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# SEX STEROIDS, GLUCOCORTICOIDS, STRESS AND AUTOIMMUNITY

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**Summary**—Interest in the field of neuroimmunoendocrinology is in full expansion. With regard to this, steroid influence on the immune system, in particular sex steroids and glucocorticoids, has been known for a long time. Sex steroids are part of the mechanism underlying the immune sexual dimorphism, as particularly emphasized in autoimmune diseases. Immunosuppressive and anti-inflammatory effects of glucocorticoids are now considered a physiological negative feedback loop to cytokines produced during an immune and/or inflammatory response. Psychosocial factors may play a role in the development of immunologically-mediated diseases, e.g. autoimmune diseases. The nonobese diabetic (NOD) mouse, that develops an immunologically-mediated insulin-dependent diabetes mellitus (IDDM) is an interesting model to study the role of endogenous steroids. Insulitis is present in both sexes, but diabetes has a strong preponderance in females. Hormonal alteration, such as castration, modulates the incidence of diabetes, whereas environmental factors, such as stress, accelerate the disease. In the present paper, we have reviewed the role of gender, sex steroid hormones, stress and glucocorticoids in autoimmunity as well as analyzed their different levels of actions and interrelationships, focusing particular attention on the immunologically-mediated IDDM of the NOD mouse.

## INTRODUCTION

Over the past 15 years, there has been a marked increase in the number of studies on the function of the immune system. It has become increasingly apparent that the immune system interacts with most, if not all of the body systems. The immune system, like the nervous and endocrine systems, plays an important role in biological adaptation, contributing to the maintenance of homeostasis and to the establishment of body integrity. The key to effective immunity is the elimination of pathogens without concomitant host injury. Indeed, finely tuned modulation of the immune system is crucial to the organism, since excessive activation can lead to autoimmune diseases, allergies, hypersensitivity and anaphylaxis, whereas oversuppression may promote infectious diseases and cancer.

In order to bring the self-regulated immune system into the same conceptual framework as the other body systems, its functioning can be considered within the context of an immune neuroendocrine network. This scheme is based upon the existence of afferent–efferent pathways between immune and neuroendocrine structures and has particular implications for certain diseases [1, 2]. For example, endocrine disorders have consequences for the immune system, such as those seen in Cushing's disease and diabetes mellitus. Moreover, psychological factors can modulate the immune response: stress, distress and a variety of psychiatric illnesses, notably affective disorders, are increasingly being related to immunosuppression [3–6]. On the other hand, autoimmune mechanisms are responsible for several endocrine diseases.

Studies on the roles of hormones in the immune response have generally involved the parenteral administration of hormones to experimental animals or to man, or the ablation or blockade of endocrine glands. Numerous reports agree that hormone administration can lead to depressed or stimulated immune responses depending upon the hormones used, the dosage and the timing of administration. Thus, such experiments demonstrate that changes in

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the levels of various hormones can considerably influence the immune response. In addition, the possibility that the immune response itself can bring about changes in hormone and/or neurotransmitter levels is also beginning to emerge [7].

Autoimmune diseases arise when the immune system fails to discriminate between self and nonself antigens. Autoimmune diseases are clearly multifactorial in origin, with genetic, immune, endocrine and environmental elements contributing to their development [8–11]. There has been considerable progress made recently in characterizing the target autoantigens against which autoimmune responses are directed, the immunological mechanisms that induce autoimmune disease and the immunogenetic background of individuals at risk. Important, but not yet well-understood, contributors to the development of autoimmune diseases may include changes in steroid hormones, such as sex steroids and glucocorticoids. On the one hand, sex steroids are part of the mechanism underlying the well-recognized sexual dimorphism, as particularly emphasized in autoimmune diseases; on the other hand, clinical and experimental observations link stressful life events and the onset of these diseases. In this regard, glucocorticoids are part of the endocrine response to stress, with well-known immunosuppressive and anti-inflammatory effects.

In the present paper we review the role of sex steroids, glucocorticoids and stress in some autoimmune processes. We provide insight into the interrelationship between glucocorticoids and sex steroid metabolism, particularly in stressful situations, in an attempt to better understand the role of endogenous steroids in an animal model of immunologically-mediated insulin-dependent diabetes mellitus (IDDM), the nonobese diabetic (NOD) mouse.

## SEX STEROIDS AND AUTOIMMUNITY

### *Sexual dimorphism in the immune response*

Physiological, experimental and clinical data substantiate differences between the two sexes, in terms of the immune response [12–17]. Briefly, the first line of evidence for a role of sex steroids in the immune response is the presence of a naturally occurring sexual–immunological dimorphism between males and females. Indeed, females seem to have a more vigorous immune response, a more developed thymus, higher immunoglobulin concentrations, stronger primary and secondary responses, more resistance to the induction of immunological tolerance and a greater ability to reject tumors and homografts. Castration of young males increases thymic weight and leads to a heightened immune response. The second line of evidence is that the majority of patients suffering from various types of autoimmune diseases is female [13, 15, 17]. For example, a higher female/male susceptibility ratio is seen in the adult form of Hashimoto's thyroiditis (25–50:1), and various other thyroid autoimmune diseases. In systemic lupus erythematosus (SLE) as well as in Sjögren's syndrome, the female/male ratio is 9:1, while it is slightly lower in rheumatoid arthritis (RA), idiopathic adrenal insufficiency, scleroderma, myasthenia gravis (MG) and multiple sclerosis. This differential sex susceptibility is also found in many animal models of autoimmune diseases: not only in the NZB/W mouse, a model of SLE, and in the NOD mouse, a model of IDDM, but also in chronic autoimmune thyroiditis induced in rats by thymectomy and irradiation (Tx-X), in experimental MG induced in susceptible C57BL/6 mice by acetylcholine receptor adjuvant injections, and in the LEW/N rat model of RA induced by injection of the cell wall peptidoglycan polysaccharide fragments from group A streptococcal bacteria. However, the female predominance is not seen in all human autoimmune diseases, particularly in IDDM, nor in all the experimental models.

### *Sex steroids in SLE and SLE-like disease of the NZB/W mouse*

The role of sex steroid hormones has been extensively investigated in human SLE as well as in the SLE-like syndrome of the NZB/W mouse [18, 19]. In NZB/W mice, the disease progresses more rapidly in females than in age-matched males, leading to earlier death of females. While ovariectomy has little influence on the evolution of the disease in females, in contrast, prepubertal orchidectomy enhances the expression of lupus in males, and gives rise to a mortality pattern similar to that observed in females. Female sex hormone replacement therapy (estradiol or progesterone) accelerates, whereas androgens (dihydrotestosterone) delay the evolution of the disease [20, 21]. Anti-DNA and anti-poly (A) autoantibody levels, intensity of glomerulonephritis and mortality are decreased by castration or androgenotherapy in females, whereas they are increased by castration or estrogenotherapy in males. Moreover,

delaying the administration of androgens to NZB/W mice with active disease is therapeutic and prolongs survival [22, 23]. Finally, sex chromosome-linked effects have not been observed to influence sex linkage of murine SLE [24, 25]. This suggests that androgens have a protective role in suppressing the disease process, whereas estrogens accelerate the immunopathogenesis, thereby accelerating mortality. According to these data, different hormonal treatments have been tested in NZB/W mice, using either synthetic androgens with weak androgenic properties, such as Danazol, or anti-estrogens, such as Nafoxidine [26–29]. While Danazol treatment of female NZB/W mice, intact or ovariectomized, has no protective effect, Nafoxidine is able to delay the expression of autoimmune symptoms.

In human SLE, several arguments also suggest a role for sex steroids in the evolution of the disease. Clinically, in addition to the important female preponderance at fertile age, one can note an aggravation of the disease symptoms during pregnancy or with administration of oral sex steroid-containing contraceptives and the association of SLE with Klinefelter's syndrome [13, 15, 17, 30]. Biologically, female SLE patients have an abnormal metabolism of androgens (increased oxidation of testosterone) and estrogens (increased 16 $\alpha$-hydroxylation of estrone), leading to a decreased androgen/estrogen ratio [31–34]. In a more recent investigation, the major plasma androgens (testosterone, androstenedione, dehydroepiandrosterone and dehydroepiandrosterone sulfate) were measured in men and women with SLE during various stages of clinical activity [35]. Decreased levels of all androgens were observed in women with SLE. Moreover, the lowest levels of plasma androgens were found in female patients, whose disease was active as determined by laboratory tests and clinical criteria. Sex steroid abnormalities also exist in male SLE patients with or without Klinefelter's syndrome [34, 36]. To the best of our knowledge, serum sex steroid levels have not been studied in NZB/W mice, but data are available on hepatic microsomal estrogen metabolism [37]. However, it appears that in premorbid female NZB/W mice this metabolism was not altered in a manner that would result in abnormal retention of hormonally active metabolites. It should be noted, however, that the estrogen receptor contents in the livers of NZW and NZB/W (F1) mice were found to be twice that of NZB mice [38], while no differences appeared to exist among receptor concentrations in the uteri, thymuses and spleens of NZB, NZW and F1 mice. The observation of an abnormal sex steroid metabolism in human SLE led, as in NZB/W mice to therapeutic regimens attempting to increase the androgen/estrogen ratio. This was achieved by using weak androgens and anti-gonadotropic drugs. Preliminary results obtained with Nandrolone (19-nortestosterone) administration did not improve patient's clinical conditions, whereas Danazol, in contrast to what has been obtained in mice, led to clinical improvement [39, 41]. In another study, the clinical efficacy and tolerance of Danazol and cyproterone acetate were tested in 11 female patients, who suffered from mildly active SLE [42, 43]. Because of its side effects, Danazol had to be withdrawn early in 2 patients, whereas cyproterone acetate appeared to be well-tolerated in all of them. On the whole, 16 clinical exacerbations of SLE were observed during a 12-month pretreatment period, vs 9 exacerbations during a 12-month treatment period. Accordingly, the average dose of prednisone could be reduced from 9.6 to 3.5 mg/day in these patients. Increased plasma testosterone levels without any change in estradiol levels were observed in patients treated with Danazol. Conversely, plasma estradiol decreased without any change in testosterone levels in women treated with cyproterone acetate. Both drugs reduced the concentration of plasma sex steroid binding protein. Thus, these results suggest that Danazol and cyproterone acetate may reduce SLE disease activity in parallel with a hormonal environment modification toward an increased androgen/estrogen ratio. A therapeutic effect of testosterone undecanoate has also been described in patients with Klinefelter's syndrome associated with SLE or Sjögren's syndrome [44]. Among various biological parameters, serum testosterone and luteinizing hormone (LH) levels, anti-nuclear antibodies, rheumatoid factor and peripheral T cell phenotypes (CD3, CD4 and CD8) were measured before treatment and after 60 days of oral testosterone undecanoate treatment. Before treatment and after placebo, compared with normal men, patients had lower serum testosterone and higher LH levels, lower percentages and absolute values of CD3 (total T lymphocytes) and CD8 (suppressor/cytotoxic T lymphocytes) and, consequently, increased CD4/CD8 ratios. All had high titers of antinuclear antibodies and rheumatoid factor. After testosterone undecanoate therapy, serum testos-

