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Sex steroids and the immune system

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Immunology in its inception was believed to be a science dealing solely with the defense of the body against infections. The Oxford dictionary defined immunology as “the study of resistance to infection in man and animals” [1]. Since then, there has been an immense growth in immunology. This growth has resulted in increased awareness of the role of the immune system in disease states, such as allergic, autoimmune, and cardiovascular diseases, and its interactions with other systems of the body.

An interaction between the immune system and reproductive hormones was observed as early as the 19th century. In 1898, Calzoari described the changes in the thymus following castration [2]. At that time, the thymus was believed to have no functional importance, and his observations failed to capture the attention of scientists. Much later, clinicians noted a higher prevalence of autoimmune diseases in women. Despite this evidence of sex hormonal involvement in autoimmune diseases, there were no studies addressing this issue. In 1972, a report by the National Advisory Committee on the Future of Arthritis Research sponsored by the Arthritis Foundation urged that “an explanation be sought for the remarkable female to male ratio in systemic lupus” [1].

A significant body of data establishes that immune responsiveness differs between men and women and that gonadal steroids are involved intimately in this immunologic dimorphism. Because autoimmune diseases result from the perturbation of normal immune function, autoimmunity also demonstrates a dimorphic pattern. This article examines the effects of sex steroids on the immune system. The sexual dimorphism that is observed in normal immune response and in susceptibility to infections and autoimmune disorders is reviewed. The effects of individual sex steroids on different aspects of the immune function are summarized.

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Sexual dimorphism in the immune response

Normal immune response

A number of aspects of immune responsiveness differ between males and females. This observation is true in many species, including humans, and applies to humoral and cellular responses. Women have higher plasma immunoglobulin M (IgM) and IgG levels than do men [3]. The most prominent difference in humoral immunity is seen in the plasma IgM levels, and this difference becomes most significant at puberty. Plasma IgG levels are also higher in women, although some studies failed to show a difference among whites [3,4–6]. Women with Turner's syndrome have lower levels of gonadal steroids associated with a lower serum IgG and IgM, compared with normal women [7]. In animal studies, females show stronger antibody responses to exogenous antigens [8]. This gender difference in humoral response is also present in humans [9].

Cell-mediated immunity is also sexually dimorphic. Compared with men, women have a higher proportion of helper and inducer T cells ($CD4^{+}$) and a lower proportion of cytotoxic and suppressor T cells ($CD8^{+}$) in the peripheral blood, which results in a significantly higher $CD4^{+}:CD8^{+}$ ratio in women [10,11]. The sexual dimorphism in the prevalence of T-cell subtypes seems to be mediated by circulating sex steroids. Comparison of the $CD4^{+}:CD8^{+}$ ratio between elderly men and postmenopausal women reveals no differences [11]. T-cell subset distribution in hypogonadal men is similar to that of women [12]. In vivo administration of estrogen alters peripheral T-cell activity in a number of model systems in ways that suggest enhancement of helper and inducer ($CD4^{+}$) activity or suppression of cytotoxic and suppressor ($CD8^{+}$) T-cell activity (discussed later).

Susceptibility to infections

Males of many species, ranging from nematodes and insects to fish, reptiles, birds, mammals, and humans, have shorter life spans than do females [13]. Death rates were higher for men than women in the United States from 1940 to 1998 [14]. There are several hypotheses for sex differences in life-span longevity. Life span may be shorter in males than in females because males are more likely to engage in risk-taking behaviors. Another possible contributing factor to shorter life span in males is their higher susceptibility to infections.

Several clinical studies of humans and field studies of animals demonstrate that males are more susceptible to infections than females. Although these studies are not controlled for factors such as exposure rates, social behavior, habitat, and diet, which may contribute to the observed differences in infection, laboratory studies of rodents under controlled settings report similar findings. As reviewed in detail by Klein, even under controlled settings, males are more susceptible to infections caused by parasites (protozoa, helminth, cestodes, arthropods), fungi, bacteria, and viruses than are females. Administration of testosterone increases susceptibility to infections, whereas castration of males or administration of estradiol has the

opposite effect, suggesting that the sexual dimorphism in susceptibility to infections is mediated by sex steroids (Table 1) [15]. For example, host resistance to *Mycobacterium* infection in mice differs between sexes: Males are more susceptible than are females. Castration causes an increase in the host resistance of males, but this effect is reversed substantially by testosterone treatment. Testosterone has a similar effect when administered to female mice [16].

Although the reasons for sexual dimorphism in immune response and susceptibility to infections is not understood entirely, it is possible that the increased immune responsiveness in females may allow them to compensate for the increased physiologic stress that accompanies reproduction. The final outcome would be the assurance of reproductive success of the species.

Autoimmunity

Autoimmune diseases result from activation of a specific humoral or cell-mediated immune response against constituents of the body's own tissues and cause significant morbidity and mortality. Although the greater immune reactivity of females may provide better protection against infectious agents, it also may predispose females to autoimmune disorders, which occur more often in females than in males.

Jacobson and colleagues reviewed the epidemiology of autoimmune diseases in the United States [17]. They identified 24 autoimmune diseases using the criteria of Rose and Bona [18], which define an autoimmune disease according to the existence of direct proof or indirect evidence of autoimmune pathogenesis. Rose and Bona defined direct proof of an autoimmune cause as "disease reproducibility through direct transfer of autoantibody or autoantigen-specific T cells to animals or into in vitro systems." Indirect evidence of an autoimmune cause was defined as "disease reproducibility in animals through experimental immunization with the implicated autoantigen or through cell transfer from animals with genetically determined models of disease." Isolation of autoantibodies or identification of self-reactive T cells from the major target tissues of patients affected by the disease also was considered indirect evidence of an autoimmune cause [18]. Using these criteria, Jacobson et al estimated that approximately 8.5 million people in the United States (1 in 31 Americans) were affected by autoimmune diseases [17]. The diseases with the highest prevalence rates were Grave's disease, insulin-dependent diabetes mellitus, pernicious anemia, rheumatoid arthritis (RA), thyroiditis, vitiligo, glomerulonephritis, multiple sclerosis (MS), and systemic lupus erythematosus (SLE), comprising more than 95% of cases. Compared with men, women more commonly are affected by many of these autoimmune diseases, and women have a 2.7 times greater risk for acquiring autoimmune disease (Table 2).

