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## **Sex Steroids, Sex Steroid Receptors, and Autoimmune Diseases**

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## I. INTRODUCTION

It is generally believed that gonadal hormones exclusively function to affect sex-related functions, including the differentiation, development, and maturation of reproductive tissues, induction or regulation of sexual behavior, and maintenance of the secondary sexual characteristics. However, this notion has recently been dispelled, as new findings have demonstrated a much broader role for sex steroid hormones. These hormones are now believed to be directly or indirectly involved with the functioning of various unrelated tissues, such as the central nervous, cardiovascular, macrophage-monocyte, skeletal, and immune systems.

The immune system, like the reproductive system, was thought to operate and function in relative isolation. However, immunologists now recognize that the immune system is influenced by many natural modulators, including hormonal factors (Ansar Ahmed, Penhale, and Talal, 1985). Interestingly, a retrospective literature survey suggested this many years ago. Calzolari (1898) and Hammar (1906) demonstrated approximately eight decades ago that manipulation of sex hormone status in animals brought about marked changes in the thymus. These novel findings did not generate much enthusiasm, because at that time the thymus was regarded as an unimportant organ. Pioneer immunologists noticed there were curious sex-related differences in the magnitude of immune responses between the two sexes. Females developed a better immune response than did males. Again, the association of sex hormones with the immune system, although seemingly obvious, was not explored. As the disciplines of medicine, endocrinology, and immunology developed, it became recognized that hormones do interact with the immune system, and the field of immunoendocrinology was born.

This chapter will deal with the effects of sex steroid hormones on the immune system, a rapidly advancing field. In order to fully understand such effects it seems pertinent to briefly review the functioning of the immune system per se. It is beyond the scope of this chapter to deal completely with all the complex intricacies involved in the generation of the immune response. Two aspects of immunology are briefly discussed: (1) the various types of cells participating in immune responses, and (2) the sequence of events leading to the development of immune responses.

## II. THE FUNCTIONING OF THE IMMUNE SYSTEM

### A. Phenotype of Immune Cells

The mouse has been employed extensively for fundamental immunologic and immunogenetic studies and serves as a prototype for man. Murine lymphocytes are phenotypically identified by the presence of characteristic cell surface proteins and are broadly divided into T cells, which possess Thy1.2 and Lyt-1 antigen, whereas B cells exhibit cell surface immunoglobulin (Beverley, 1977; Yamamura

and Tada, 1983). These cells differ in their functional abilities as well. T cells mediate cytotoxic and hypersensitivity reactions, whereas B cells produce and secrete immunoglobulins (antibodies). T and B cells are further divided into many subsets either morphologically (by the presence of unique surface protein markers) or functionally. For example, T cells have either L3T4 or Lyt-2 antigens. In most systems, L3T4 positive cells mediate helper function, while Lyt-2 positive cells perform cytotoxic/suppressor function. B cells may have either IgM, IgG, IgD, or IgA class of antibodies on their surface. In addition, a small subpopulation of B cells exhibiting surface IgM/IgD immunoglobulins also manifest low-density Lyt-1 antigen and are referred to as  $Lyt^+B$  cells (Manohar et al., 1982; Hayakawa et al., 1983). These cells are unique in that they produce IgM auto-antibodies to many self-antigens (single-stranded DNA, erythrocytes pretreated with the enzyme, bromelain, and thymocytes).

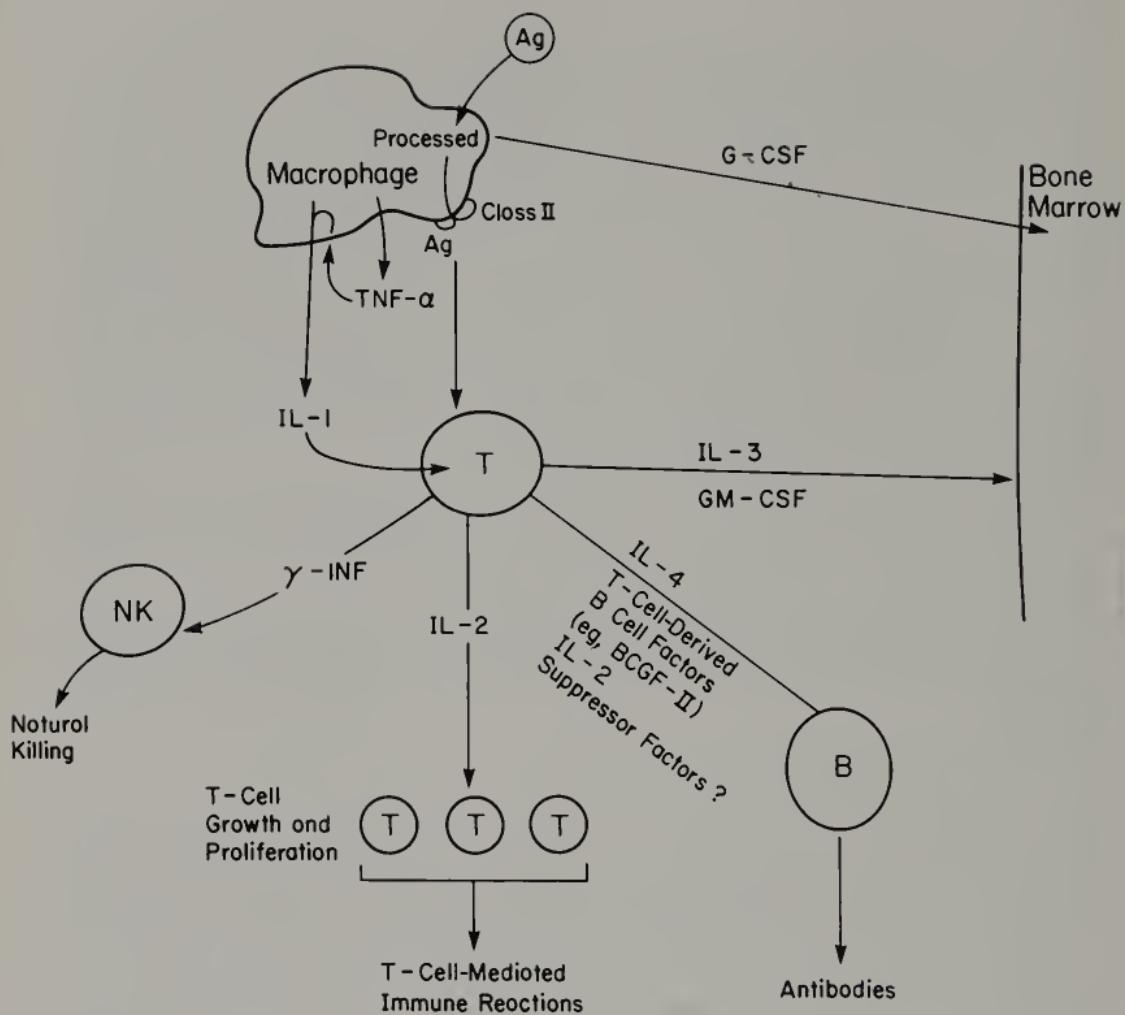
Also involved in the immune response are cells that belong to the macrophage-monocyte system. These function not only as antigen presenting cells but also to elaborate important immunoregulatory cytokines.