terone levels increased and LH levels decreased, CD3 and CD8 cell numbers and the CD4/CD8 ratio became normal and antinuclear antibodies and rheumatoid factor titers decreased.

*Sex steroids and other autoimmune diseases*

Abnormalities of sex steroid levels, particularly of androgens, have been reported in other autoimmune diseases, mainly RA, but also IDDM and MG [45–51]. A decreased concentration of androgens either basal or stimulated, was usually observed in male patients with RA, whereas conflicting data were obtained in women, with, nevertheless, a tendency toward normal values [45–49].

*Dissociation between female preponderance in autoimmunity and sex steroid effects*

It should be emphasized that, since the disease is preponderant in the females, the effects of physiological variations in sex hormone levels or of castration and sex steroid hormone treatment are extremely logical in SLE and its murine model, the NZB/W mouse; this is not always the case, when considering other autoimmune diseases. RA is also more common in women, with a sex ratio (3:1) that is not as high as that for lupus (9:1) [13, 17]. However, in contrast to SLE, estrogens seem to ameliorate clinical status rather than aggravate it [17, 24]. Indeed, rheumatoid disease symptoms decrease during pregnancy and during the postovulatory phase of the menstrual cycle. Moreover, oral contraceptives or substitutive estrogen therapy may reduce the incidence of RA [52]. Thus, estrogens, despite the increased female susceptibility, may protect humans from rheumatoid disease. This is also observed in animal models of RA, for example, in type II collagen-induced arthritis (CIA): in the mouse, males are more susceptible than females, whereas in the rat, females are more susceptible than their male counterparts [24]. However, it is clear that estrogens suppress both the incidence and the severity of CIA in mice and rats [24, 53–57]. Female mice are to a large degree protected from CIA during pregnancy. Treatment of castrated DBA/1 mice with physiological doses of 17 $\beta$-estradiol suppresses both the incidence and the severity of the disease [56,57]. Progesterone has no effect by itself, but enhances the effect of estradiol. Moreover, mouse T cell proliferative responses and delayed-type hypersensitivity reactions are suppressed by estradiol, as is the T-dependent IgG response to type II collagen, whereas the IgM response (T cell-independent) is enhanced. Adjuvant arthritis (AA) represents another model of polyarthritis. It is induced by injection of complete Freund's adjuvant into susceptible rat strains. There is little difference between the sexes, but pharmacological doses of estrogens diminish the severity of symptoms [24]. Male rats with AA have reduced testosterone levels, while LH levels are increased [58]. With regard to other autoimmune diseases in humans, it should be noted that pregnancy reduces the clinical and biological activity of Graves' and Hashimoto's diseases [24, 59]. In MG, crises are also noted less frequently during pregnancy. In experimental autoimmune thyroiditis induced in Tx-X rats, females are more susceptible than males and prepubertal ovariectomy increases this susceptibility. Estrogen treatment induces a partial suppression of thyroiditis with a concomitant reduction in autoantibodies to thyroglobulin [60]. Estrogens also reduce the incidences of both thyroiditis and autoantibody production in orchidectomized Tx-X rats. However, androgens also have beneficial effects in this model: they induce significant regression of established thyroiditis without affecting serum levels of autoantibody to thyroglobulin [61]. The differential effect of estrogens on the evolution of different types of autoimmune diseases may be linked to their different immunopathophysiological mechanisms, as discussed below.

## STRESS AND AUTOIMMUNITY

The historical basis for studying the influence of stress on the immune response comes from a legacy of centuries of clinical observations of individuals who became sick following stressful situations. Indeed, stress, distress and a variety of psychiatric illnesses, notably affective disorders, such as depression, have been increasingly reported in the last two decades to be associated with immunosuppression [3–6]. Thus, part of the concept of interactions between the central nervous system (CNS) and the immune system arose from clinical observations indicating that mood states may affect susceptibility to physical illness. On the contrary, the feedback loop from the immune system to the CNS, as already mentioned, is now demonstrated by the fact that soluble immune mediators may affect CNS functions and possibly adaptative behavior during the course of illness.

With regard to autoimmune diseases, numerous clinical observations have noted stressful life events before the onset of the disease, but most of them are subjective and there are only a few well-controlled studies in the literature [62]. Indeed, the evaluation of stress may be easy in some circumstances, such as physical exercise, surgery, hypoglycemia, but more difficult in the case of psychological stress. In humans, assessment of stress is qualitative (i.e. Andrew's questionnaire) or quantitative (i.e. the Holmes–Harris questionnaire). As recently emphasized questionnaires have to be adapted to each country in view of social and cultural particularities [62]. Moreover, stress can vary in duration, frequency, content as well as intensity and the delay between life events and the onset of autoimmune diseases is of critical importance. Despite methodological difficulties associated with the assessment of stress and, hence, the association of stress with the onset of diseases, the concept of stress facilitating the emergence of autoimmune diseases is tempting in the context of the new field of psychoneuroimmunoendocrinology.

*Autoimmune thyroid diseases*

Although a survey of the literature since 1825 shows a reciprocal relationship between emotions and the onset of thyroid diseases, objective evidence has been more difficult to obtain [62–64]. Both physical stress, such as trauma or major illness, and psychological stress, such as bereavement have been implicated. More recently, Forteza found that most of his 116 patients experienced stressful events just before the first signs of Graves' disease [65]. Moreover, an epidemiological study was able to prove a higher incidence of hyperthyroidism in the Danish population during the 1941–1945 German occupation, compared to the 1845–1948 period, i.e. during a period of difficult life conditions [62]. Lastly, in a prospective study, an increased uptake of $^{131}I$ during periods of stress coincided with the development of thyroid hyperfunctioning nodules [66].

*Rheumatoid arthritis (RA)*

The literature concerning RA has been reviewed with regard to the empirical evidence for the widely held view that the onset and course of disease are influenced by stress variables [67]. Different studies indicate that personality factors seem to predispose the individual to the development of RA and suggest that stress and/or the failure of physiological defenses and adaptations to compensate for it are related to the onset of disease [68]. A number of investigations in humans have been conducted to determine whether a relationship exists between the onset or aggravation of rheumatoid symptoms and psychological disturbances. They have led to contradictory results. While the earlier investigations were based upon unsystematic clinical observations, the later ones used control groups and objective methods to assess stressful events [67]. Two well-controlled studies did not find an association, whereas a third one showed a preponderance of stressful life events in only one subgroup of RA patients [69–71]. A study using pairs of female monozygotic twins discordant for RA showed that, in the adult twin pairs, the affected sibling had experienced considerably more stressful life events before the onset of the disease than the healthy one during the corresponding period [72]. Some studies on juvenile RA tend to show a preponderance of stressful events before the onset of the disease [73–75]. Studies on animal models of RA (CA or AA) are scarce and contradictory [67]. Few data are available in SLE, but they mention psychosocial and emotional disturbances [76, 77].

*Diabetes*

Finally, in human IDDM, many studies have suggested a possible role for traumatic or stressful events in precipitating the disease. First, diabetic patients who have undergone surgery, exhibit dramatic deterioration [78]. In this case, the stimulation of the hypothalamo–pituitary–adrenal (HPA) axis induced by surgery mobilizes energy substrates that the insufficient amount of available insulin is not able to balance. Second, psychological stress has also been associated with diabetes in humans [62, 79]. Third, abnormally high frequencies of depressive behavior were reported in children recently diagnosed as having IDDM [80]. Nevertheless, the role of psychogenic factors was questioned in a critical review of these earlier studies [79]. In another study, 25 new diabetic adults were psychiatrically evaluated using a standard personality profile (MMPI); abnormal scores were obtained in 14/25 diabetics, but IDDM and noninsulin-dependent diabetes mellitus (NIDDM) were not separated [81]. A triggering effect of emotional stress was pointed out by Stein and Charles's series, where the delay between the stressful events and overt disease was

very long, but the series lacks a control group [82]. More recent and well-controlled studies underline the role of stress in precipitating IDDM. Kisch found that life events, such as febrile disease, accident, pregnancy, problems at home or at work and others, were mentioned by 74% of the patients of his series in the last year preceding the onset of diabetes [83]. Robinson and Fuller compared ratings of life events occurring during the 3 years prior to diagnosis of diabetes between diabetic and nondiabetic siblings [84]. They found that during the 6 months prior to diagnosis, 46% of the diabetics had experienced at least one severely disturbing life event compared with 18% of the siblings. During the 2.5-year period prior to diagnosis, 73% of the diabetics had one or more dramatic life events compared with 36% of the siblings. In a prospective longitudinal study, Linn *et al.* [85] compared stressful life events in IDDM and NIDDM. They found that IDDM patients reported more stressful events and more perceived stress, anticipation of stress and responsibility for events. Lastly, Vialettes *et al.* [86] showed that recently diagnosed IDDM patients had a significantly higher frequency of stress during the 12-month period preceding the onset of diabetes as compared to age-matched controls. Psychological analysis defined this stress as unpredictable and short, leaving the patient without resources. Finally, two recent studies have shown that stress is able to accelerate the onset of the disease in two animal models of IDDM, the BB rat and the NOD mouse [87, 88].