Several clinical observations suggest that sexual dimorphism in autoimmunity results from different levels of circulating sex steroids. Altered hormonal states, such as pregnancy, oral contraceptive use, and menopause, change the course of autoimmune diseases in humans. RA usually improves during pregnancy, whereas the effect of pregnancy on SLE is more variable [19]. Hypogonadal males have

Table 1

Viral, bacterial, fungal, and macroparasite infections for which males are more susceptible than females

Pathogen	Host	Measure	Effects of hormone manipulation ^a	Reference
Viruses				
Arbovirus	Mus musculus	I	T↑	[143]
Coxsackievirus	Mus musculus	P	T↑	[144]
Encephalomyocarditis virus	Mus musculus	I	T↑, C↓, E↓	[145]
Equine arteritis virus	Equus caballus	I	T↑, C↓	[146]
Friend virus	Mus musculus	I	E↓	[147]
Hantaviruses	Homo sapiens	P	—	[148]
	Peromyscus boylii	P	—	[149]
	Peromyscus maniculatus	P	—	[149]
	Rattus norvegicus	I	NE	[150]
	Reithrodontomys megalotis	P	—	[151]
	Sigmodon hispidus	P	—	[152]
Polyoma virus	Mus musculus	I	T↑, C↓	[153]
Vesicular stomatitis virus	Mus musculus	I	—	[154]
Bacteria				
<i>Corynebacterium kutscheri</i>	Mus musculus	I	C↓	[155]
<i>Leptospira</i> spp	Apodemus sylvaticus	P	—	[156]
<i>Mycobacterium marinum</i>	Mus musculus	I	T↑, C↓	[16]
<i>Salmonella typhimurium</i>	Mus musculus	I	E↓	[157]
Fungi				
<i>Candida albicans</i>	Homo sapiens	I	E↓	[158]
	Mus musculus	I	C↓	[159]
<i>Paracoccidioides brasiliensis</i>	Mus musculus	I	E↓	[160]
Protozoa				
<i>Brugia malayi</i>	Mus musculus	I	T↑	[161]
	Meriones unguiculatus	I	—	[162]
<i>B. pahangi</i>	Mus musculus	I	—	[163]
<i>Giardia muris</i>	Mus musculus	I	—	[164]
<i>Leishmania donovani</i>	Homo sapiens	P	—	[165]
<i>Leishmania mexicana</i>	Mus musculus	I	E↓	[166,167]
<i>Plasmodium mexicanum</i>	Sceloporus occidentalis	P	—	[168]
<i>Plasmodium</i> spp.	Parus major	P	—	[169]
<i>Trypanozoma cruzi</i>	Mus musculus	I	E↓	[170]
Helminths				
<i>Alaria taxideae</i>	Martes Americana	I	—	[171]
<i>Angiostrongylus malaysiensis</i>	Rattus norvegicus	I	T↑, C↓	[172]
<i>Capillaria muris</i>	Clethrionomys glareolus	P	—	[173]
<i>Crepidostomum farionis</i>	Salmo trutta L.	I,P	—	[174]
<i>Dirofilaria immitis</i>	Canis familiaris	P	—	[175]
<i>Discocotyle sagittata</i>	Salmo trutta L.	I,P	—	[176]
<i>Elaphostrongylus rangiferi</i>	Rangifer tarandus	I	—	[177]
<i>Gorgoderina vitelliloba</i>	Rana temporaria	P	E↓	[178]
<i>Heterakis spumosa</i>	Mus musculus	I	T↑, C↓	[179]

(continued on next page)

Table 1 (continued)

Pathogen	Host	Measure	Effects of hormone manipulation ^a	Reference
<i>Nematospiroides dubius</i>	Apodemus sylvaticus	P	—	[156]
<i>Neoechinorhynchus rutili</i>	Salmo trutta L.	I,P	—	[174]
<i>Nippostrongylus brasiliensis</i>	Millardia meltada	I	C↓	[180]
<i>Polystoma integerrimum</i>	Rana temporaria	P	E↓	[178]
<i>Pseudodiploorchis americanus</i>	Scaphiopus couchii	P	—	[181]
<i>Rhabdias bufonis</i>	Rana temporaria	P	E↓	[178]
<i>Schistosoma mansoni</i>	Homo sapiens	I	—	[182]
<i>Strongyloides ratti</i>	Mus musculus	I	T↑, C↓	[183]
<i>Syphacia obvelata</i>	Apodemus sylvaticus	P	—	[156]
<i>Syphacia stoma</i>	Apodemus sylvaticus	I	—	[184]
<i>Trichinella spiralis</i>	Microtus pennsylvanicus	I	—	[185]
	Rattus norvegicus	I	T↑, C↓, E↓	[186]
<i>Wuchereria bancrofti</i>	Homo sapiens	P	—	[187]
Cestodes				
<i>Echinococcus multilocularis</i>	Mus musculus	I	—	[188]
Arthropods				
<i>Amblyomma hebraeum</i>	Tragelaphus strepsiceros	I	—	[189]
<i>Hypoderma tarandi</i>	Rangifet tarandus tarandus	I	C↓	[190]

Abbreviations: C↓, male castration reduces infection; E↓, administration of estadiol reduces infection in males, females, or both; I, intensity; NE, no effect; P, prevalence; T↑, administration of testosterone increases infection in females, males, or both.

^a Only studies using physiologic (not supraphysiologic) concentrations of hormones are included. From Klein SL. The effects of hormones on sex differences in infection: from genes to behavior. Neurosci Biobehav Rev 2000;24:627–38; with permission from Elsevier Science.

lower circulating androgen levels and a higher prevalence of autoimmune diseases, including SLE compared to normal men. In case reports of patients with Klinefelter syndrome and autoimmune disorders (such as SLE and Sjögren's syndrome), testosterone therapy ameliorated the symptoms and hematologic and immunologic parameters of the diseases [12,20]. Another observation suggesting a role for sex steroids in susceptibility to autoimmune disorders is the altered steroid metabolism in men and women with SLE. Androgen oxidation is increased in women with SLE, resulting in lower levels of plasma androgens [21]. Similarly, men with SLE have higher estrogen and lower androgen levels [22].