A separate class of lymphocytes referred to as natural killer (NK) cells function in defense by lysing tumors and abnormal cells in a genetically unrestricted fashion.

## B. Generation of Immune Response

Immune responses are regulated by a complex set of genes referred to as the Major Histocompatibility Complex (MHC; H-2 on murine chromosome 17 and HLA on human chromosome 6). The genes in this region encode two major classes of cell surface proteins, which are closely involved in antigen presentation and cell-cell interaction (Yamamura and Tada, 1983). These include the class I molecules (H-2 K, D, and L in mouse; HLA A, B, and C in man), which are classic transplantation antigens. These antigens function as targets for specifically sensitized cytotoxic T cells. The class II molecules (Ia in the mouse and DP, DR, and DQ in humans) are encoded by the I region of the H-2 and HLA DP, DR, and DQ regions, respectively. These antigenic molecules are present primarily on macrophages and B cells and serve as genetic restriction molecules, which are required to mediate T helper cell activation. These molecules are also present in certain epithelial and endothelial tissues.

An abridged and simplified sequence of events leading to the induction of immune responses is outlined below (Figure 1). Macrophages/monocytes and other specialized antigen presenting cells (APC) engulf complex antigens, which are then enzymatically digested, processed, and presented on the cell surface as antigenic determinants in conjunction with class II molecules. These APCs present the antigen to T cells, which recognize the antigen in the context of these class II molecules. Also, macrophages secrete a number of protein factors, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (Old, 1985; Chang and Lee, 1986; Old, 1987)



**Figure 1.** The complex interactions of macrophages, T cells, and B cells leading to the production of various immunoregulatory cytokines.

and interleukin-1 (IL-1) (Oppenheim et al., 1986). IL-1 serves as an important second signal to activate T cells, while TNF- $\alpha$  induces IL-1 production and may induce Ia antigen expression on certain cells (Chang and Lee, 1986). The activation of T cells brings about the induction of a series of membrane events leading to the production of secondary messengers (e.g., inositol 1,4,5 triphosphate, intracellular calcium ( $\text{Ca}^{++}$ ), diacylglycerol, etc.), which in turn activate specific genes. T cells then elaborate a number of lymphokines, including  $\gamma$ -interferon ( $\gamma$ -INF), B-cell stimulatory factor-1 (BSF-1), or B-cell growth factor-II, interleukin-2 (IL-2), and interleukin-3 (IL-3), interleukin-4 (IL-4). These lymphokines serve as messengers to activate diverse types of cells, including B and NK cells. Lymphokines,  $\gamma$ -interferon, and BSF-1 are inducers of Ia antigen on

**Table 1.** Predominant Female Autoimmunity and Autoimmune Diseases

	Spontaneous	Induced	Animal
Systemic lupus erythematosus	Systemic lupus erythematosus/ Rheumatoid arthritis/ Autoantibodies to DNA, Br-MRBC		
Sjogren's syndrome			Thyroiditis
Rheumatoid arthritis			Thymectomy-irradiation Neonatal-thymectomy Chemical administration Adjuvant-antigen administration
Autoimmune thyroiditis			
Type 1b insulin-dependent diabetes	NZB		
Scleroderma	NZB × NZW (F <sub>1</sub> )		
Multiple sclerosis	NZB × SJL/J (F <sub>1</sub> )		
	NZB × DBA/2 (F <sub>1</sub> )		
	NZB × CBA (F <sub>1</sub> )		
	PN (palmerston north)		
	NZB × PN		
	PN × NZB (F <sub>1</sub> )		
	C57BL/6-1pr		Polyarthritis LEW/N
	Autoimmune thyroiditis		
	BUF Rats		
			Autoimmune thrombocytopenia

Table modified from Ansar Ahmed, Penhale, and Talal, 1985.

cells surface and have marked immunoregulatory influences (Dubois, 1974; Noelle et al., 1984; Unanue et al., 1984; Todd et al., 1985). Interleukin-2, in addition, is a T-cell growth factor, which induces the proliferation of T cells with various functions.

### III. AUTOIMMUNE DISEASES—PREPONDERANCE IN FEMALES

Under normal conditions, immune cells are able to discriminate self from nonself and to remain tolerant to the former. However, for reasons poorly understood, immunological reactivity (mediated by lymphocytes and/or autoantibodies) against self tissue components can develop, leading to structural and/or functional damage of target organs. Such pathological states are referred to as autoimmune diseases. The concept that self-immunological attack can cause disease has only been introduced in comparatively recent times. When introduced approximately four decades ago, this concept provided the unifying basis for the etiology of many conditions that had previously been obscure and thus revolutionized the approach of both clinicians and researchers alike to the investigations of these diseases. With ever-improving sophisticated diagnostic and analytical tools, the fact that these diseases affect a host of tissues is rapidly becoming recognized. Although the pathogenic and triggering mechanisms will be found to differ from one condition to another, there has been one strikingly common feature—women are highly susceptible to the development of these conditions compared with their male counterparts (Table 1; Ansar Ahmed, Penhale, and Talal, 1985). For example, the female-to-male susceptibility ratio to systemic lupus erythematosus (SLE) and Sjogren's Syndrome is 9–13 to 1 and 9 to 1, respectively (Dubois, 1974; Inman, 1978; Whaley and Buchanan, 1980).

Analogous to the human situation, a similar sex-related susceptibility to autoimmune disease is also evident in many experimental animal models (Table 1; Ansar Ahmed, Penhale, and Talal, 1985). For example, in murine SLE, females have an earlier expression of autoantibodies or autoimmune diseases. Female B/W mice develop autoantibodies to DNA and Poly A, immune complexes, and glomerulonephritis and die earlier than males (Roubinian et al., 1978). Similarly, the incidence and severity of autoimmune thyroiditis induced in normal rats by early thymectomy and sublethal irradiation is greater in females than in males (Ansar Ahmed and Penhale, 1980; Penhale and Ansar Ahmed, 1981; Ansar Ahmed and Penhale, 1982). Another organ-specific disease, polyarthritis, induced in LEW/N rats by the injections of peptidoglycan-polysaccharide derived from streptococcal bacteria, can be more easily induced in females than in males (Allen et al., 1983).