## STEROIDS AND DIABETES

### *Steroid hormone metabolism in diabetes*

The importance of steroid metabolism in diabetes has been recently emphasized in genetic models of IDDM and NIDDM [89]. In some of these models, the relation between genetic mutations and enzymes of the sulfurylation/desulfurylation cycle in determining the severity of diabetes by controlling intrahepatic balance of free androgens and estrogens has been emphasized. It has also been shown in rodents that insulin helps to maintain sex differences in hepatic sterol metabolism so that insulin deficiency (such as seen in streptozotocin-induced diabetes) leads to altered regulation of hepatic steroid metabolizing enzymes, changes that include increased tissue metabolism of estrogens and increased plasma levels of testosterone [90–92]. With regard to glucocorticoids, administration of diabetogenic doses of streptozotocin is able to increase glucocorticoid sulfotransferase activity in the livers of male rats in a fashion that can be prevented or reversed by insulin [93]. A time course study suggested that the process appears to be a consequence of the disease rather than a diabetes-initiating factor. Moreover, diurnal rhythms of corticosterone have been studied in genetic and chemical diabetes [94]. In genetic models of NIDDM, the normal diurnal pattern of corticosterone secretion is preserved, but, diabetic animals have higher levels of corticosterone throughout the night–day cycle [95, 96]. In streptozotocin-induced diabetes in the rat, a model of IDDM, corticosterone levels are significantly higher during the light cycle [97]. Abnormal diurnal rhythms of cortisol have also been observed in human IDDM, in both children and adults [98–101]. The elevation of circulating glucocorticoids in diabetes is an apparent paradox. Corticosteroids cause insulin resistance and amplify the physiological hyperglycemic effects of catecholamines [102]. Glucocorticoid inhibition by dexamethasone is decreased or absent in both animal and human diabetes [94, 103, 104]. This suggests a decreased sensitivity of the hypothalamo–pituitary axis to the negative feedback effect of glucocorticoids. The mechanism of this hypercorticism remains to be investigated in more detail, but it appears to be a specific response to altered energy regulation and is mediated by the brain. Moreover, these alterations in the HPA axis of diabetic rodents may be responsible for behavioral changes [105, 106]. These data led to a study on the effect of adrenalectomy in streptozotocin-treated rats [107]. When compared to intact diabetic rats, adrenalectomized diabetic rats had less pronounced elevations of plasma glucose and higher insulin levels. To date, the latter have not been clearly explained. To the best of our knowledge, no data are available on glucocorticoid secretion in animal models of genetic, immunologically-mediated IDDM, such as the BB rat or the NOD mouse, in particular before the emergence of the disease symptoms, but, as already mentioned, stress has been shown to accelerate the onset of the disease in both models.

### *Steroids and the NOD mouse*

Between the 12th and 30th weeks of age, the inbred NOD mouse, first described in

Japan, spontaneously develops an autoimmune-induced diabetes which is now recognized as a model of immunologically-mediated IDDM [108–111]. The involvement of the immune system is implicated in this disease in the following ways: (1) mononuclear cell infiltration of the islets of Langerhans, preceding the onset of diabetes, consisting primarily of T cells [112]; (2) experimental transfer of the disease into newborn healthy mice or irradiated adult male NOD recipients by injection of $CD4^+$ and $CD8^+$ T cells from diabetic mice [113, 114]; (3) prevention of diabetes by neonatal thymectomy, introduction of the *nu* gene, treatment of female NOD mice with anti-CD4 or anti-Ia monoclonal antibodies or cyclosporin A [111, 115, 116]; and (4) acceleration of the disease by altering T cell regulation (thymectomy at weaning, cyclophosphamide) [117, 118].

Onset of the disease is characterized by polydipsia, polyuria, glycosuria, rapid weight loss, hyperglycemia and ketoacidosis. Insulitis is characterized by invasion of islets of Langherans, not only by T cells but also by macrophages, and leads to progressive $\beta$-cells destruction and decreased insulin levels, preceding the onset of hyperglycemia. Insulitis begins at 4–5 weeks of age and is present in 100% of females and more than 90% of males at 30 weeks of age [119]. However, there is a sexual dimorphism in the incidence of diabetes: the expression of autoimmune symptoms occurs earlier and is more frequently observed in females, with an incidence reaching 30–70% depending upon the breeding colony, compared to males, in whom the incidence stays below 20% [111]. The characteristics of insulitis also differ between the two sexes, females exhibiting a higher percentage of islets of Langerhans with destructive lesions. However, in contrast to the clear-cut effects of castration and hormone replacement observed in the NZB/W mice, the exact role of sex steroids in the incidence of diabetes remains confused, due to conflicting data [120, 121]. It is well-recognized that the incidence of diabetes may vary from colony to colony depending upon environmental factors, such as diet or stress [111, 122]. The metabolic consequences of stress hormones, catecholamines and glucocorticoids, are well-known: catecholamines simultaneously exert a negative action on insulin production and a positive one on glucagon secretion, thereby increasing circulating glucose and lipolysis. These effects are further amplified by glucocorticoids [123]. As mentioned above, stress accelerates the onset of diabetes in animal models of IDDM [87, 88]. Moreover, adrenalectomy has been shown to counteract the diabetogenic effect of streptozotocin in the rat [107]. These data led us to investigate: (1) the effect of castration at weaning on the incidence of the disease and on the degree of insulitis, at 12 weeks of age, in female and male NOD mice from our own colony; (2) any sex differences in various immune parameters and any modulation of these parameters induced by changes in the sex hormone environment; and (3) serum glucocorticoid and sex steroid concentrations in both sexes of NOD mice under basal and stress conditions, since stress and glucocorticoids are also known to interfere with sex steroid metabolism.

The major conclusions which could be drawn from this work are the following:

1. The sexual dimorphism takes place at the level of the expression of the autoimmune process (incidence, insulitis), with regard to the spontaneous evolution of the disease and the effect of castration (Fig. 1). In agreement with the results of Makino *et al.* [120] ovariectomy tends to exert a protective effect, while orchidectomy has a strong deleterious effect on the incidence of diabetes. The effect of surgery was also studied *in situ* on insulitis. While ovariectomy induced an 11% reduction in the percentage of damaged islets, orchidectomy increased it by 17% [124].
2. In contrast to the clear-cut and opposite effect of castration on the autoimmune process evolving in female and male NOD mice, its effect on the various nonspecific immunological parameters tested (Table 1) gives little information. Data concerning spleen T cell proliferation and IL-2 production as

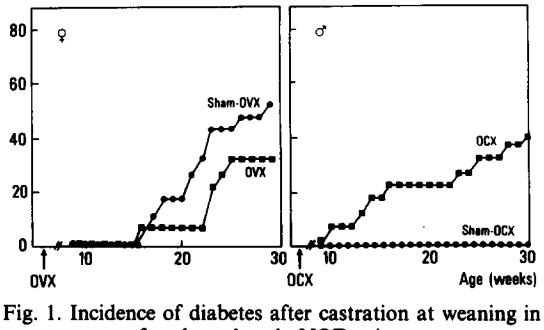


Fig. 1. Incidence of diabetes after castration at weaning in female and male NOD mice.

Table 1. Studies of various immunological parameters in female and male NOD mice and effects of castration on these parameters

| |
|---|
| *Thymus* |
| Weight, cellularity |
| Cell phenotypes |
| *Spleen* |
| Weight, cellularity |
| Cell phenotypes |
| Mitogen-induced cell proliferation (Con A, PHA) |
| IL-2 production |
| Plaque forming cell (PFC) production |

well as antibody production to sheep red blood cells do not reveal in NOD mice any spontaneous sex difference or any effect of castration while phenotype analysis gives only minor information [124]. These negative results of the effect of sex hormone deprivation on some classical immune parameters give rise to an interesting paradox: the autoimmune process is clearly modulated by sex hormones, partly acting via the thymus (thymectomy at weaning strongly modifies the effect of castration in both sexes (data not shown)); however, the impact of sex steroids, via the immune system, on the pancreas, could not be detected either phenotypically or functionally within the limits of the immune parameters measured. This suggests that a numerically minor immune cell subpopulation is involved, which was not detected by the methods used.

3. When measuring serum glucocorticoid levels under basal conditions, a sex difference was noted in NOD mice, as in other rodents, with the females having higher values than the males. In immobilization stress conditions, female nondiabetic NOD mice failed to adapt, whereas males did (data not shown).
4. With regard to sex steroids, male NOD mice exhibited very high testosterone levels when compared to other strains of mice, taken as controls. Estrone and estradiol levels did not differ. Finally, acute or repeated stress significantly reduced serum testosterone levels in male NOD mice (data not shown).

## DIFFERENT LEVELS OF ACTION OF STEROID HORMONES IN AUTOIMMUNITY

With the increasing knowledge of the immunological mechanism underlying the various autoimmune processes, in the biological reactions induced by stress (and especially by different types of stress) and in the interrelationship between stress, glucocorticoids and sex steroids, some thoughts may arise concerning the complexity of the interactions between these different elements. It is obvious that several structures are concerned: the nervous system, responsible for the reactions to stress; the immune system, effector of the aggressivity against self antigens; the endocrine target organ or cells, where the autoimmune process takes place; and, finally, the different organs involved in the production or metabolism of steroid hormones. All these structures are potential targets for different hormones, including steroids, neuromediators and cytokines, which may play a role in autoimmune processes.