Direct evidence for the role of sex steroids in the pathogenesis of autoimmune diseases comes from animal models of autoimmune diseases. In mice, spontaneous autoimmune syndromes are more prevalent and more severe in females than in males. The best-studied example is the NZB/NZW F1 hybrid mouse, which develops a generalized autoimmune disorder similar to SLE in humans [23]. Female NZB/NZW F1 mice generally die within the first year of life because of glomerulonephritis, whereas male mortality rates are 10% or less [23,24]. Pre-pubertal castration of males results in an acceleration of the disease course similar to that in females, and administration of androgens to ovariectomized females

Table 2

Estimated prevalence of autoimmune diseases in the United States and percentage of female patients

Autoimmune diseases	Prevalence rate/100,000	Female patients (%)
Grave's disease	1152	88
Insulin-dependent diabetes mellitus	192	48
Pernicious anemia	151	—
Rheumatoid arthritis	860	75
Hashimoto's thyroiditis	792	95
Vitiligo	400	52
Glomerulonephritis	40	32
Multiple sclerosis	58	64
Systemic lupus erythematosus	24	88
Sjögren's syndrome	14	94
Myasthenia gravis	5	73
Polymyositis/dermatomyositis	5	67
Addison's disease	5	93
Scleroderma	4	92.2
Primary biliary cirrhosis	3	88.7
Uveitis	2	50

largely prevents presentation of the disease. Replacement with the male sex hormone dihydrotestosterone (DHT) markedly delays the onset of lupus, whereas estrogen administrations accelerates the disease process and increases mortality [23–25]. Female predominance also is observed in other mouse strains that develop spontaneous lupus-like autoimmune syndromes [26,27] and in animal models of diabetes and RA [28]. Similarly, the protective effects of androgens are not limited to lupus models. In mouse models of Sjögren's syndrome, testosterone administration induces an improvement in the disease course [29,30]. Orchiectomy potentiates the development of autoimmune thyroiditis that is induced by thymectomy and irradiation in male rats. Testosterone administration markedly reduces the development of this condition [31]. In male, nonobese, diabetic mice, castration accelerates the development of diabetes, whereas the administration of androgens to female mice decreases the rate of development of overt diabetes [32].

Although androgens in general improve and estrogens worsen manifestations of autoimmune disorders, the effects of the same steroid hormone on different autoimmune disorders and on different manifestations of a single autoimmune disorder may differ. Treatment with physiologic doses of estradiol exerts dichotomous effects on different manifestations of the lupus disease in mice. Immune complex-mediated glomerulonephritis is accelerated significantly because of polyclonal B-cell activation, and production of antibodies and formation of circulating immune complexes are increased. In contrast, T-cell-mediated lesions, such as focal sialadenitis, renal vasculitis, and periarticular inflammation, are ameliorated significantly in mice that are exposed to estrogen. Within one subject and even within one organ, administration of estrogen leads to differential outcome of SLE morbidity. The differential effect of estrogen on the manifestations of autoimmune disease in mice may be explained by estrogen's dichotomous effects on B- and T-cell-mediated immune responses [33].

Autoimmune disorders with strong female predominance, such as SLE, RA, and MS, respond differently to pregnancy. Disease severity is decreased significantly during pregnancy in women with MS and RA, but returns to prepregnancy levels following delivery. SLE seems to worsen during pregnancy. These observations are best explained by a dichotomous effect of pregnancy (a high-estrogen, high-progesterone state) on helper T cell subtypes (eg, T_H1 , T_H2). Helper T cells express $CD4^+$ and recognize antigens presented in the presence of MHC class II proteins that are expressed by specialized antigen-presenting cells of the macrophage lineage. Undifferentiated naïve precursor $CD4^+$ T cells proliferate on primary activation and produce interleukin 2 (IL-2), but have a limited effector spectrum. On reactivation, these cells differentiate into T_H1 or T_H2 effector cells, depending on the cytokine signal received by the cell during reactivation. Activated T_H1 cells secrete IL-2, interferon (IFN- γ), and tumor necrosis factors (TNFs), resulting in activation of macrophages and the defense against intracellular microorganisms. Activated T_H2 cells secrete a multitude of cytokines, including IL-4, IL-5, IL-6, IL-10, and IL-13, and assist B lymphocytes in generating antibodies. Pregnancy is believed to favor a T_H2 response and to halt the progression of the T_H1 immune response associated with MS and RA. In SLE, pregnancy further enhances the ongoing T_H2 (antibody-promoting) response (Fig. 1) [34].

Effects of sex steroids on immune function

The observed sexual dimorphism in immune response and autoimmune disorders led to *in vitro* and *in vivo* studies investigating the effects of sex steroids on these processes. In the last 30 years, thousands of studies were conducted on this subject, including: (1) descriptive studies in humans that investigated the changes in immune response and autoimmune disease activity during physiologic states associated with altered hormonal status such as pregnancy and puberty, (2) animal studies that investigated the effects of castration and hormone therapy on immune response and autoimmune disease activity, and (3) *in vitro* studies that investigated the effects of sex steroids on immune cells under controlled conditions, such as cell cultures.

Each of these approaches has caveats. Although the changes observed in pregnancy likely result from elevated estrogen and progesterone levels, other factors also may be involved. It is not possible to attribute all the changes observed in pregnancy to elevated female sex steroids, and animal or *in vitro* studies are necessary. The results of *in vitro* studies also should be evaluated with caution, because they may not reflect the effects of sex steroids on cells that interact with immune cells *in vivo*. Estradiol has been reported to enhance macrophage activity in cultures; however, estradiol also down-regulates expression of endothelial leukocyte adhesion molecules [35] and secretion of monocyte chemoattractants by vascular endothelial and smooth muscle cells, [36] resulting in overall inhibition of macrophage activity in the vascular wall. Moreover, a single hormone may have