We found that female normal (C57BL/6J, C3H/Hej, and NZW) as well as autoimmune (NZB and C3H/1pr) mice produce more autoantibodies to bromelain-treated mouse red blood cells than age-matched males (Ansar Ahmed,

Dauphinee, and Talal, in preparation [a]). Similarly, C57BL/6-1pr female mice make more autoantibodies to DNA than their male counterparts (Warren et al., 1984).

In parallel to the above situation, the heightened immunity of females is reflected by their ability to respond better to a variety of natural and synthetic antigens (Ahlquist, 1981; Grossman, 1984; Ansar Ahmed, Penhale, and Talal, 1985), higher serum immunoglobulin levels, better resistance to infections, and earlier rejection of certain types of tumors. The general increased longevity of females may have, in part, an immunological basis. However, depressed cell-mediated immunity has been noted in females.

#### IV. SEX HORMONES AND AUTOIMMUNE DISEASES

##### A. Animal Studies

The use of appropriate animal models for autoimmune disease provides an excellent opportunity to investigate the direct role of sex hormones in the pathogenesis of autoimmune diseases as they allow sex hormonal manipulations that are not possible in humans.

###### 1. Systemic Lupus Erythematosus Like Diseases

NZB × NZW(F<sub>1</sub>) or B/W mice have served as a reliable model for study of the above aspects. Definitive studies of sex hormonal involvement of autoimmune diseases were reported from our laboratory about a decade ago (Roubinian et al., 1978). Prepubertal orchidectomy of B/W mice accelerated the lupus disease to the level occurring in females. Further, estrogen administration significantly worsened the disease. In contrast, the male hormone, 5- $\alpha$ -dihydrotestosterone, markedly ameliorated the disease, thereby suggesting that estrogens are detrimental, while androgens are protective. Importantly, 5- $\alpha$ -dihydrotestosterone was beneficial even when administered at a time when the disease was already manifest, indicating the therapeutic value of this male sex hormone (Roubinian et al., 1979b). Other types of androgens were also found to be highly beneficial. For example, dehydroisoandrosterone administered orally to B/W mice delayed mortality and reduced anti-DNA autoantibodies (Lucas et al., 1985). Nandrolone (19-nortestosterone), an anabolic steroid with minimal virilizing effects, markedly reduced anti-DNA antibodies, improved renal function, and prolonged survival when administered to B/W mice (Verheul et al., 1981).

NZB × SJL/J (F<sub>1</sub>) or N/S mice also develop a lupuslike disease, although less severe than B/W mice (Dumont and Monier, 1983). At 12 months, lupus nephritis (immunoglobulin deposits in the renal glomeruli and proteinuria) is evident in 80–90% of females as opposed to only 10–20% of males. Further, only NS female mice exhibit thymic abnormalities characterized by the expansion of a mature thymocyte population and the presence of plasma cells and B cells.

Orchidectomy increased, whereas 5- $\alpha$ -dihydrotestosterone treatment reduced, autoantibody levels.

Androgens similarly reduced, whereas estrogens enhanced, the spontaneous autoantibody production in NZB  $\times$  CBA mice, NZB  $\times$  C3H mice (Steinberg et al., 1979), and NZB  $\times$  DBA/2 mice (Raveche, Tijo, and Steinberg, 1980). Further, in MRL/lpr mice that develop a severe lupuslike disease with massive generalized lymphadenopathy, the 5- $\alpha$ -dihydrotestosterone treatment reduced the lupuslike symptoms without affecting the lymphoproliferation (Steinberg et al., 1980).

## 2. Autoimmune Thyroiditis

Autoimmune thyroiditis, characterized by moderate to severe mononuclear cellular infiltration of the thyroid and serum autoantibodies to thyroglobulin, can be regularly induced in normal rats by early thymectomy and sublethal irradiation (Tx-X) (Penhale and Ansar Ahmed, 1981; Ansar Ahmed and Penhale, 1982). Female Tx-X rats have a higher incidence and levels of autoantibodies to thyroglobulin than males (Penhale and Ansar Ahmed, 1981; Ansar Ahmed, Young, and Penhale, 1983). The severity of the lesions and levels of autoantibodies to thyroglobulin are also higher in female compared with male Tx-X rats. Prepubertal orchidectomy increased these disease parameters to a degree comparable to female Tx-X rats. Testosterone administration abrogated the development of the disease (Ansar Ahmed and Penhale, 1982) and was found to be beneficial even in animals with established severe disease (Ansar Ahmed, Young, and Penhale, 1986). Prepubertal ovariectomy and progesterone administration further augmented the disease (although not statistically significant because of severe disease in ovariectomized Tx-X rats) (Ansar Ahmed, Young, and Penhale, 1983). Estrogen, in contrast to that noted in the lupus model, reduced the expression of the disease (Ansar Ahmed, Young, and Penhale, 1983).

Both testosterone and estrogen suppressed autoimmune thyroiditis induced in guinea pigs by the administration of thyroid extract and adjuvant. Testosterone had comparable effects in rats with induced autoimmune thyroiditis (Kappas, Jones, and Roitt, 1963).

Autoimmune thyroiditis can be readily induced in responder C3H mice by injections of thyroglobulin in adjuvant or lipopolysaccharide. Orchidectomy and/or estrogen treatment augmented, whereas 5- $\alpha$ -dihydrotestosterone reduced, the production of autoantibodies to thyroglobulin in these mice (Okayasu, Kong, and Rose, 1981). In this acute and self-limiting model of autoimmune thyroiditis, the effects of sex hormones were noted on autoantibodies rather than lesions.

Obese strain (OS) chickens develop spontaneous autoimmune thyroid lesions and autoantibodies to thyroglobulin. Persistent levels of autoantibodies to thyroglobulin were manifested only in female OS chickens, suggesting hormonal influence (Gause et al., 1985). Testosterone treatment has been shown to prevent the development of thyroiditis (Cole, Kite, and Witebsky, 1968).

### 3. Autoimmune Hemolytic Anemia and Autoantibodies to Bromelain-treated Autologous Mouse Erythrocytes

Spontaneous autoimmune hemolytic anemia, which develops in NZB mice, although not in a female-predominant fashion, can be modulated by sex hormones. Testosterone and dehydroepiandrosterone (DHEA) reduced autoantibodies (Steinberg, Smathers, and Boegel, 1980). Autoimmune hemolytic anemia, inducible in mice by xenogeneic erythrocyte immunization, can be abrogated or delayed by testosterone administration (Milch and Gershwin, 1981).