### *Sexual dimorphism, stress hormones, neurotransmitters and the nervous system*

Stress represents the reaction of the body to stimuli that disturb its normal physiological equilibrium or homeostasis, often with detrimental effects [2, 6, 18]. There is a common biological response to stress, regardless of its nature. Briefly, different areas of the CNS are involved, which successively release noradrenaline (NA) and acetylcholine (AC) from the vegetative nerve terminals, then adrenaline (A) from the adrenal medulla and later activate the HPA [including corticotrophin releasing factor (CRF), ACTH, $\beta$-endorphin and glucocorticoids] [123, 125]. Other hormones may also be implicated in more specific responses to stress, such as growth hormone (GH), prolactin, glucagon... [126]. On the one hand, it is interesting to note that, in animals, the biological reactions to stress may vary in intensity according to individual factors, such as genetic components [127]. On the other hand, it has become obvious that sex differences exist in the brain, which contribute to sex differences in physiology and behavior [128]. Neurochemical studies have revealed that in certain areas, neurotransmitter content and metabolism are sexually differentiated and under the influence of sex steroids in adulthood. In the context of stress, it is interesting to note that women have a higher risk of depression, that sex differences in 5-hydroxytryptamine (5-HT or serotonin) in rat brain content have been reported, as well as sex differences in the central serotonergic regulation of cortisol, ACTH and prolactin in humans [129, 130]. In contrast, there is increasing evidence that the nervous system is a target for cytokines, produced by the immune system.

Cytokines, such as IL-1, not only affect behavior, but are also able to stimulate the HPA or gonadal axis [2, 5, 131]. This had led to the concept that the immunological challenge itself is able, via the production of cytokines, to generate glucocorticoids, which in turn regulate the immune response [2, 132]. This phenomenon may have implications in some autoimmune processes. First, in highly active forms of RA, the circadian pattern of cortisol secretion is altered [133]. Moreover, a significant correlation between cortisol levels and the inflammatory activity of the disease has been found. In RA, numerous inflammatory mediators are produced, in particular cytokines, which in turn, are able to stimulate the HPA. Second, Lewis rats with experimental allergic encephalomyelitis (EAE), induced either by the s.c. injection of guinea pig myelin basic protein (MBP) or by the adoptive transfer of MBP-primed spleen cells, experienced a single episode of paralysis from which they recovered spontaneously [134]. Animals developing EAE were found to have greatly elevated levels of circulating corticosterone. Adrenalectomized rats given s.c. implants of corticosterone to maintain basal steroid levels invariably died when EAE was induced. However, if the steroid replacement therapy was adjusted to mimic the high hormone levels observed in intact rats developing EAE, then the disease followed a nonfatal course, closely resembling that seen in the non-adrenalectomized controls. It was concluded that endogenous corticosterone release in rats with EAE plays an essential role in the spontaneous recovery observed.

This work on EAE points out the immunosuppressive role of glucocorticoids, which, together with their anti-inflammatory effects, had led to their use as therapeutic agents. However, with regard to stress, glucocorticoids are not the only hormones that may affect lymphoid cells. Many other hormones and neuromediators are altered during stress and there is a growing body of evidence supporting that they have immunomodulatory properties [3, 5, 6]. Moreover, it should be underlined that the immune system is closely related to the nervous system, as demonstrated by the innervation of the lymphoid organs and the demonstration of specific receptors for several neurotransmitters on the lymphoid cells. Noradrenergic, acetylcholinergic fibers have been described as well as fibers immunoreactive for enkephalins, vasoactive intestinal peptide (VIP), neuropeptide Y, cholecystokinin (CCK) and neurotensin [135]. Most, if not all of these neurotransmitters have immunomodulatory properties [136]. Another degree of complexity is represented by the fact that cells of the immune system are able to produce, under some conditions a wide variety of neuropeptides and neuroendocrine hormones, including proopiomelanocortin, preproenkephalin, ACTH endorphins, VIP, somatostatin, thyroid stimulating hormone (TSH), HCG, GH, FSH, LH, oxytocin and neurophysin [137]. The meaning of these endocrine activities remains to be established, but a local action on a neuroendocrine organ or function cannot be excluded.

*Glucocorticoid action on the immune system*

With regard to autoimmunity, the role of glucocorticoids appears to be important, even if sometimes ambiguous. This is clearly indicated not only by the work on EAE [134], but also by a recent report on women with Cushing's syndrome due to an adrenal adenoma, in whom symptomatic autoimmune thyroid disease developed after unilateral adrenalectomy [138]. All the patients, who had detectable but low levels of anti-thyroid microsomal antibody activity before surgery, exhibited increases in anti-thyroid microsomal-antibody titers and changes in thyroid function after surgery. It was concluded that reductions in supranormal glucocorticoid secretion may exacerbate subclinical autoimmune thyroid disease. The immunosuppressive effects of adrenal steroids are, indeed, well-known, although small amounts of glucocorticoids are necessary for *in vitro* immune reactions [2, 11, 132, 139, 140]. Corticosteroids are cytolytic to T cells and thymocytes both *in vivo* and *in vitro*. In addition, inhibitory effects of adrenocorticoids on virtually every aspect of the immunological response have been described. In terms of glucocorticoids, the human system differs from those of the animal models (particularly rodents, which are extremely corticosensitive) only in the degree of responsiveness. Most of the effects of glucocorticoids which have been studied in lymphoid tissue are thought to be mediated by an initial step of hormone interaction with specific receptors. In fact, glucocorticoid addition is followed by several events, including inhibition of transport (glucose, amino acids), decrease in cell ATP content, modulation of macromolecule synthesis (proteins, nucleic acids), modifications at the membrane level; these may be followed by

cell lysis or cell selection [132, 140, 141]. However, the final effector role of glucocorticoids should be considered as the result of complex cellular and/or hormonal interactions, involving various cytokines and other factors, whose production and/or secretion can be regulated by glucocorticoids [132, 140].

It is nearly impossible to determine a primary target for the immunosuppressive effects of glucocorticoids. They are able to inhibit the activation, the proliferation, the differentiation and the functions of virtually all cells involved in the immune response [2, 11, 139, 140]. Moreover, inasmuch as they have differential effects on regulatory T cells according to dose, glucocorticoids may favor either T suppressor or T helper cell function. It has been demonstrated that glucocorticoids act by inhibiting the production of cytokines, such as IL-1 and IL-2, which are involved in lymphocyte proliferation [132]. They also inhibit the production of colony-stimulating factors (CSFs), and that of $\gamma$-interferon; this latter factor is thought to be involved in the regulation of the macrophage Fc receptor, and Ia antigen expression. The different cytokines affected by glucocorticoids as well as how they affect their production is shown in Table 2 [142–149]. The predominant effect of glucocorticoids is the inhibition of T cell proliferation. In contrast, a precise definition of the glucocorticoid effect on B cells remains elusive, due to the extremely complex nature of the immune regulation of B cell responses. Immunoglobulin production is generally suppressed by pharmacological doses of glucocorticoids administered *in vivo*, whereas *in vitro* immunoglobulin production is either unaffected or augmented by physiological doses [139, 140, 150]. *In vitro*, glucocorticoids may inhibit T cell suppressive activity or act directly on B cells. An interesting theory concerning the mechanism of action of glucocorticoids consists of the observation that they may allow the selection of immune cells [141].

*Sex steroid action on the immune system*

In addition to the sexual dimorphism observed in the immune response, clinical observations have now clearly established that while some autoimmune diseases, such as SLE, are aggravated during pregnancy, others, such as RA, may be reduced [15, 24]. Moreover, transient changes of thyroid dysfunction and their immunological basis have been extensively studied during the postpartum period [11]. This emphasizes that sex steroid hormones, in particular, not only influence the immune system, but also directly affect the natural history of autoimmune diseases. It appears that it is the androgen deficiency rather than the presence of physiological estrogens that is more critical to suppression of antibody responses, which are under thymic control [14].

Androgens, by stimulating hematopoiesis and the generation of an appropriate precursor cell, may activate thymus-derived suppressor T cells, leading to decreased antibody production. In birds, androgens directly inhibit the development of the bursa of *Fabricius*, and thus B cell differentiation and antibody production [14]. *In vitro*, androgens are able to inhibit in a dose-dependent manner, the proliferation of normal human lymphocytes, induced by T and B cell mitogens, but some individuals are completely insensitive [151]. *In vivo*, androgens have been shown to increase mouse spleen and lymph nod $CD8^+$ cells (suppressor/cytotoxic), whereas in humans they increase this population only during the prepubertal period [152, 153]. As already mentioned, the beneficial effect of androgens has been well-documented *in vivo*, in NZB/W mice, a murine model of SLE. In the Tx-X rat model of autoimmune thyroiditis, testosterone administration frequently produced complete resolution of chronic lesions involving the entire gland: normal thyroid architecture reappeared and the mononuclear cellular infiltrate disappeared completely [61].

Table 2. Corticosteroid effects on cytokines

| Inhibition | | Induction | | |
|---|---|---|---|---|
| Transcription (mRNA) | Protein synthesis and/or secretion | Receptor number and/or affinity | Inhibitory protein | Receptors |
| IL-1 | IL-1 | | | IL-1 (↗nb) |
| IL-6 | IL-6 | | | |
| TNF | TNF | TNF (↘aff.) | | |
| IL-2 | IL-2 | | IL-2 | |
| IFN$\beta$,$\gamma$ | IFN$\beta$ | | | |
| IL-3 | | | | |
| GM-CSF | | | | |

However, no inhibitory effect on circulating concentration of antibodies to thyroglobulin was noted. Additional evidence of the beneficial effect of androgens comes from an animal model of Sjögren's syndrome (the MRL/Mp–lpr/lpr mouse), where androgen implantation was able to reduce lymphocytic infiltration in lacrimal and submandibular glands [154]. This could be compared with the finding that the percentages of infiltrated islets and islets with destructive lesions in NOD mice at 12 weeks are lower in males than in females and that castration of males increases these percentages to values comparable to those found in females. At this point, it should be stressed that, compared to other strains male NOD mice have very high serum testosterone levels. On the contrary, the low concentrations of androgens in SLE as well as in men over 60 years with RA may be part of the pathophysiological process favoring the evolution of the autoimmune process [47–49]. Moreover, we will see below that stress is able to decrease androgen production.