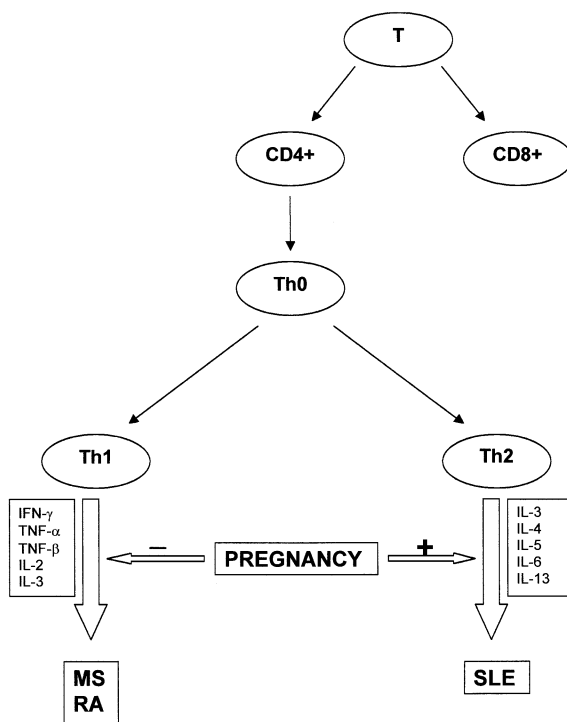


Fig. 1. Development of T-lymphocyte subtypes, their products, and their possible role in the pathogenesis of MS, RA, and SLE. Pregnancy is believed to cause a worsening in the course of SLE by enhancing a T_H2 response.

variable effects on the same aspect of immune response in different tissues. Estrogen increases IgA levels in the uterus, decreases IgA levels in the vagina, and has no effect in lacrimal tissues [37]. The effects of sex hormones on the immune system must be evaluated in an organ- and cell-dependent manner and in conjunction the evaluation of their effects on nonimmune cells.

Overall, available human, animal, and in vitro studies suggest that sex hormones affect the function of T cells, B cells, natural killer (NK) cells, and macrophages. Sex hormones also modulate the expression, severity, and course of autoimmune diseases in animal models. In the following sections the known effects of individual sex steroids on specific aspects of the immune response are summarized (Table 3).

Estrogen

Estrogen interacts with the immune system directly and indirectly and contributes to its sexual dimorphism. The indirect effects mostly result from estrogen's modulation of other hormones. Elevated estrogen levels lead to

Table 3

Effects of estrogens and androgens on acquired immune response

Response	Estrogens	Androgens
T-cell development in the thymus	Thymic atrophy, increased CD4 ⁺ :CD8 ⁺ ratio	Thymic atrophy, decreased CD4 ⁺ :CD8 ⁺ ratio
Peripheral T-cell activity	Increased CD4 ⁺ activity, especially T _H 2	Increased suppressor CD8 ⁺ activity
B-cell development in bone marrow	Reduction of developing B cells	Reduction of developing B cells
Peripheral B-cell activity	Increased antibody and autoantibody production	Decreased antibody and autoantibody production

increased growth hormone (GH), prolactin secretion, and thymosin release, all of which affect lymphocyte development. The direct effects of estrogen on the immune system are discussed.

Estrogen and acquired immune response

Thymus and peripheral T lymphocytes. Estrogens induce thymic atrophy. In mice, thymic involution is accelerated during pregnancy when estrogen and progesterone levels are high, and thymic size gradually returns to normal in the first month after delivery [38,39]. Evidence from animal studies suggests that at least part of the thymic involution observed in pregnancy results from a direct effect of estrogen on the thymus. In mice, a single subcutaneous injection of 1 mg of estrogen induces significant thymic atrophy within 10 days [40]. The thymic atrophy induced by estrogen in mice is associated with a decrease in thymocytes that are in earlier stages of maturation and a relative increase in more mature phenotypes. This finding is observed in female and male mice [40,41]. Thymic atrophy caused by estrogen treatment also is associated with an increase in the CD4⁺ subset of T cells (helper and inducer) relative to the CD8⁺ subset (cytotoxic and suppressor) [42]. This observation is contrary to the effects of androgens, which cause an increase in CD8⁺ subset associated with thymic atrophy [43]. Although male and female sex steroids cause thymic atrophy, they have different effects on thymocyte subsets. Estrogen treatment also enhances extrathymic T-cell maturation, mainly in the liver [40]. T cells that mature outside the thymus escape selective depletion and may express specificities to self-antigens, resulting in a predisposition to autoimmune disorders.

Thymic effects of estrogen seem to be receptor mediated. In the thymus, the stromal cells and thymocytes express estrogen receptors [44]. Estrogen receptor α (ER- α) [45] and estrogen receptor β (ER- β) [46] are present in the human thymus. The role of individual estrogen receptors in thymic development and estrogen-induced thymic alterations has been studied in ER- α (ERKO), ER- β (BERKO), and $\alpha\beta$ -double knockout (DERKO) mice [47,48]. Female ERKO mice display significantly higher production of estrogens, making the interpretation of data difficult. Experiments in ERKO mice were conducted in males. Four-month-old, ER- α -deficient mice (ERKO, DERKO) display significantly lower thymic

cellularity compared with mice expressing ER- α (BERKO and wild type). This finding is associated with a smaller thymic size and suggests that ER- α expression is essential for the development of a normal-sized thymus in male mice, whereas ER- β has no such role. In male ERKO mice, estrogen treatment does not change the CD4⁺ and CD8⁺ profile, but it causes loss of thymocytes and reduced thymic size (50%), although to a lesser extent than that seen in estrogen-treated, wild-type mice (80%). ER- α mediates at least part of estrogen-induced thymic atrophy and is necessary for estrogen-induced phenotypic shifts [47,48]. Estrogen treatment of female BERKO mice results in thymic atrophy similar to that seen in wild-type mice, but it does not affect T-cell phenotypes and leads to a less pronounced reduction in cortex area fraction. ER- β is not required for reduction of thymic weight and cellularity but mediates changes in the thymus gland's gross morphology and is necessary for alteration of T-cell phenotypes in response to estrogen treatment [47].