Murine spleen cells, peritoneal cell exudates, and bone-marrow cells spontaneously produce autoantibodies to bromelain-treated mouse red blood cells (Br-MRBC). These autoantibodies are markedly increased in autoimmune mice and are produced by Lyl<sup>+</sup>B cells. We found that female mice produce more autoantibodies to Br-MRBC than do males. Estrogen administration to autoimmune and normal males greatly increased these autoantibodies (Ansar Ahmed, Dauphinee, and Talal in preparation).

### 4. Autoimmune Arthritis

Autoimmune arthritis induced in LEW/N rats by the injection of peptidoglycan-polysaccharide fragments obtained from group A streptococci can be readily induced in females compared with males (Allen et al., 1983). The resistance to induction of the disease is due to male sex hormones, since estrogen treatment or orchidectomy enhanced the susceptibility. Further, estrogen treatment induced the disease in nonresponder F-334/N. Genetic studies further established hormonal rather than X-chromosomal influence.

Collagen-induced arthritis in rats derived from susceptible DA and resistant B/W rats can be easily induced in female hybrids compared with males, again suggesting sex hormone involvement (Griffiths and DeWitt, 1984).

### 5. Experimental Autoimmune Myasthenia Gravis

Autoantibodies to acetylcholine receptors induced in normal C57BL/6 mice by acetylcholine receptor and complete Freund's adjuvant injection can be modulated by sex hormone manipulations. Orchidectomy increased, whereas testosterone administration or ovariectomy reduced, autoantibodies to acetylcholine receptors (Talal et al., 1983).

### 6. Sjogren's Syndrome-like Disease

Autoimmune-prone B/W mice, in addition to lupus, also develop a Sjogren's syndrome-like disease characterized by mononuclear cellular infiltration in the submandibular salivary glands (Kessler, 1968). Nandrolone deconate had both beneficial prophylactic and therapeutic effects on established disease (Schot, Verheul, and Schuurs, 1984). Other steroids, such as tibolone (org OD14; with less potent androgenic, progestational, and estrogen activities), ethylestranol (progestational anabolic steroid with minimal virilizing effects), and lynestrenol (progestational effects with no androgenic or little estrogenic activities) were shown

to reduce Sjogren's syndrome as well as lupus occurring in B/W mice (Verheul, Schot, and Schuurs, 1986).

### 7. Experimental Autoimmune Encephalomyelitis

Estrogen-containing oral contraceptives abrogated the induction of experimental autoimmune encephalomyelitis, perhaps by suppressing the effects on T cells (Arnason and Richman, 1969).

Overall, the above studies clearly demonstrate that sex hormones markedly influence a variety of autoimmune diseases, which perhaps differ in initiating pathogenic mechanisms. The mechanisms of such effects are indeed complex and are discussed in selected models in a latter part of this chapter.

## B. Human Studies

1. Association of Sex Hormonal Changes with Autoimmune Diseases  
Although the precise reasons underlying this sex-related phenomenon in humans is not known, there is an accumulating body of evidence to indicate that sex hormones influence autoimmune diseases (Ansar Ahmed, Penhale, and Talal, 1985). The fact that the female-to-male susceptibility ratio for systemic lupus erythematosus varies with age, particularly during child-bearing years is strongly suggestive of sex hormonal influence. Further, flare of lupus in pregnant patients, usually a few weeks postpartum, has been reported (Mund, Swison, and Rothfield, 1963). Pregnancy also modulates the course of many autoimmune diseases, including rheumatoid arthritis (Hench, 1949), autoimmune thyroiditis (Amino et al., 1977), Graves' disease (Amino, Miyai, and Yamamoto, 1977), polymyositis/dermatomyositis (Gutierrez, Dagnino, and Mintz, 1984), and idiopathic thrombocytopenic purpura (Lorz and Frumin, 1961). Pregnancy has been reported infrequently in patients with systemic sclerosis (Ballou, Morley, and Kushner, 1984). Menses also alters the severity of lupus (Rose and Pillsbury, 1944). Estrogen-containing oral contraceptives are often associated with exacerbation of lupus (Chapel and Burns, 1971). Conversely, withdrawal of these contraceptives may result in disease remission. Normal women with no history of autoimmune disease on oral contraceptives can develop rheumatic symptoms or LE cells, a salient feature of lupus (Schleicher, 1968; McKenna, Weiman, and Schulman, 1969; Spiera and Plotz, 1969).

The role of sex hormones in autoimmunity is also indicated by findings in alcohol-induced cirrhosis patients. There is an inverse relationship of serum testosterone levels with the development of antinuclear and anti-smooth muscle autoantibodies in such patients (Gluud et al., 1981).

Autoimmune diseases (SLE, myasthenia gravis) have been observed in Klinefelter's syndrome, in which males (XXY karyotype) manifest hypogonadism (Rozenbaum, 1965; Vallotton and Forbes, 1967; Stern et al., 1977). Finally,

reduction of estrogen-to-androgen ratio in female SLE patients by the administration of cyproterone acetate minimizes the frequency of exacerbations (Jungers et al., 1985).

A monozygotic twin who underwent oophorectomy (presumably resulting in depleted female hormones) did not develop lupus compared with her counterpart, thus supporting the contention that sex hormones influence the pathogenesis of the disease (Jungers et al., 1985).

## 2. Sex Hormone Metabolism Abnormalities

Abnormal sex hormone metabolism occurs in SLE patients, leading to the production of estrogen metabolites, which have persistent estrogenic effect. Female lupus patients have increased 16- $\alpha$ -hydroxyestosterone and estriol (Lahita et al., 1979). It must be noted, however, that a direct correlation with clinical status is not evident. In male patients there is an increase of 16- $\alpha$ -hydroxyestosterone only. Abnormal 16- $\alpha$ -hydroxylation has also been reported in Klinefelter's patients with lupus (Lahita, 1987). Interestingly, in lupus, which tends to exhibit familial aggregation, there was an increased level of 16- $\alpha$ -hydroxylation in first-degree relatives (Lahita et al., 1981).

Female patients with SLE have increased oxidation of testosterone at C-17 (Lahita, Kunkel, and Bradlow, 1983). Further, these female patients have lowered levels of testosterone, particularly dehydroepiandrosterone and dehydroepiandrosterone sulfate, which correlates with disease activity (Jungers et al., 1982; Lahita et al., 1987). Whether there is a conversion to estrogenic compounds in these patients is not readily apparent. Studies in male SLE patients suggests that basal testosterone levels may be lower (Lavalle et al., 1987), although this was not confirmed in a second study (Lahita et al., 1987). However, it appears that dehydroepiandrosterone and dehydroepiandrosterone sulfate may be reduced, suggesting abnormalities in androgen metabolism similar to that in female lupus (Lahita et al., 1987). Increased serum levels of prolactin were also reported in male SLE patients (Lavalle, et al., 1987). Whether this is a consequence of estrogen-induced stimulation of prolactin is not known.