Estrogens have also been shown to modify various immune parameters in animals and humans, but their effects vary according to the dose given [12–14]. Physiologically, they modulate lymphocyte and monocyte numbers, increase B cell differentiation, decrease $CD8^+$ cells (suppressor/cytotoxic) in thymus, spleen and lymph nodes, decrease T cell suppressor activity and increase antibody production [14, 155–157]. Recently, estrogens have been shown to induce normal murine $CD5^+$ B cells to synthesize natural autoantibodies [158]. They also stimulate the reticuloepithelial system, increase phagocytic activity and modulate IL-1 production [14, 159, 160]. When considering the opposing effects of estrogens in autoimmunity, it has been suggested that autoimmune diseases could be divided into two categories on the basis of the effects of these hormones [24]: (1) a first group, in which autoimmunity involves polyclonal B cell activation and/or circulating immune complexes, as in SLE and in NZB/W mice, and on which estrogens have deleterious effects; and (2) a second group, in which T cell mechanisms prime in autoimmune process and for which estrogen therapy may be beneficial. Certain experimental animal models such as CIA, AA, Tx-X induced autoimmune thyroiditis may belong to this group as well as RA, Hashimoto's thyroiditis and multiple sclerosis [24]. As already mentioned, both types of sex steroids have beneficial effect in Tx-X autoimmune thyroiditis [60, 61]. The reasons for these parallel effects of estrogens and androgens remain to be elucidated. Finally, if most of the effects of sex steroids are receptor-mediated, it should be underlined that sex steroid receptors are not generally found in lymphocytes (except in some subpopulations), but merely in other cells that are able to indirectly modulate lymphocytes, such as reticuloepithelial cells of the thymus or macrophages (Table 3) [12, 161–173]. Some of the sex steroid effects, particularly at pharmacological doses, may be nonreceptor-mediated acting directly at the cell membrane level [14].

### *Neuromediator and steroid hormone interaction in endocrine target organs*

The effects of steroid hormones and neuromediators must also be considered at the level of

Table 3. Sex steroid receptors and the immune system

| | Cytosol assay | | Cytosol/autoradiography | |
|---|---|---|---|---|
| | Whole organ | Lymphocytes | Reticuloepithelial cells | Whole cell assay |
| *Thymus* | | | | |
| Estradiol | + | ± | + | |
| Testosterone | + | ± | + | |
| Progesterone | + | ? | + | |
| *Bursa of Fabricius* | | | | |
| Estradiol | + | − | | |
| Testosterone | + | − | | |
| Progesterone | + | − | | |
| *Peripheral lymphocytes* | | | | |
| Estradiol | | ± (CD8) | | |
| Testosterone | | − | | |
| Progesterone | | ± | | |
| *Macrophages* | | | | |
| Estradiol | | | | + |
| Testosterone | | | | − |
| Progesterone | | | | ? |

the endocrine target organ or cells, in which the autoimmune process takes place. Here, we will focus our attention only on the islets of Langerhans and the puzzling interactions between their hormonal and neurohormonal environments as well as their possible relevance to diabetes.

The innervation of islets of Langerhans by sympathetic network, parasympathetic nerves and peptidergic structures, including VIP, substance P, neurotensin and CCK is now well-documented [174]. In the context of stress, these neurotransmitters may affect insulin secretion and glucose metabolism. With regard to adrenergic substances (A and NA), it has been shown that stimulation of the splanchnic nerves in different species inhibits insulin secretion, while reflex stimulation of the sympathetic system by exercise in man or stress in rats depresses the release of insulin [175, 176]. Moreover, surgical splanchnic denervation and chemical sympathectomy in the rat elevate basal insulin levels. In addition, a dual effect of catecholamines on the insulin-secreting mechanisms exists: at physiologically high concentrations of A and NA, such as those seen in stress, the predominant effect observed is the inhibition of insulin secretion via the activation of $\alpha_2$-receptors; in contrast, at low concentrations ($10^{-9}$ M or higher), when $\alpha_2$-inhibitory receptors are blocked, catecholamines stimulate insulin secretion, via $\beta_2$-adrenoreceptor stimulation [174, 175]. Thus, the involvement of the sympathoadrenal system is important in insulin secretion, both in basal and stimulated conditions, and may be dependent upon the nature of the secretagogue [175]. It has also been reported that catecholamines stimulate glucagon secretion [174]. In the context of IDDM of the NOD mouse, it is interesting to note that increased glucagon levels have been noted before the onset of overt diabetes, i.e. when no hyperglycemia or decreased in plasma insulin is observed [177]. Parasympathetic influence on the islets of Langerhans results in an increased production of insulin and glucagon [174]. While the role of pancreatic neuropeptides remains to be defined [174], it should be emphasized that strain and sex differences exist in pancreatic (as well as pituitary) peptide contents of obese and diabetic mutant mice [178]. This suggests that somewhat different metabolic control mechanisms may operate in the two sexes. Finally, these neuromediators may also affect, particularly during stressful events, the presence and/or functions of immune cells, responsible for insulitis. Indeed, there is increasing evidence for the presence of specific receptors for most of the neurohormones and neurotransmitters on lymphoid cells [136].

It is also well-known that adrenocortical hyperactivity or chronic administration of glucocorticoids produces diabetogenic effects: hyperglycemia, negative nitrogen balance, potentiation of lipolysis [132]. The mechanism of glucocorticoid action in the control of glucose homeostasis takes place, directly or indirectly, at different levels: not only on glucose production (increased gluconeogenesis); and glucose utilization (impaired peripheral glucose utilization); but also on pancreatic $\beta$-cells by influencing insulin production. Glucocorticoid action may be seen as impairing the effect of insulin, generally accompanied by increased insulin production [179]. Indeed, numerous studies, using adrenalectomized or hypophysectomized animals, given corticosteroid replacement therapy showed that glucocorticoids increase the pancreatic insulin content, insulin output by isolated perfused pancreas and insulin plasma levels [180–182]. Moreover, it was recently suggested that glucocorticoid stimulation of pancreatic proinsulin mRNA levels is mediated indirectly through its regulation of glucose metabolism [183]. However, important and immediate *in vitro* effects of glucocorticoids have also been noted, resulting in inhibition of insulin and stimulation of glucagon secretion [184, 185]. These effects have been suggested to take place at the plasma membrane (i.e. nonreceptor-mediated), in relation with a potentiation of $\alpha$-adrenergic receptors [184].

Since the demonstration that subtotal pancreatectomy-induced diabetes was more frequent in males than in females [186], the mechanism of the protective influence of the ovaries has been carefully investigated [187–191]. Whereas ovariectomy was shown to increase the incidence and severity of diabetes in female animals, natural estrogens caused islet hypertrophy and hyperplasia as well as $\beta$-cell degranulation. In ovariectomized animals, *in vivo* treatment with physiological amounts of estrogens or progesterone lowered blood glucose concentrations and increased circulating insulin levels, while decreasing those of glucagon [191]. Because of the effect of estradiol on the concentrations of other plasma steroids (in particular, glucocorticoids and progesterone, see below), the possibility of an indirect effect has been suggested [190, 191]. Indeed, whereas studies of the estradiol action on castrated (progesterone-

deprived) rats tended to exclude progesterone as a mediator of estradiol effect, the presence of adrenal glands appeared to be essential for the expression of estradiol action on glucoregulation and islets: adrenalectomy not only suppressed the estradiol-induced hyperinsulinism, but caused a decrease in basal plasma insulin and in pancreatic insulin content. At this point, it is interesting to note that, despite this beneficial effect of estradiol on insulin secretion, female NOD mice spontaneously develop IDDM, suggesting that other targets are involved for the deleterious effects of physiological amounts of estradiol, and/or the importance of the absence of testosterone. Moreover, if the beneficial effect of estradiol treatment on the incidence in female NOD mice is confirmed [121], one has to consider that: IDDM is a T cell-dependent autoimmune process, where estradiol may improve the disease, such as in Tx-X thyroiditis [60]; and also that estrogens have a well-recognized stimulatory effect on insulin production. Both effects may be partly mediated by an estradiol-induced increase in glucocorticoids.

*Estrogen dependence of glucocorticoid metabolism: possible relationship with the immune system*

The notion of sexual dimorphism in pituitary adrenal function has been around for a long time, at least in animals and particularly in rodents. Indeed, compared to males, female rats have higher basal levels of plasma corticosterone, higher diurnal rise in plasma corticosterone, higher corticosteroidogenesis by adrenal slices *in vitro* or corticosterone levels in the adrenal vein, and twice the circulating concentrations of transcortin [192–197]. ACTH administration or stress (ether anesthesia) produced higher and more persistently elevated plasma corticosterone levels in female than in male rats [192]. Moreover, plasma corticosterone concentrations have been shown to fluctuate during the estrous cycle and are higher in proestrous [197–199]. This sexual dimorphism in the rat cannot be only explained in terms of differences of steroid in clearance and metabolism of steroid, but takes place at different levels of the HPA axis, not only in the adrenals, but also in the pituitary [192]. Indeed, the pituitary appears to be functionally dimorphic in different ways: secretory capacity of specific cell types; modulation of hormone secretion by androgens and estrogens; activities of various pituitary proteins. For example, the sex difference in rat pituitary glucocorticoid receptors (with lower levels in female) is estrogen-dependent and may reflect an *in vivo* down-regulation due to the higher circulating corticosterone levels in females [200]. This may lead to a reduced sensitivity to the negative feedback effects of glucocorticoids in the pituitary. Comparable sex-related differences in glucocorticoid receptor levels have also been observed in the rat liver and thymus [201]. Moreover, after adrenalectomy or ovariectomy, the concentration of glucocorticoid receptors increased, suggesting a dependence upon changes in plasma corticosterone influenced by the ovaries. Thus, such interactions between estrogens and glucocorticoids must be kept in mind, and may exist at the level of the immune system.