The effects of estrogen on circulating T-cell subsets reflect its thymic effects. In premenopausal women, surgically induced hypoestrogenism results in a decrease in the CD4⁺:CD8⁺ ratio, although CD8⁺ and CD4⁺ lymphocytes increase in number [49]. This finding is consistent with the previously mentioned effects of estrogen on the thymus in which the CD4⁺:CD8⁺ ratio is increased. In vivo administration of estrogen alters peripheral T-cell activity in a number of model systems in ways that suggest enhancement of helper and inducer (CD4⁺) activity or suppression of cytotoxic and suppressor (CD8⁺) activity. Estrogen therapy in men with prostate cancer or Peyronie's disease and in transsexuals suppresses blastogenic response of peripheral blood lymphocytes to phytohemagglutinin, a nonspecific T-cell mitogen [50]. In postmenopausal women, estrogen replacement therapy for 6 months had a dose-dependent inhibitory influence on the mixed lymphocyte reaction [51]. Animal experiments show similar results. Estrogen treatment of male rats in vivo results in a suppression of mixed lymphocyte reaction [52]. Leukocytes from female Fischer rats have a lower response to mitogens than do leukocytes from males. Ovariectomy resulted in an increased response, raising it to the level of response in normal males. Estradiol valerate treatment at physiologic levels reverses the mitogen response of gonadectomized animals of both sexes and decreases to the level of females [53].

The β_2 -microglobulin-deficient mouse is an interesting murine model that has a defect in MHC class I molecules which results in low levels of functional CD8⁺ cytotoxic T lymphocytes. In this model, viral infection induces CD4⁺ cytotoxic T lymphocytes and a MHC class II restricted immune response. The immune response mediated by these cells increases mortality in response to infection with lymphocyte choriomeningitis virus. This response is weaker in males, and estrogen treatment of castrated males results in enhanced immune response similar to that of females [54]. The findings in β_2 -microglobulin-deficient mice confirm that estrogen enhances the activity of CD4⁺ T lymphocytes and suggest that the suppression of response to mitogens and mixed lymphocyte reaction is caused by a preferential suppression of the activity of CD8⁺ T lymphocytes. Although these

findings suggest that estrogen affects peripheral T-cell function in vivo, they do not prove a direct effect.

To elucidate the direct effects of estrogen on peripheral T lymphocytes, in vitro studies have been conducted with conflicting results. Investigators have shown that estrogen inhibits lymphocyte transformation when added to cultures of human lymphocytes that are stimulated with antigens [55–57], suppresses the mixed lymphocyte reaction [58], and blocks the negative regulatory effect of suppressor T lymphocytes on immunoglobulin production by B lymphocytes [59]; however, other investigators did not show a consistent suppressive effect of estrogen [60,61]. The reported effects of sex hormones that are administered in vivo on the immune system may be mediated by precursors of peripheral lymphocytes; hence, the in vitro system may not be a reproducible test for determining immunoendocrine events [60,61]. Although estrogen receptors were detected in peripheral T cells, available data suggest that they may be limited to the CD8⁺ subset [62]. The peripheral effects of estrogen may be explained by the inhibition of suppressor T cells by estrogen, which leads to increased helper and inducer T-cell activity and promotion of B-cell maturation. The exact mechanism by which estrogen exerts its CD8⁺-independent effects on peripheral T lymphocytes remains to be explained.

Bursa, bone marrow, and peripheral humoral immunity. Pregnancy causes a reduction of developing B-cell subpopulations in the bone marrow of mice [63]. The alteration in B lymphopoiesis during pregnancy seems to result from hormonal action. When nonpregnant female mice are given supplemental estrogen to produce serum estrogen levels slightly higher than those attained in pregnant mice, relative numbers of developing B cells in bone marrow change similar to those observed in pregnant mice [64]. Ovariectomy in nonpregnant female mice results in an increase in the number of bone marrow B cells, an effect which is reversed by estrogen replacement [65]. Similarly, the hypogonadal mouse, with partial deletion of the gene for gonadotropin-releasing hormone, shows greatly increased numbers of B-lymphocyte precursors in the bone marrow. Estrogen replacement results in a dose-dependent decrease in the numbers of these cells [66].

These findings suggest a role for estrogen in B-cell development; however, controversy over early differentiation pathways has made it difficult to identify cellular targets. Newly formed B lymphocytes and pre-B cells are normally abundant in the bone marrow and noticeably are depressed in pregnant or estrogen-treated mice [63,64]. Evidence suggests that very early lymphoid precursors (eg, early pro-B cells and common lymphoid progenitors) also are depleted in response to estrogen treatment in mice [67]. The effects of estradiol on bone marrow B-cell precursor do not require the presence of a thymus. Stromal cells in bone marrow have been found to express estrogen receptors (ER- α and ER- β) [68] and, until recently, were believed to be the sole mediators of estrogen's effects on B lymphopoiesis. This theory was based on findings that expansion of lymphocytes in bulk cultures that were initiated with lymphoid cells

and stromal cells always were depressed by estrogen addition, whereas many B lymphocyte subsets were not sensitive to estrogen when placed in stromal-cell-free cultures [69]. Conditioned medium from estrogen-treated stromal cells showed suppressive activity [69]; however, Medina and colleagues reported dramatic reductions in B-cell lineage differentiation and expansion with estrogen treatment in stromal-cell-free cultures of early pro-B cells and multipotential progenitors [67]. Although stromal cells may be necessary to observe all of the estrogen-induced effects on B lymphopoiesis, early pro-B cells may represent an estrogen-sensitive subset, in which estrogen exerts its effects independent of stromal cells.

In the periphery, estrogen enhances B-cell response. Estradiol administered to castrated mice augments polyclonal B-cell activation [70], causes an increase in the production of peripheral antibody by the B-cell population [71], and results in an earlier, increased production of specific antibodies. Autoantibody-producing cells increase significantly in normal male and female mice following estrogen treatment, whereas total numbers of B cells remain the same. Autoantibodies to cardiolipin [72] and to double-stranded DNA [73] are increased in the estrogen-treated animals. Although these findings show that estrogen increases antibody production by B cells, the relative specificity of estrogen for autoantibodies is not clear.

Overall, addition of estrogens or removal of androgens enhances peripheral B-cell response and production of autoantibodies, even in normal animals. Mechanisms responsible for these effects on B cells are not understood completely, but the thymus and T lymphocytes seem to mediate at least part of estrogen's action on peripheral humoral immunity. Physiologic concentrations of estradiol enhances the proliferation and differentiation of B lymphocytes in response to pokeweed mitogen (a nonspecific, T-cell-dependent, B-cell mitogen) in peripheral blood mononuclear cell (PBMC) cultures. Enhancement of *in vivo* humoral immunity by estradiol requires the secretion of thymic factors by thymus, exerting a permissive influence on the action of estradiol to increase the specific humoral immune response [74].