Male rheumatoid arthritis patients have decreased concentrations of serum testosterone and dehydroepiandrosterone (Cutolo et al., 1984), providing further evidence for sex hormonal involvement in autoimmune diseases.

Gonadal-autoimmune association is also suggested in male Graves' disease patients, who may develop gynecomastia and have an imbalance of estrogen and androgens (Chopra and Tulchnsky, 1974). However, it is noteworthy that the precise involvement with the pathogenesis of the disease remains unclear. Collectively, the data suggests that both female and male sex hormone levels are altered in autoimmune patients. It appears that the ratio of estrogen (estriol) to androgen (dehydroepiandrosterone or dehydroepiandrosterone sulfate) may be more meaningful than individual hormone levels.

### 3. Sex Hormone Therapy

Early studies, though preliminary and unconfirmed, hinted at the therapeutic benefits of testosterone esters in diseases such as SLE and Sjogren's syndrome (Fromer, 1950; Schoonhoven Van Beurden, 1953). Nandrolone-deconate (19-nortestosterone), an androgen with minimal androgenizing effects, has been shown to have promising effects in SLE (Lahita and Kunkel, 1984) and RA (Pipitone and Carrozzo, 1976).

Unlike in SLE, pregnancy and estrogen-containing oral contraceptives have a beneficial effect in RA in most, but not all, studies (Kay and Wingrave, 1983). This apparently conflicting result reflects the complex effects of sex hormones in these two different autoimmune diseases. It is highly conceivable that estrogens may have anti-inflammatory effects, perhaps by retarding the production of certain cytokines with bone-resorbing abilities. If the beneficial effects of this steroid in RA is substantiated, then it may prove beneficial to chemically modify the estrogen to retain its anti-inflammatory effects without adverse side effects.

Androgens, such as testosterone or 5- $\alpha$ -dihydrotestosterone, cannot be utilized clinically because of obvious male virilizing effects. Further, testosterone may be converted to estrogen. Danazol, an attenuated male sex hormone, has been employed in the clinical treatment of SLE, with varied results (Morley, Parke, and Hughes, 1982). It is of relevance that no beneficial effects of danazol were evident in animal studies (Roubinian et al., 1979a). Further studies are warranted, particularly since this drug has been shown to have a marked therapeutic effect in the treatment of idiopathic thrombocytopenia (Ahn et al., 1982). Nonetheless, the use of chemically synthesized androgenlike drugs (by appropriate substitution and/or conjugation), which possess their immunological effects but are devoid of virilizing effects, is a feasible and attractive clinical approach. It may prove advantageous to administer the androgenlike drugs with antiestrogens, thus correcting the androgen-estrogen balance.

## V. SEX HORMONE-MEDIATED CELLULAR CHANGES IN THE IMMUNE SYSTEM OF AUTOIMMUNE INDIVIDUALS

Although sex hormones affect all lymphoid organs, the thymus appears to be the major target organ through which they bring about their action. Early classic experiments revealed that the thymus must be present in order to demonstrate the immunopotentiating effects of orchidectomy (Castro, 1974). A similar effect has been noted in B/W mice (Ansar Ahmed, Penhale, and Talal, 1985). Thymic hyperplasia, which occurs after orchidectomy, may be qualitatively different in autoimmune and normal individuals. Flow cytometry analysis in SLE-prone NS mice revealed a selective expansion of dull Thy 1.2, Lyt-1, and dull PNA<sup>+</sup> thymocytes, as opposed to an equal expansion of all subsets in normal mice (Dumont, Barrois, and Haberset, 1982).

Sex hormone administration modulates T-cell-mediated delayed-type hypersensitivity reactions (Wyle and Kent, 1977; Bullock, Anderson, and Golding, 1980; Dumont, Barrois, and Haberset, 1982; Ansar Ahmed, Penhale, and Talal, 1985; Ansar Ahmed, Talal, and Christadoss, 1987). We found that in vivo testosterone treatment reduced lymphoproliferative response to purified protein derivative, a T-dependent antigen (Ansar Ahmed, Talal, and Christadoss, 1987). Autoimmune thyroiditis occurring in Tx-X rats, believed to be a T-cell-mediated disease, can be therapeutically treated with testosterone (Ansar Ahmed, Young, and Penhale, 1986). Androgens maintain suppressor cell activity and the numbers of Lyt-2 positive cells (Weinstein and Berkovich, 1981; Ansar Ahmed, Dauphinee, and Talal, 1985; Bruley-Rosset, Dardenno, and Schuurs, 1985). Physiological concentrations of endogenous androgens are also believed to affect lymphocyte subpopulations in prepubertal boys (Dunkel et al., 1985). Androgens can maintain or increase IL-2 levels, whereas estrogens induce hypoactivity of T cells (Weinstein and Berkovich, 1981; Ansar Ahmed, Dauphinee, and Talal, 1985; Bruley-Rosset, Dardenno, and Schuurs, 1985). We measured the activity of ornithine decarboxylase, an early enzyme in polyamine synthesis which correlates with DNA and RNA synthesis, in lymphocytes from estrogen- and sham-treated mice. Splenic lymphocytes from these mice were stimulated, with Con A and the ODC activity measured subsequently. We found that estrogen-treated mice had reduced ODC activity compared to controls (Ansar Ahmed, Talal, and Fischbach, in preparation).

Estrogen alters T-cell subsets in lymphoid organs. In general, estrogens reduce Lyt-2 positive cells (Ansar Ahmed, Dauphinee, and Talal, 1985; Bruley-Rosset, Dardenno, and Schuurs, 1985) and Lyt-1 positive cells (Olde, 1987). Further, in vitro studies using human lymphocytes indicate that estrogens affect OKT-8 positive T cells, which have suppressor/cytotoxic function. Estrogens reduce IL-2 and  $\gamma$ -interferon production (Reinicke, 1965; Ansar Ahmed, Dauphinee, and Talal, 1985; Pung et al., 1985) by T cells. Finally, estrogens reduced T-cell-mediated autoimmune thyroiditis (Kappas, Jones, and Roitt, 1963; Ansar Ahmed, Young, and Penhale, 1983) and experimental encephalomyelitis (Arnason and Richman, 1969).