*Effect of stress on the different classes of steroid hormones: possible relationship with the immune system*

When analyzing the effect of stress on the immune system and more particularly on autoimmunity, its influence on sex hormone metabolism should be underlined. For example, after physical exercise high serum cortisol concentrations are observed with concomitant decrease in testosterone levels [202]. Intense mental stress over several days also decreases plasma testosterone in men, possibly reaching female levels [203]. Critical illness such as surgery, brain injury, myocardial infarction or septic shock induce transient fall in serum testosterone in men and estradiol variations in men and women [204, 206]. In animals, acute or chronic immobilization stress in rats drastically lowers the plasma concentration and/or testicular testosterone content [202, 207–210]. It should be noted that a species sensitivity exists: mice seem to be more sensitive than rats [208]. Moreover, glucocorticoids may be involved, or not, as possible underlying endocrine effectors of such regulation [209]. An interesting model is represented by the endotoxin-induced changes in steroid hormone levels in male rats [211]. Acute i.v. administration of *E. coli* endotoxin induced dramatic time- and dose-dependent increases in corticosterone, progesterone, 17$\alpha$-progesterone, as well as estradiol and estrone, contrasting with a profound decrease in testosterone. Endotoxin administered in similar conditions to adrenalectomized or orchidectomized male rats did not provoke such hormonal changes. These results suggest that an adrenal-

testicular cooperation and potentiation of aromatization of adrenal and testicular androgens exist in the hormonal response to acute endotoxin administration. These observations suggest that the HPA–testicular axis is activated during shock: cytokines such as IL-1, IL-6 and tumor necrosis factor (TNF) may stimulate corticosteroidogenesis [2, 131], which potentiates the aromatization of adrenal or testicular androgens, leading to decreased androgen and increased estrogen levels [212].

In conclusion, we have tried to review the role of gender, sex steroid hormones, stress and glucocorticoids in autoimmunity as well as to analyze their different levels of actions and interrelationships. In the model of immunologically-mediated IDDM of the NOD mouse, sex hormones and stress are implicated in the development of the disease, but their precise mechanism of action remains to be determined. With regard to the immunologically-mediated IDDM in humans, stress may represent a precipitating factor, while sexual dimorphism does not appear to represent a risk factor. However, a possible interaction between stress hormones and sex steroids, accelerating the pathogenesis of diabetes cannot be excluded.

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## REFERENCES

1. Ader R., Cohen N. and Felten D. L.: Brain, behavior and immunity. *Brain Behav. Immunity* **1** (1987) 1–6.
2. Bateman A., Singh A., Kral T. and Solomon S.: The immune-hypothalamic–pituitary–adrenal axis. *Endocr. Rev.* **10** (1989) 92–112.
3. Tecoma E. S. and Huey L. Y.: Psychic distress and the immune response: minireview. *Life Sci.* **36** (1985) 1799–1812.
4. Dorian B. and Garfinkel P. E.: Stress, immunity and illness: a review. *Psychol. Med.* **17** (1987) 393–407.
5. Dantzer R. and Kelley K. W.: Stress and immunity: an integrated view of relationships between the brain and the immune system. *Life Sci.* **44** (1989) 1995–2008.
6. Khansari D. N., Murgo A. J. and Faith R. E.: Effects of stress on the immune system. *Immun. Today* **11** (1990) 170–175.
7. Weighent D. A. and Blalock E.: Interactions between the neuroendocrine and immune systems: common hormones and receptors. *Immun. Rev.* **100** (1987) 79–108.
8. Rose N. R.: Current concepts of autoimmune diseases. *Transpl. Proc.* **20** (1988) 3–10.
9. Talal N.: Cyclosporine as an immunosuppressive agent for autoimmune disease: theoretical concepts and therapeutic strategies. *Transpl. Proc.* **20** (1988) 11–15.
10. Rose N. R.: Pathogenic mechanisms in autoimmune diseases. *Clin. Immun. Immunopath.* **53** (1989) S7–S16.
11. McGregor A. M.: Immunoendocrine interactions and autoimmunity. *New Engl. J. Med.* **322** (1990) 1739–1741.
12. Grossman C. J.: Regulation of the immune system by sex steroids. *Endocr. Rev.* **5** (1984) 435–455.
13. Ansar Ahmed S., Penhale W. J. and Talal N.: Sex hormones, immune responses, and autoimmune diseases. *Am. J. Path.* **121** (1985) 531–551.
14. Luster M. I., Pfeifer R. W. and Tucker A. N.: Influence of sex hormones on immunoregulation with specific reference to natural and environmental estrogens. In *Endocrine Toxicology* (Edited by J. A. Thomas *et al.* Raven Press, New York (1985) pp. 67–83.
15. Talal N. and Ansar Ahmed S.: Sex hormones and autoimmune diseases: a short review. *Int. J. Immunotherapy* **3** (1987) 65–70.
16. Grossman C. J.: Possible underlying mechanisms of sexual dimorphism in the immune response, fact and hypothesis. *J. Steroid Biochem.* **34** (1989) 241–151.
17. Schuurs A. H. W. M. and Verheul H. A. M.: Effects of gender and sex steroids on the immune response: general review. *J. Steroid Biochem.* **35** (1990) 157–172.
18. Siiteri P. K.: Sex hormone production and action. *Arthritis Rheum.* **22** (1979) 1284–1294.
19. Siiteri P. K., Jones L. A., Roubinian J. and Talal N.: Sex steroids and the immune system. I. Sex difference in autoimmune disease in NZB/NZW hybrid mice. *J. Steroid Biochem.* **12** (1980) 425–432.
20. Roubinian J. R., Papoian R. and Talal N.: Androgenic hormones modulate autoantibody responses and improve survival in murine lupus. *J. Clin. Invest.* **59** (1977) 1066–1070.
21. Roubinian J. R., Talal N., Greenspan J. S., Goodman J. R. and Siiteri P. K.: Effect of castration and sex hormone treatment on survival, anti-nucleic acid antibodies and glomerulonephritis in NZB/NZW F1 mice. *J. Exp. Med.* **147** (1978) 1568–1583.
22. Roubinian J. R., Talal N., Greenspan J. S., Goodman J. R. and Siiteri P. K.: Delayed androgen treatment prolongs survival in murine lupus. *J. Clin. Invest.* **63** (1979) 902–911.
23. Melez K. A., Boegel W. A. and Steinberg A. D.: Therapeutic studies in new zealand mice. VII. Successful androgen treatment of NZB/NZW F1 females of different ages. *Arthritis Rheum.* **23** (1980) 41–47.
24. Holmdahl R.: Estrogen exaggerates lupus but supresses T-cell-dependent-autoimmune disease: an hypothesis. *J. Autoimmunity* **2** (1989) 651–656.
25. Raveche E. S., Tjio J. H. and Steinberg A. D.: Genetic studies in NZB mice. IV. The effect of sex hormones on the spontaneous production of anti-T cell autoantibodies. *Arthritis Rheum.* **23** (1980) 48–56.
26. Verhuel H. A. M., Stimson W. J., Den Hollander F. C. and Schuurs A. H. W. M.: The effects of nandrolone, testosterone and their decanoate esters on murine lupus. *Clin. Exp. Immun.* **44** (1981) 11–17.
27. Verheul H. A. M., Schot L. P. C. and Schuurs A. H. W. M.: Therapeutic effects of nandrolone decanoate, tibolone, lynestrol and ethylestrenol on Sjögren's syndrome-like disorder in NZB/W mice. *Clin. Exp. Immun.* **64** (1986) 243–348.
28. Verheul H. A. M., Deckers G. H. J. and Schuurs A. H. W. M.: Effects of nandrolone decanoate or testosterone decanoate on murine lupus: further evidence for a dissociation of autoimmunosuppressive and endocrine effects. *Immunopharmacology* **11** (1986) 93–99.
29. Duvic M., Steinberg A. D. and Klassen L. W.: Effect of the anti-estrogen nafoxidine, on NZB/W autoimmune disease. *Arthritis Rheum.* **21** (1978) 414–417.
30. Jungers P., Dougados M., Pelissier C., Kuttenn F., Tron F., Lesavre P. and Bach J. F.: Influence of