Estrogen and innate immune response

Estrogens reduce NK cell activity in normal and autoimmune mice [70,75,76]. Neonatal treatment of mice with diethylstilbestrol permanently reduces the number of NK cells [77]. Similarly, in women with endometriosis (an estrogen-dependent disease), there is a significant decrease in NK cell activity with increased stages of disease [78]. Estradiol does not down-regulate [79,80] and may enhance [81] NK cell activity *in vitro*, suggesting that the *in vivo* effect of estradiol on NK cells may be indirect or involve NK cell precursors.

Bactericidal activity of peripheral blood neutrophils does not differ during the estrous cycle or pregnancy [82,83]. Estradiol reduces chemotaxis and random migration of neutrophils by an estrogen receptor-mediated effect [84]. Preincubation with estrogen antagonists clomiphene or tamoxifen eliminates the inhibitory effect of estradiol on neutrophil chemotaxis [85].

Multiple studies suggest that estrogens stimulate macrophage phagocytic activity. Estradiol enhances clearance by macrophages in mice [86] and guinea pigs [87]. Gonadectomy produces a decrease in the macrophage phagocytic activity in mice. Estradiol treatment returns the phagocytic and metabolic capacities of macrophages to normal levels [88]. On the other hand macrophage function does not depend solely on phagocytic activity. Other factors, such as monocyte adhesion to vascular endothelium, and monocyte and macrophage chemoattraction, also affect macrophage-mediated immune response. Estradiol inhibits expression of vascular adhesion molecules [35] and production of monocyte chemotactic protein 1 (MCP-1) in vascular endothelial [89] and smooth muscle cells [36], resulting in an overall inhibition of macrophage function. In vivo effect of estrogen on macrophage activity depends on location and cause of immune response and the other cell types involved.

Estrogen and cytokines

Cytokines are proteins that are produced locally in a wide variety of tissues, and they work by paracrine or autocrine mechanisms. Although these peptides are named after their originally observed biologic action, they are involved in a wide range of other actions, including stimulation of cell growth, inhibition of cellular proliferation, and alterations of cell functions. Sex steroid hormones and cytokines affect each other's expression and action. Estrogen affects the production and secretion of many cytokines in a multitude of tissues. This section briefly reviews estrogen's modulation of expression and activity of cytokines that are believed to be mediators of disease processes affected by sex steroids.

Interleukin 1 is produced predominantly by monocytes and macrophages and acts primarily as a mediator of innate immunity. IL-1 is also a known stimulator of bone resorption. Acceleration of bone resorption is the principal contributing factor to osteoporosis in postmenopausal women. The effects of menopause and hormone replacement therapy on IL-1 release by monocytes have been studied. IL-1 activity in supernatants of monocyte cultures derived from untreated postmenopausal women (nonosteoporotic and osteoporotic) is higher than that in supernatants of monocyte cultures derived from premenopausal women and from postmenopausal women treated with estrogen and progesterone [90]. Treatment with estrogen and progesterone for 1 month caused a decrease in IL-1 activity in nonosteoporotic and osteoporotic postmenopausal women [90]. Its two forms (IL-1 α , IL-1 β) and the IL-1 receptor antagonist (IL-1RA) determine IL-1 activity in the medium. Because IL-1 β is secreted consistently and in much higher amounts, the IL-1 β :IL-1RA ratio often is used to measure IL-1 activity. Hormone replacement therapy with estrogen alone or estrogen and progesterone in osteoporotic and nonosteoporotic postmenopausal women results in a decrease in the IL-1 β :IL-1RA ratio [91,92]. Moreover, when cultures of peripheral blood from postmenopausal women are treated with estradiol at physiologic concentrations, decreases in spontaneous secretion of IL-1 β , secretion of IL-1RA, and the IL-1 β :IL-1ra ratio are observed [93]. Alterations in IL-1 production may underlie the postmenopausal acceleration in bone loss and its inhibition by hormone replacement therapy.

Tumor necrosis factor α (TNF- α) also is produced by cells of monocyte and macrophage lineage and has biologic functions similar to those of IL-1. TNF- α is present in the local bone microenvironment and is a potent stimulator of bone resorption. Estradiol inhibits TNF- α release from unstimulated PBMCs in culture derived from postmenopausal women with osteoporotic vertebral fractures, but has no consistent effect on PBMCs from men or premenopausal women [94]. These findings are confirmed by similar experiments in whole-blood cultures derived from postmenopausal women [93]. Because TNF- α is a potent stimulator of bone resorption, the inhibitory effect of estrogen on TNF- α release may be part of the mechanism by which it exerts a protective effect on the skeletons of postmenopausal women.

Interleukin 6 is another example monocyte-derived cytokine that modulates innate immunity. It is produced by a multitude of other cell types, including fibroblasts, vascular endothelial and smooth muscle cells, bone marrow cells, and stromal cells. Osteoclasts, the tissue macrophages of the bone, are controlled by factors that are produced in their microenvironment. The production of IL-6, a cytokine that promotes hematopoiesis and osteoclastogenesis, is suppressed by estradiol in vitro. In mice, oophorectomy increases the number of granulocyte and macrophage colonies, enhances osteoclast development in ex vivo bone marrow cultures, and increases the number of osteoclasts in the trabecular bone. These changes are prevented by treatment with estradiol or antibodies against IL-6. Another mechanism for the increased bone resorption in postmenopausal osteoporosis is IL-6-mediated stimulation of osteoclastogenesis, induced by hypoestrogenism [95].

Monocyte chemotactic protein 1 is a chemotactic cytokine that is produced by a multitude of cell types. It attracts and activates monocytes and macrophages and is believed to be involved in the pathogenesis of atherosclerosis by mediating the recruitment of macrophages to the arterial wall early in the process. Estradiol down-regulates MCP-1 expression in the vascular wall [39,89], resulting in decreased macrophage recruitment. The down-regulation of monocyte and macrophage chemotaxis may be the mechanism by which estrogen suppresses arterial response to injury [96].