Sex hormones also modulate the autoantibody levels, thus suggesting that B cells are targets of sex hormones. Autoantibodies to bromelain-treated mouse red blood cells are produced largely by Lyl<sup>+</sup>B cells and are modulated by sex hormones. We found that these autoantibodies are increased in female autoimmune as well as normal mice (Ansar Ahmed, Dauphinee, and Talal, in preparation [a]). Estrogen administration further increased this response. One mechanism of estrogen effects on autoimmune disease is by inducing hypoactivity of T cells and hyperactivity of B cells.

Macrophages-monocytes are considered another target for sex hormones. The activity of macrophages is altered by the natural estrous cycle. Estrogens modulate

macrophage phagocytic activity (Vernon-Roberts, 1969), bring about cytostasis of malignant cells, and the secretion of plasminogen activators. Estrogens stimulate the reticuloendothelial cells (Kelly, Brown, and Dobson, 1962; Dean et al., 1986), the number of circulating monocytes (Boorman et al., 1980), and the division of Kupffer cells (Dulk, Crofton, and Van Furth, 1979). Estrogens also enhance clearance of immunoglobulin-G-coated erythrocytes (Friedman, Neltl, and Schreiber, 1985).

Peritoneal and alveolar macrophages themselves can convert testosterone to  $5\alpha$ -reduced metabolites (Lofthus, Marthinsen, and Eik-Nes, 1984; Milewich et al., 1985). Incubation of guinea pig alveolar macrophages with radiolabeled androstenedione resulted in the metabolites testosterone,  $5\alpha$ -dihydrotestosterone,  $5\alpha$ -androstane-3 $\beta$ , 17 $\beta$ -diol (Milewich et al., 1983). Macrophages contain a variety of steroid-metabolizing enzymes, including 17 $\beta$ -hydroxysteroid dehydrogenase,  $5\alpha$ -reductase, and 20 $\alpha$ -hydroxysteroid dehydrogenase (Hapel et al., 1985).

The production of cytokines by macrophages is also affected by sex hormones. Estradiol stimulated IL-1 production by macrophagelike cells derived from the placenta (Flynn, Finke, and Loftus, 1985). Estrogen (and progesterone) administration to mice increased the production of IL-1 and Ia expression on peritoneal macrophages (Flynn, 1986).

Thymic macrophages display class II antigens (Ia) and possibly account for all the Ia-positive cells in this organ (Epstein et al., 1985). Testosterone or dehydrotestosterone treatment of normal and autoimmune mice profoundly reduced Ia positive cells in the thymus, whereas estrogens did not (Ansar Ahmed and Talal, in preparation). The phenotype of Ia-positive cells is currently being studied. Whether the modulation of Ia antigen by sex hormones is the indirect consequence of changes in cytokines (e.g.,  $\gamma$ -INF, TNF) is not known. Sex hormones have been shown to modulate Ia antigen expression by the mammary gland epithelium. Estrogen, prolactin, or progesterone administered to guinea pigs and mice increased Ia expression on these cells (Klareskog, Forsum, and Peterson, 1980).

Natural killer cells are important cells participating in spontaneous cytotoxicity against a variety of malignant and viral-infected cells. Natural killer cell activity in the spleen is reduced in autoimmune mice (Pan et al., 1986). Estrogens reduce splenic NK cell activity both in normal as well as autoimmune mice (Seaman and Gindhart, 1979). The mechanism of action is not precisely understood. Several possibilities exist. First, estrogens bring about occlusion of the bone-marrow cavity of long bones, thus severely diminishing the precursors of NK cells. Estrogen may reduce the production of T-cell lymphokines, such as  $\gamma$ -INF (or IL-2), which promote NK cell activity. Finally, estrogen may affect the suppressor cells regulating NK cell activity, although this possibility remains unproven.

Sex hormones may also influence nonlymphoid cells that interact closely with

lymphoid cells. For example, chemical bursectomy induced by testosterone is due to the effects of steroids on the epithelial cells but not the lymphoid cells (Cole, Kite, and Witebsky, 1968). This affects the maturation and development of B cells.

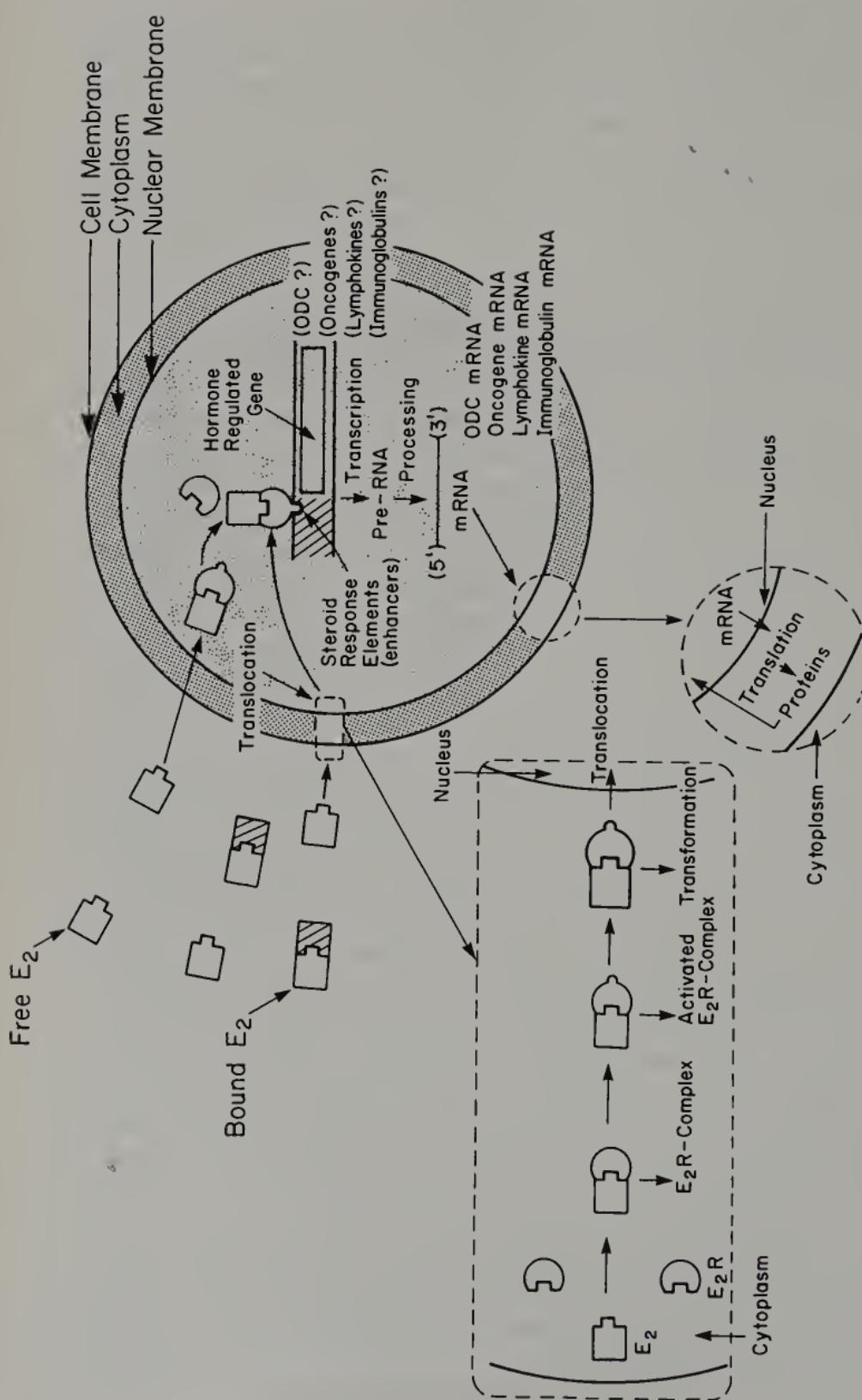
Sex steroid receptors found in thymic epithelial cells suggest that these cells are targets for sex hormones. Sex hormones may bring about alterations of thymic hormones and the internal thymic microenvironment and thereby influence T-cell differentiation and proliferation.