oral contraceptive therapy on the activity of systemic lupus erythematosus. *Arthritis Rheum.* **25** (1982) 618–623.
31. Lahita R. G., Bradlow H. L., Kunkel H. G and Fishman J.: Increased 16α-hydroxylation of estradiol in systemic lupus erythematosus. *J. Clin. Endocr. Metab.* **53** (1981) 174–178.
33. Lahita R. G., Kunkel H. G. and Bradlow H. L.: Increased oxidation of testosterone in systemic lupus erythematosus. *Arthritis Rheum.* **26** (1983) 1517–1521.
33. Lahita R. G., Bucala R., Bradlow H. L., and Fishman J.: Determination of 16α-hydroxyestrone by radioimmunoassay in systemic lupus erythematosus. *Arthritis Rheum.* **28** (1985) 1122–1127.
34. Carraba M., Giovine C., Chevallaro M., Angelini M., Ambrosi B. and Travaglini P.: Abnormalities of sex hormones in men with systemic lupus erythematosus. *Clin. Rheum.* **4** (1985) 420–425.
35. Lahita R. G., Bradlow H. L., Ginzler E., Pang S. and New M.: Low plasma androgens in women with systemic lupus erythematosus. *Arthritis Rheum.* **30** (1987) 241–248.
36. Lahita R. G. and Bradlow H. L.: Klinefelter's syndrome: hormone metabolism in hypogonadal males with systemic lupus erythematosus. *J. Rheum.* **14** (1987) 154–157.
37. Baer A. N. and Green F. A.: Estrogen metabolism in the NZB/NZW F1 murine model of systemic lupus erythematosus. *Arthritis Rheum.* **33** (1990) 107–112.
38. Athreya B. H., Moore W. C., Wadsworth S. A., Gupta C. and Goldman A. S.: Estrogen receptor levels in a murine model of systemic lupus erythematosus. *Clin. Exp. Rheum.* **7** (1989) 589–593.
39. Morley K. D., Parke A. and Hughes G. R.: Systemic lupus erythematosus: two patients treated with danazol. *Br. Med. J.* **284** (1982) 1431–1432.
40. Hazelton R. A., McCruden A. B., Sturrock R. D. and Stimson W. H.: Hormonal manipulation of the immune response in systemic lupus erythematosus: a drug trial of an anabolic steroid, 19-nortestosterone. *Ann. Rheum. Dis.* **42** (1983) 155–157.
41. Agnello V., Pariser K., Gell J., Gelfand J. and Turksoy R. N.: Preliminary observations on Danazol therapy of systemic lupus erythematosus: effects on DNA antibodies, thrombocytopenia and complement. *J. Rheum.* **10** (1983) 682–687.
42. Jungers P., Kuttenn F., Liote F., Pelissier C., Athea N., Laurent M. C., Viriot J., Dougados M. and Bach J. F.: Hormonal modulation in systemic lupus erythematosus. Preliminary clinical and hormonal results with cyproterone acetate. *Arthritis Rheum.* **28** (1985) 1243–1250.
43. Jungers P., Liote F., Pelissier C., Viriot J., Laurent M. C., Athea N., Dougados M., Lesavre P., Kuttenn F. and Bach J. F.: The hormonomodulation in systemic lupus erythematosus: preliminary results with Danazol and cyproterone acetate. *Ann. Med. Intern.* **137** (1986) 313–319.
44. Bizzaro A., Valentini G., Di Martino G., Daponte A., De Bellis A. and Iacono G.: Influence of testosterone therapy on clinical and immunological features of autoimmune diseases associated with Klinefelter's syndrome. *J. Clin. Endocr. Metab.* **64** (1987) 32–36.
45. Feher K. G., Feher T. and Meretey K.: Interrelationship between the immunological and steroid hormone parameters in rheumatoid arthritis. *Exp. Clin. Endocr.* **87** (1986) 38–42.
46. Cutolo M., Balleari E., Guisti M., Monachesi M. and Accardo S.: Sex hormone status in women suffering from rheumatoid arthritis. *J. Rheum.* **13** (1986) 1019–1023.
47. Gordon D., Beastall G. H., Thomson J. A. and Sturrock R. D.: Androgenic status and sexual function in males with rheumatoid arthritis and ankylosing spondylitis. *Q. J. Med.* **60** (1986) 671–679.
48. Spector T. D., Perry L. A., Tubb G., Silman A. J. and Huskisson E. C.: Low free testosterone levels in rheumatoid arthritis. *Ann. Rheum. Dis.* **47** (1988) 65–68.
49. Cutolo M., Balleari E., Guisti M., Monachesi M. and Accardo S.: Sex hormone status in male patients with rheumatoid arthritis: evidence of low serum concentrations of testosterone at baseline and after human chorionic gondadotrophin stimulation *Arthritis Rheum.* **31** (1988) 1314–1317.
50. Arreola F., Paniagua R., Herrera S., Diaz-Bensussen S., Mondragon L., Bermudez J. A., Perez Pasten E. and Villalpando S.: Low plasma zinc and androgen in insulin-dependent diabetes mellitus. *Archs Androl.* **16** (1986) 151–154.
51. Papatestas A. E., Mulvihill M., Genkins G., Kornfeld P., Aufses A. H., Wang D. Y. and Bulbrook R. D.: Thymus and breast cancer: plasma androgens, thymic pathology and peripheral lymphocytes in myasthenia gravis. *J. Natn Cancer Inst.* **59** (1977) 1583–1588.
52. Vandenbrouke J. P., Witteman J. C. M., Valkenburg H. A., Boersma J. W., Cats A., Festen J. J. M., Hartman A. P., Huber-Bruning O., Rasker J. J. and Weber J.: Non contraceptive hormones and rheumatoid arthritis in perimenopausal and postmenopausal women. *Rheum. Arthritis* **255** (1986) 1299–1303.
53. Holmdahl R., Jansson L. and Andersson M.: Female sex hormones suppress development of collagen-induced arthritis in mice. *Arthritis Rheum.* **29** (1986) 1505–1509.
54. Larsson P. and Holmdahl R.: Oestrogen-induced suppression of collagen arthritis. II. Treatment of rats suppresses development of arthritis but does not affect the anti-type II collagen humoral response. *Scand. J. Immun.* **26** (1987) 579–583.
55. Larsson P., Goldschmidt T. J., Klareskog L. and Holmdahl R.: Oestrogen-mediated suppression of collagen-induced arthritis in rats: studies on the role of the thymus and of peripheral $CD8^+$ T lymphocytes. *Scand. J. Immun.* **30** (1989) 741–747.
56. Holmdahl R., Jansson L., Meyerson B. and Klareskog L: Oestrogen-induced suppression of collagen-arthritis. I. Long-term oestradiol treatment of DBA/1 mice reduces severity and incidence of arthritis and decreases the anti type II collagen immune response. *Clin. Exp. Immun.* **70** (1987) 372–378.
57. Jansson L., Mattsson A., Mattsson R. and Holmdahl R.: Estrogen induced suppression of collagen arthritis. V. Physiological level of estrogen in DBA/1 mice is therapeutic on established arthritis, suppresses anti-type II collagen T-cell dependent immunity and stimulates polyclonal B-cell activity. *J. Autoimmunity* **3** (1990) 257–270.
58. Bruot B. C. and Clemens J. W.: Effect of adjuvant-induced arthritis on serum luteinizing hormone and testosterone concentrations in the male rat. *Life Sci.* **41** (1987) 1559–1565.
59. Scott J. R. and Ward K.: Autoimmune diseases in pregnancy. *Immun. Allergy Clin. Am.* **10** (1990) 119–131.
60. Ansar Ahmed S., Young P. R. and Penhale W. J.: The effects of female sex steroids on the development of autoimmune thyroiditis in thymectomized and irradiated rats. *Clin. Exp. Immun.* **54** (1983) 351–358.
61. Ansar Ahmed S., Young P. R. and Penhale W. J.: Beneficial effect of testosterone in the treatment of chronic autoimmune thyroiditis in rats. *J. Immun.* **136** (1986) 143–147.
62. Leclere J. and Weryha G.: Stress and autoimmune endocrine diseases. *Horm. Res.* **31** (1989) 90–93.