MCP-1 also plays a role in adhesion formation. Estradiol replacement reduces talc-induced, connective-tissue deposition, reduces the number of macrophages and MCP-1 expression in intraperitoneal adhesions in mice, and inhibits adhesion formation [97]. At high physiologic concentrations (equivalent to pregnancy levels), estradiol inhibits MCP-1 expression in stimulated fibroblasts in vitro, but has no significant effect at concentrations seen in cycling female. Estradiol may down-regulate MCP-1 expression in fibroblasts, leading to a decrease in macrophage recruitment and inflammation, which in turn would cause inhibition of adhesion formation [98].

Progesterone

Progesterone has demonstrable immunosuppressive properties, such as anti-inflammatory and graft-sparing effects, when administered locally in animals. It

inhibits lymphocyte activation and production of monocyte-macrophage oxygen radicals in human and murine cells. Many authors suggest that progesterone, at concentrations produced locally at the placental–maternal interface, may be involved in the prevention of pregnancy rejection [99].

Progesterone and acquired immune response

A marked decrease in the size of the thymus occurs during pregnancy and reverses in the postpartum period [38]. Whether this involution is mediated by progesterone and whether it is involved in the tolerance of the fetus are not clear. One study reported that, contrary to the effects of testosterone and estrogen, progesterone inhibits spontaneous and glucocorticoid-induced apoptosis of murine thymocytes in vitro [100]. The thymus, however, is known to express specific high-affinity, progestin-binding receptors [101,102], and progesterone receptors are required for thymic involution during pregnancy [103]. Progesterone receptors have been shown to be present in thymic epithelial cells, but not in thymocytes [101,104,105].

Progesterone down-regulates peripheral T-cell activity. It inhibits in vitro human lymphocyte transformation [56,57,106] and mixed lymphocyte reaction [58]. It prolongs the survival of hamster-to-rat and mice-to-mice skin grafts [107] and inhibits stimulation of human lymphocytes by prostaglandin $F_{2\alpha}$ [108].

In the bone marrow, pregnancy causes a reduction of the subpopulations of developing B cells, except for the pro-B cells [63]. Ovariectomy in nonpregnant female mice results in an increase in bone marrow B cells, which is reversed by estrogen replacement [65]. Progesterone that is administered alone has no effect on B-cell precursors in the bone marrow, but when progesterone is combined with estradiol, there is synergism, in which lower doses of estradiol are effective in reducing the number of B-cell precursors [64]. Stromal cells and B lymphocytes express progesterone receptors in the bursa of Fabricius in chicken [104].

Progesterone also seems to down-regulate peripheral antibody production. Administration of progesterone to ovariectomized rats resulted in a significant, dose-dependent decline in the cervicovaginal content of IgA, IgG, and the secretory component [109]. The mechanism of this action remains to be elucidated, because studies investigating progesterone receptor in lymphocytes report conflicting results [110–112].

Progesterone and innate immune response

Progesterone at concentrations observed during human pregnancy and the menstrual cycle inhibits nitric oxide production [113] and the clearance of IgG-coated erythrocytes by macrophages [86]. Progesterone and its antagonist mifepristone (RU486) synergistically inhibit the proliferation of macrophages [114]. The mechanism of this action has not been determined. Human peripheral blood lymphocytes and monocytes (PBMCs) do not seem to express the classical progesterone receptor, even when exposed to the elevated concentrations of circulating estrogen in pregnancy. Although the glucocorticoid receptor is

expressed in PBMCs, the immunomodulatory effects of progesterone in these cells are not mediated through this receptor [111].

Progesterone has no direct effect on NK cell activity [80]. Progesterone-induced blocking factor (PIBF), a protein that mediates the immunologic effects of progesterone, blocks NK cell activity in vitro and prevents the abortive effect of high NK cell activity in mice. Increased NK cell activity may have a role in pregnancy loss; the NK cell inhibitory effect of PIBF may contribute to the maintenance of normal gestation [115]. Progesterone seems to enhance chemotaxis and random migration of neutrophils [84]. It has no effect on neutrophil antistaphylococcal activity [82].

Progesterone and cytokines

Progesterone affects cytokine production by many different cell types. It inhibits expression of IL-1 β mRNA in cultured human peripheral monocytes [116] and reduces IFN- γ production by human leukocytes that are subjected to mitogens. IL-6 production of human decidual stromal cells in culture [117] and IL-8 production by human endometrial explants also are inhibited by progesterone. Progesterone at physiologic concentrations also suppresses the constitutive and IL-1-mediated expression of IL-8 in uterine cervical fibroblasts in rabbits. These suppressive actions of progesterone are offset by cotreatment of cells with the progesterone antagonist mifepristone [118]. Progesterone enhances production of macrophage colony-stimulating factor in human endometrial stromal cells [119].

Testosterone

Testosterone suppresses most aspects of immune activity. In many species, males are more susceptible to infections than are females, and androgen treatment leads to an increase in the incidence or intensity of infections [15]. Autoimmune disorders are less common in males than in females, and androgen treatment causes a decrease in the activity of autoimmune disorder in animal models. This section reviews the effects of testosterone, which is the main male sex steroid, on different aspects of the immune response.

Testosterone and acquired immune response

Thymus and peripheral T lymphocytes

In males, castration results in enlargement of the thymus size. This change has been documented in many species, including cattle [120], rats [121], and mice [43], and is reversed by androgen replacement [43,122]. Thymic enlargement similar to that seen in castrated males also is observed in mice with testicular feminization mutation (Tfm/Y) that express a mutant androgen receptor (AR), rendering the animal insensitive to the effects of androgens [123].

Thymic enlargement following castration mostly is caused by increased thymocyte proliferation. Increased proliferation becomes apparent shortly after castration and reaches its peak on the fourth day following surgery. Thymic en-

largement becomes apparent by day 7 and reaches its plateau on day 10. Increased proliferation following androgen withdrawal is most prevalent in more immature thymocytes. At 10 days, after the thymus almost doubles its size, thymocyte proliferation slows down to the baseline rate, and thymic size is maintained [124].