## VI. SEX STEROID RECEPTORS IN THE IMMUNE SYSTEM

The precise mechanisms by which sex hormones interact with lymphocytes are complex. Whether lymphocytes are direct targets for sex hormones is a fundamental question which remains unanswered. If so, then the hormones must act through specific binding with steroid receptor protein. However, these receptor proteins have never been definitively demonstrated in murine lymphocytes. This may be due to a very low capacity of receptors, the use of whole lymphocyte populations rather than sex hormone-sensitive cells, and the technical difficulties encountered by adapting classic autoradiography and exchange assays. Based on these traditional assays, estrogen and androgen receptors have been reported in the thymus gland, lymph nodes, and spleen (Stumpf and Sar, 1976; Kalland, 1980; McCruden and Stimson, 1981; Grossman, Sholiton, and Helmsworth, 1983). It is not clear whether these receptors are present on lymphocytes, nonlymphoid cells, or both. Studies involving the fractionation of lymphocytes suggested that estrogen receptors were present on OKT-8<sup>+</sup> cells and thymomas in humans that have suppressor/cytotoxic function (Ranelletti et al., 1980; Cohen et al., 1983). These studies, if confirmed, suggest that a specific T-cell subset may be sensitive to sex hormones.

In any event, the addressing of the fundamental question of whether or not sex steroid hormone receptors are present in lymphocytes has been hampered by technological flaws. However, two recent significant developments should aid in the precise identification of estrogen receptors (King and Greene, 1984), i.e., the availability of monoclonal antibodies to estrogen receptors and a cDNA for estrogen receptors which is successfully cloned and sequenced (Green et al., 1986; Greene et al., 1986). We are currently attempting to employ these two approaches, in collaboration with Dr. Sheridan, in order to identify both the message for estrogen receptors and the protein product in lymphocytes.

Extrapolating from available data on the classic reproductive organs (Knowler and Beaumont, 1984; Yamamoto, 1985), we believe that sex hormones may act on lymphocytes in a similar manner. A hypothetical model suggests that biologically active free estrogen in the circulation which is lipophilic passively enters the lymphocytes (Figure 2) and specifically binds to receptors to form estrogen receptor complexes ( $E_2R$  complexes) within the nucleus. A minor pathway may involve



**Figure 2.** A hypothetical mechanism of action of estrogen ( $E_2$ ) on lymphocytes. Free  $E_2$  binds to its receptor in the nucleus to form  $E_2R$  complex, which binds to nuclear acceptor sites to regulate expression of genes. A minor mechanism of sex steroid entry may be through binding of  $E_2$  with cytosolic R, which then is translocated into the nucleus. If sex steroids directly affect lymphocytes, they may influence the regulation of lymphokines, oncogenes, and immunoglobulins. This aspect still needs to be investigated.

specific E<sub>2</sub>R complexes in the cytoplasm, which translocate into the nucleus. Estrogen receptor complexes acquire increased binding affinity for a particular sequence of DNA, i.e., the nuclear matrix or chromosome-binding region (steroid response elements). This results in selective modulation of specific gene transcription. Perhaps, as in the classical model, these receptor elements reside in multiple copies in close proximity to the genes that they regulate. We would postulate that the DNA binding of estrogen receptor complexes facilitates the selective transcription of genes involved in cellular proliferation and immunoregulation including proto-oncogenes, c-fos and c-myc, the ornithine decarboxylase gene, and lymphokines and monokines, interleukin-2,  $\gamma$ -interferon, and TNF- $\alpha$ . Another possibility that warrants consideration is that E<sub>2</sub>R complex binding to specific DNA regions may induce the production of certain polypeptides (e.g., prolactin), which may in turn influence the production of lymphokines. These aspects are being actively considered in our laboratory.

Macrophages/monocytes that are important in the immune system may also contain these receptors. Sex hormones may then regulate the production of important lymphokines, such as IL-1 and TNF- $\alpha$  and class II antigen expression.

## VII. FACTORS INFLUENCING THE MODULATION OF THE IMMUNE SYSTEM BY SEX HORMONES

### A. Sex Steroid Receptors in Nonclassic (Nonreproductive) Target Sites

Independent and collaborative work among various subspecialties of medicine led to the conclusion that diverse body tissues communicate and mutually influence function. For example, gonadal hormones influence the thymic state, and the thymus in turn affects gonadal function. Neonatal thymectomy of mice results in ovarian dysgenesis (Michael, 1979). Furthermore, thymic hormones decrease sex hormones like progesterone. A third system, the CNS, may also be involved. Thymic hormones may influence the release of pituitary hormonal neuropeptides, which in turn influence the immune system and the reproductive system (Proceedings of a conference on neuromodulation of immunity and hypersensitivity, 1985).

The current evidence suggests that sex hormones influence the immune system through multiple pathways. The presence of intracellular sex steroid receptor proteins is indicative of potential action sites. These receptors have been found not only in classical reproductive tissues, but also in tissues not directly related to the reproductive system. We believe that the presence of sex hormone receptors in these tissues may have an indirect relevance to the immune system, including the following:

#### 1. Central Nervous System

Sex steroid receptors are located in various anatomic regions of the brain, including the ventromedial nucleus (VMN) of the hypothalamus, the brain stem,

allocortical and mesocortical regions of the phallum, the preoptic region, and the olfactory lobe (Stumpf and Sar, 1976; McEwen et al., 1979). These receptors are also found in the pituitary gland.

