell biology of interferon-gamma and its receptor. Annu Rev Immunol 11:571–611
154. **Sarvetnick N, Fox HS** 1990 Interferon-gamma and the sexual dimorphism of autoimmunity. Mol Biol Med 7:323–331
155. **Huygen K, Palfliet K** 1984 Strain variation in interferon gamma production of BCG-sensitized mice challenged with PPD. II. Im-

portance of one major autosomal locus and additional sexual influences. Cell Immunol 85:75–81
156. **McFarland HI, Bigley NJ** 1989 Sex-dependent, early cytokine production by NK-like spleen cells following infection with the D variant of encephalomyocarditis virus (EMCV-D). Viral Immunol 2:205–214
157. **Fox HS, Bond BL, Parslow TG** 1991 Estrogen regulates the IFN-gamma promoter. J Immunol 146:4362–4367
158. **Van Vollenhoven RF, McGuire JL** 1994 Estrogen, progesterone, and testosterone: can they be used to treat autoimmune diseases?. Cleve Clin J Med 61:276–284
159. **Cutolo M, Balleari E, Giusti M, Intra E, Accardo S** 1991 Androgen replacement therapy in male patients with rheumatoid arthritis. Arthritis Rheum 34:1–5
160. **Agnello V, Pariser K, Gell J, Gelfand J, Turksoy RN** 1983 Preliminary observations on danazol therapy of systemic lupus erythematosus: effects on DNA antibodies, thrombocytopenia and complement. J Rheumatol 10:682–687
161. **Lahita RG, Cheng CY, Monder C, Bardin CW** 1992 Experience with 19-nortestosterone in the therapy of systemic lupus erythematosus: worsened disease after treatment with 19-nortestosterone in men and lack of improvement in women. J Rheumatol 19:547–555
162. **Van Vollenhoven RF, Engleman EG, McGuire JL** 1994 An open study of dehydroepiandrosterone in systemic lupus erythematosus. Arthritis Rheum 37:1305–1310
163. **Jost A, Vigier B, Prepin J, Perchellet JP** 1973 Studies on sex differentiation in mammals. Recent Prog Horm Res 29:1–41