The enlarged thymus in the castrated male mouse has a normal architecture, with intact cortical and medullary areas; however, the animal's relative proportion of thymocyte phenotypes is altered, and its $CD8^+$ population is relatively decreased. Androgen replacement in these animals promotes thymic atrophy, changes the balance toward the more mature cell types (partly by depleting immature $CD4^+CD8^+$ cells), and results in a relative increase in $CD8^+$ subset [43]. Consistent with these findings, androgen treatment of 1-month duration in postmenopausal osteoporotic women was found to cause an increase in the peripheral $CD8^+:CD4^+$ ratio [125].

The observed effects of androgens on the thymus likely are receptor mediated. Thymocytes and thymic epithelial cells express ARs [126,127]. The cellular targets of androgens that lead to the observed involution have been investigated using C57BL/6 mice expressing AR, Tfm/Y mice lacking AR, and chimeric mice expressing AR selectively in thymocytes or thymic epithelial cells. As expected, Tfm/Y mice had a large thymus and showed no changes following DHT administration. Chimeric mice with AR-positive thymocytes transplanted into the AR-negative microenvironment had significantly larger thymuses than did intact controls and did not show any involutional response to DHT. Chimeric mice with AR-negative thymocytes and AR-positive epithelium preserved the involutional response to DHT, suggesting that thymic atrophy in response to androgens is mediated by thymic epithelial cells. The mechanisms by which AR-positive thymic epithelial cells mediate androgen-induced thymic involution are unknown. A potential mechanism is the acceleration of thymocyte apoptosis by androgens [128].

In the periphery, androgens enhance the net functional capacity of $CD8^+$ cytotoxic and suppressor T cells. The activity of these cells can be measured by determining their ability to suppress mitogen-induced proliferation of syngeneic splenocytes. Spleen cells from castrated male mice are not as effective at inhibiting proliferation of other spleen cells as are cells from intact males [129]. This effect partly results from the presence of fewer mature T cells in the spleen of castrated male mice [130]. When castrated male animals are treated with testosterone, the inhibitory capacity of spleen cells is increased [129]. Testosterone also down-regulates the delayed-type hypersensitivity response in mice [131] and inhibits human lymphocyte transformation in vitro [56,57]. The mechanism of a direct effect of androgens on peripheral T cells is not clear, because multiple investigators have failed to show the presence of androgen receptors in these cells [126,132].

Bursa, bone marrow, and peripheral humoral immunity. Androgens have suppressive effects on B lymphopoiesis. In Japanese quail, castration slows and testosterone implants accelerate the normal age-related bursal involution

[133]. In mammals, B lymphopoiesis occurs in the bone marrow. Bone marrow lymphocytes rapidly decrease in number after puberty in normal males, but they increase if testes are removed. Castration of male mice results in expansion of the pre-B-cell population in the bone marrow [134]. This population can be depleted by treatment with testosterone or DHT [64,135]. These androgen-induced changes are unaltered in mice that have undergone previous thymectomy; therefore, androgens act directly on the bone marrow [136]. In the bone marrow, stromal cells and B-cell precursors express ARs [136]. It is not known whether androgens act directly on developing B lymphocytes or act indirectly through AR-positive stromal cells. Data derived from in vitro experiments and animal models suggest that the stromal cell compartment has a critical role in mediating androgen effects on developing B cells [137].

The effects of androgen depletion on peripheral B cells have been investigated. Similar to its effect in the bone marrow, castration of male mice results in a 40% increase in spleen weight, largely accounted for by expansion of the B-cell population [130]. Androgen replacement in castrated mice reverses this effect in the bone marrow, but not in the spleen [136]. Whereas developing B lymphocytes and stromal cells in the bone marrow express AR [136], mature B lymphocytes and stromal cells in the spleen do not [138]. These findings suggest that cells in the bone marrow, but not in the spleen, are likely targets of androgen action. These androgen-induced changes are unaltered in mice that have undergone previous thymectomy [136].

Testosterone treatment suppresses the antibody response [131]. Spleen cells from castrated animals show an increased autoantibody production that cannot be attributed to polyclonal stimulation [130], suggesting a modulating role for androgens in autoimmunity. The increased autoantibody production in castrated male mice is observed only with the addition of pokeweed mitogen (but not lipopolysaccharide) to the cultures, suggesting T-cell dependence of the enhanced response.

An exception to testosterone's suppressive effect on humoral immune system is its regulation of immunoglobulin content in tear. The secretory-component concentration in tears of male rats is five-fold greater than that in tears of females. Castration of male, but not female, rats results in a time-dependent decrease in the secretory-component content of tears. Administration of testosterone to orchietomized rats reverses this decline and induces a time- and dose-dependent increase in the secretory-component concentration in tears [139]. Androgen administration also increases the tear levels of IgA [140].

Innate immune system and cytokines

Testosterone has no significant effect on proliferation or cytotoxic activity of the NK cells [79]. Langerhans' cells are bone marrow-derived immune cells in the epidermis; density of these cells in male mice is lower than that in females, and orchietomy results in an increase in density, though ovariectomy has no significant effect. Testosterone results in a significant decrease in the density of Langerhans' cells in orchietomized males and normal females [141].

Dihydrotestosterone reduces the amount of production of IL-4, IL-5, and IFN- γ by activated murine T cells without affecting the production of IL-2. Exposure of T cells to androstenedione or testosterone (the metabolic precursors of DHT) does not affect the biosynthesis of these cytokines. Macrophages possess 5 α -reductase and are competent to metabolize testosterone to DHT [142].

Summary

Immune responsiveness and susceptibility to infections and autoimmune disorders suggest sexual dimorphism. Women have higher immune reactivity, resulting in better protection against infections but a predisposition to autoimmune diseases. Evidence suggests that sex steroids mediate the sexual dimorphism in immune response.

Estrogens induce thymic atrophy and alter the proportion of thymic cells, promoting an increase in the CD4⁺:CD8⁺ ratio. In the periphery, they enhance helper and inducer T-cell activity and production of antibodies. Estrogens also reduce cytotoxic and suppressor T-cell activity, impair the activity of NK cells, and suppress aspects of neutrophil function.

Androgens suppress most aspects of the immune system. They cause involution of the thymus and bursa of Fabricius, decrease cellular cytotoxicity and humoral immunity, enhance suppressor T-cell activity, and, in animal models, ameliorate many autoimmune diseases.

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