Solid evidence is now emerging to show that the central nervous system and the immune system interact closely (Proceedings of a conference on neuromodulation of immunity and hypersensitivity, 1985). The brain and the pituitary can influence the regulation of the immune system. Examples include: (1) Hypothalamic lesions profoundly modulate immune responses, (2) the immune response to antigens is associated with the electrical activity in the VMN of the hypothalamus, (3) the direct innervation of lymphoid organs, and (4) the production of neuropeptides and polypeptides by lymphocytes, including prolactin, adrenocorticotropic hormone, and endorphins.

Thus it is conceivable that sex hormones can act in appropriate areas of the CNS (and pituitary) to release immunomodulatory peptides.

## 2. Macrophage-Monocyte System

Sex steroid receptors are believed to be present on these antigen-presenting cells (Stumpf and Sar, 1976). Sex hormones may thus influence the presentation of antigen and production of important cytokines.

## 3. Other Systems

Sex hormone receptors are present on endocrine glands, such as the thyroid, and on submandibular and parotid salivary glands (Stumpf and Sar, 1976). Thus, they may influence the release of thyroxine or other factors, which can have immunoregulatory effects.

These receptors are also present in the liver (Stumpf and Sar, 1976; Ansar Ahmed, and Talal, 1985), perhaps influencing the clearance of immune complexes and macrophage function.

Sex steroid receptors have not been unequivocally demonstrated on bone cells. However, it is commonly observed that prolonged estrogen treatment of mice leads to osteopetrosis and obstruction of the marrow cavity. Since bone marrow is filled with stem cells, the availability of these cells to the peripheral organs is severely diminished and can result in decreased NK function in the spleen (Eidinger, Genant, and Connock, 1987). Estrogen effects on the prevention of osteoporosis in women is well known. However, it is highly likely that estrogen may influence bone cells indirectly rather than directly.

Thus, taken together, it is clearly evident that sex hormones can influence the immune system through many routes.

## B. Genetic Regulation of Sex Hormonal Modulation of Immune Responses

The major histocompatibility complex genes in the mouse regulate several immune functions as well as diverse aspects of sex-related physiological functions.

These include the H-2 regulation of serum testosterone levels and androgen metabolism (Ivanyi et al., 1973), testicular weights (Ivanyi, 1978), sensitivity to testosterone (Chai, 1960; Ivanyi et al., 1973), levels of testosterone binding globulins (Ivanyi et al., 1973), estrogen receptors in the uterus (Palumbo and Vladutiu, 1979), production of sex-limited proteins in serum of males (Hansen, Shin, and Shreffler, 1975), blocking of pregnancy, and mating preferences (Yamazaki et al., 1976). Moreover, genes controlling an enzyme involved in steroid conversion, 21-hydroxylase, have been mapped within the MHC of both man (Carroll, Campbell, and Porter, 1985) and mouse (White et al., 1984). We found that the genetic haplotype of mice influenced testosterone induced T-cell immune suppression (Ansar Ahmed, Talal, and Christadoss, 1987). The MHC supratype, including C4AQ0 allele, is associated with 21-hydroxylase deficiency, depleted testosterone levels, and increased manifestations of autoimmune disease (Dawkins et al., 1983). Sex hormonal levels modulate HLA-antigenic expression and development of autoantibodies (Gluud et al., 1981). Autoimmune mice belonging to H-2<sup>b</sup> haplotype (C57BL/6-Ipr), but not those belonging to H-2<sup>k</sup> haplotype (C3H-1pr, MRL/pr and AKR-1pr), displayed sex differences in autoantibodies to DNA and rheumatoid factors (Warren et al., 1984). H-2-associated differences in glucocorticosteroid and glucogen receptors in lymphoid and nonlymphoid organs have been reported (Lafuse and Eddin, 1980; Gupta and Goldman, 1982).

The exact mechanisms underlying the relationship of MHC genes, sex hormones, and immune responses are not known. Could the right genetic make-up render the individual susceptible to deleterious effects of sex hormones (e.g., estrogen) leading to the development of autoimmune diseases? This question will hopefully be answered as we progress in the field of molecular immunology.

### VIII. CONCLUSION

We believe for the following reasons that studies pertaining to the interplay of endocrine products (sex hormones) and the immune system are important from both biologic and immunopathologic standpoints:

1. Sex hormones are produced throughout life, including prenatal life, although the quantities vary at different stages of life. We believe that sex hormones influence the immune system in terms of differentiation, maturation, maintenance, or deletion and migration of lymphocytes during all phases of life.
2. Sex hormones markedly affect immunoregulation leading to the development of autoimmune diseases.
3. Sex hormones may be used as a natural biological tool to study the normal and abnormal regulation of the immune system, thus facilitating understanding of the complex functioning of lymphocytes at the subcellular and molecular level.

4. Pharmacologically modified sex hormones may be used as immunomodulators and hence may be of potential therapeutic benefit.

The foregoing studies established that sex hormones directly or indirectly affect T and B cells and macrophages by manipulating the production of lymphokines and cytokines and alter expression of critical surface molecules thereby regulating the development of autoimmunity and autoimmune diseases.

Sex hormones influence the immune system at many levels by affecting multiple organs and cells through mechanisms that are complex and imperfectly understood. Investigative studies in the fields of immunology, endocrinology, neuroendocrinology, and molecular biology should lead to a better understanding. Molecular biological studies will aid in the understanding of fundamental questions such as: Do cells of the immune system possess sex steroid receptor proteins? Do sex hormones specifically activate lymphokines and MHC antigens and other antigenic molecules? Do sex hormones modulate "activation" of proto-oncogenes c-fos, c-myc, and an oncogene v-erb-A, which share strong homology with steroid receptor proteins and thyroid hormone? In this context, the development of an in vitro culture system specifically suitable for sex hormones and lymphocytes would be enormously helpful in elucidating whether lymphocytes are direct targets of sex hormone actions.

## ACKNOWLEDGMENTS

This work was supported, in part, by grants from the National Institutes of Health Multipurpose Arthritis Center, General Medical Research Council of the Veteran's Administration, and the Lupus Foundation of America, Inc.

The expert secretarial assistance of Mrs. Ann Franklin is gratefully acknowledged. The excellent technical assistance of Miss Iris Montoya is greatly appreciated.

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