63. Mandelbrote B. M. and Wittkower E. D.: Emotional factors in Graves' disease. *Psychosom. Med.* **17** (1955) 109–123.
64. Safran M., Paul T. L., Roti E. and Braverman L. E.: Environmental factors affecting autoimmune thyroid diseases. *Endocr. Metab. Clin. N. Am* **16** (1987) 327–342.
65. Forteza M. E.: Precipitating factors in hyperthyroidism. *Geriatrics* **28** (1973) 123–126.
66. Voth H. M., Holzman P. S., Katz J. B. and Wallerstein R. S.: Thyroid "hot spots": their relationship to life stress. *Psychosom. Med.* **32** (1970) 561–568.
67. Koehler T.: Stress and rheumatoid arthritis: a survey of empirical evidence in human and animal studies. *J. Psychosom. Res.* **29** (1985) 655–663.
68. Solomon G. F.: Emotional and personnality factors in the onset and course of autoimmune disease, particularly rheumatoid arthritis. In *Psychoneuroimmunology* (Edited by R. Ader). Academic Press, New York. (1981) pp. 159–182.
69. Empire Rheumatism Council: A controlled investigation into the etiology and clinical features of rheumatoid arthritis. *Br. Med. J.* **1** (1950) 799–805.
70. Lewis-Faning E.: Report on an enquiry into the etiology factors associated with rheumatoid arthritis. *Ann. Rheum. Dis.* **9** (Suppl.) (1950) 94–99.
71. Hendrie H. C., Paraskevas F., Baragar F. D. and Adamson J. D.: Stress, immunoglobulin levels and early polyarthritis. *J. Psychosom. Res.* **15** (1971) 337–342.
72. Meyerowitz S., Jacox R. F. and Hess D. W.: Monozygotic twins discordant for rheumatoid arthritis: a genetic, clinical and psychological study of 8 sets. *Arthritis Rheum.* **11** (1968) 1–21.
73. Heisel J. S.: Life changes as etiologic factors in juvenile rheumatoid arthritis. *J. Psychosom. Res.* **16** (1972) 411–420.
74. Rimon, R., Belmaker R. H. and Ebstein R.: Psychosomatic aspects of juvenile rheumatoid arthritis. *Scand. J. Rheum.* **6** (1977) 1–10.
75. Henoch M. J., Batson J. W. and Baum J.: Psychosocial factors in juvenile rheumatoid arthritis. *Arthritis Rheum.* **21** (1978) 229–233.
76. Otto R. and Mackay I. R.: Psychosocial and emotional disturbance in systemic lupus erythematosus. *Med. J. Aust.* **2** (1967) 488–493.
77. Wallace D. J.: The role of stress and trauma in rheumatoid arthritis and systemic lupus erythematosus. *Semin. Arthritis Rheum.* **16** (1987) 153–157.
78. Bargero G., Capra Marzani M., Fasano M., Guazzo G., Minoglio A. and Rosso A.: Diabete e interventi chirurgici. *Minerva Med.* **77** (1986) 1369–1375.
79. Hauser S. T. and Pollets D.: Psychological aspects of diabetes mellitus: a critical review. *Diabetes Care* **2** (1979) 227–232.
80. Cowie D. M., Parson J. P. and Raphael, T.: Insulin and mental depression. *Archs Neurol. Chicago* **12** (1924) 522–529.
81. Slawson P. F., Flynn W. R. and Kollar E. J.: Psychological factors-associated with the onset of diabetes mellitus. *J. Am. Med. Ass.* **185** (1963) 166–170.
82. Stein S. P. and Charles E. S.: Emotional factors in juvenile diabetes mellitus: a study of early life experience of adolescent diabetes. *Am. J. Psychol.* **128** (1971) 700–704.
83. Kisch E. S.: Stressful events and the onset of diabetes mellitus. *Israel J. Med. Sci.* **21** (1985) 356–358.
84. Robinson N. and Fuller J. H.: Severe life events and their relationship to the etiology of insulin-dependent (type I) diabetes mellitus. *Pediat. Adolesc. Endocr.* **15** (1986) 129–133.
85. Linn M. W., Linn B. S., Skyler J. S. and Jensen J.: Stress and immune function in diabetes mellitus. *Clin. Immun. Immunopath.* **27** (1983) 223–233.
86. Vialettes B., Ozanon J. P., Kaplansky S., Farnarier C., Sauvaget E., Lassman-Vague V., Bernard D. and Vague P.: Stress antecedents and immune status in recently diagnosed type 1 (insulin-dependent) diabetes mellitus. *Diabete Metab. (Paris)* **15** (1989) 45–50.
87. Carter W. R., Herrman J., Stokes K. and Cox D. J.: Promotion of diabetes onset by stress in the BB rat. *Diabetologia* **30** (1987) 674–675.
88. Villemain F., Lepault F., Fitzpatrick F., Leboulenger F., Bach J. F. and Dardenne M.: Effects of restraint stress in nonobese diabetic mice. Immunology of diabetes. Presented at the *10th Int. Wkshp*, Jerusalem, Israel (March 1990) (Abstr.).
89. Leiter E. H.: The genetic of diabetes susceptibility in mice. *FASEB Jl* **3** (1989) 2231–2241.
90. De Hertog R., Vanderheyden I., Delait A. M. and Ekka E.: Enhanced metabolism [2, 4, 6, 7-$^3$H]estradiol-17$\beta$ in the diabetic rat. *J. Steroid Biochem.* **21** (1984) 433–438.
91. Leaming A. B., Mathur R. S. and Levine J. H.: Increased plasma testosterone in streptozotocin-diabetic female rats. *Endocrinology* **111** (1982) 1329–1333.
92. Skett P.: Sex-dependent effect of streptozotocin-induced diabetes mellitus on hepatic steroid metabolism in the rat. *Acta Endocr.* **111** (1986) 217–221.
93. Singer S. S., Martin V. and Federspiel M.: Enzymatic sulfation of steroids: XII. The effect of streptozotocin on hepatic cortisol sulfation and on the individual hepatic glucocorticoid sulfotransferases in male rats. *Horm. Metab. Res.* **13** (1981) 45–49.
94. Meehan W.P., Leedom L. J. and Henry J.P.: Diabetes mellitus, adrenal function and the defeat response. *Acta Physiol. Scand.* **133** (1988) 117–128.
95. Coleman D. L. and Burkart D. L.: Plasma corticosterone concentrations in diabetic (db) mice. *Diabetologia* **13** (1977) 25–26.
96. Saito M. and Bray G. A.: Diurnal rhythm for corticosterone in obese (ob/ob) diabetes (db/db) and gold-thioglucose-induced obesity in mice. *Endocrinology* **113** (1983) 2181–2185.
97. Oster M. H., Castonguay T. W., Keen C. L. and Stern J. S.: Circadian rhythm of corticosterone in diabetic rats. *Life Sci.* **43** (1988) 1643–1645.
98. Klein R., Weigand F. A., Iunes M. and Greenman L.: Corticoids in serum of children with treated diabetes mellitus. *Pediatrics* **17** (1956) 214–220.
99. McGillivray M. H., Voorhess M. L., Putnam T. I., Li P. K., Schaefer P. A. and Bruck E.: Hormone and metabolic profiles in children and adolescents with type I diabetes mellitus. *Diabetes Care* **5** (Suppl 1) (1982) 38–47.
100. Lebinger T. G., Saenger P., Fukushima D. K., Kream J., Wu R. and Finkelstein J. W.: Twenty-four hour cortisol profiles demonstrate exaggerated nocturnal rise in diabetic children. *Diabetes Care* **6** (1983) 506–509.
101. Cameron O. G., Thomas B., Tiongco D., Hariharan M. and Greden J. F.: Hypercortisolism in diabetes mellitus. *Diabetes Care* **10** (1987) 662–664.
102. Baxter J. D. and Forsham P. H.: Tissue effects of glucocorticoids. *Am. J. Med.* **53** (1972) 573–589.
103. Hudson J. I., Hudson M. S., Rothschild A. J., Vignati L., Schatzberg A. F. and Melby J. C.: Abnormal results of dexamethasone suppression tests in nondepressed patients with diabetes mellitus. *Archs Gen. Psychiat.* **41** (1984) 1086–1089.
104. Cameron O. G., Kronfol Z., Greden J. F. and Carroll B. J.: Hypothalamic-pituitary-adrenocortical activity in patients with diabetes mellitus. *Archs Gen. Psychiat.* **41** (1984) 1090–1095.
105. Leedom L. J. and Meehan W. P.: The psychoneuroendocrinology of diabetes mellitus in rodents. *Psychoneuroendocrinology* **14** (1989) 275–294.

106. Rowland N. E. and Bellush L. L.: Diabetes mellitus: stress, neurochemistry and behavior. *Neurosci. Biobehav. Rev.* **13** (1989) 199-206.
107. Albrecht W., de Gasparo M. and Marki F.: Role of the adrenals in the development of streptozotocin (STR)-induced diabetes in male albino rats. *Horm. Metab. Res.* **16** (1984) 71–76.
108. Leiter E. H., Prochazka M. and Coleman D. L.: The nonobese diabetic (NOD) mouse. *Am. J. Path.* **128** (1987) 380-383.
109. Tochino Y.: The NOD mouse as a model of type 1 diabetes. *CRC Crit. Rev. Immun.* **8** (1987) 49–81.
110. Kolb H.: Mouse models of insulin dependent diabetes: low dose streptozocin-induced diabetes and nonobese diabetic (NOD) mice. *Diabetes Metab. Rev.* **3** (1987) 751–778.
111. Lampeter E. F., Signore A., Gale E. A. M. and Pozzilli P.: Lessons from the NOD mouse for the pathogenesis and immunotherapy of human type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* **32** (1989) 703–708.
112. Signore A., Pozzilli P., Gale E. A. M., Andreani D. and Beverley P. C. L.: The natural history of lymphocyte subsets infiltrating the pancreas of NOD mice. *Diabetologia* **32** (1989) 282–289.
113. Bendelac A., Carnaud C., Boitard C. and Bach J. F.: Syngeneic transfer of autoimmune diabetes from diabetic NOD mice to healthy neonates. Requirement for both L3T4$^+$ and Lyt-2$^+$ T cells. *J. Exp. Med.* **166** (1987) 823–832.
114. Bedossa P., Bendelac A., Bach J. F. and Carnaud C.: Syngeneic T cell transfer of diabetes into NOD newborn mice: in situ studies of the autoimmune steps leading to insulin-producing cell destruction. *Eur. J. Immun.* **19** (1989) 1947–1951.
115. Ogawa M., Maruyama T., Hasegawa T., Kanaya T., Kobayashi F., Tochino Y. and Uda H.: The inhibitory effect of neonatal thymectomy on the incidence of insulitis in nonobese diabetic (NOD) mice. *Biomed. Res.* **6** (1985) 103–105.
116. Boitard C., Bendelac A., Richard M. F., Carnaud C. and Bach J. F.: Prevention of diabetes in nonobese diabetic mice by anti-Ia monoclonal antibodies: transfer of protection by splenic T cells. *Proc. Natn Acad. Sci. U.S.A.* **85** (1988) 9719–9723.
117. Dardenne M., Lepault F., Bendelac A. and Bach J. F.: Acceleration of the onset of diabetes in NOD mice by thymectomy at weaning. *Eur. J. Immun.* **19** (1989) 889–895.
118. Charlton B., Bacelj A., Slattery R. M. and Mandel T. E.: Cyclophosphamide-induced diabetes in NOD/WEHI mice. Evidence for suppression in spontaneous autoimmune diabetes mellitus. *Diabetes* **38** (1989) 441–447.
119. Fujita T., Yui R., Kusomoto Y., Serizawa Y., Makino S. and Tochino Y.: Lymphocytic insulitis in a "nonobese diabetic" (NOD) strain of mice: an immunohistochemical and electron microscope investigation. *Biomed. Res.* **3** (1982) 429–443.
120. Makino S., Kunimoto K., Muraoka Y. and Katagiri K.: Effect of castration on the appearance of diabetes in NOD mouse. *Exp. Anim.* **30** (1981) 137–140.
121. Kanazawa Y., Tochino Y. and Komeda K.: Nonobese diabetic mice: solved and unsolved problems of the pathogenesis. In *The Immunology of Diabetes Mellitus* (Edited by M. A. Jaworski *et al.*). Elsevier, Amsterdam (1989) pp. 113–119.
122. Coleman D. L., Kuzawa J. E. and Leiter E. H.: Effect of diet on incidence of diabetes in nonobese diabetic mice. *Diabetes* **39** (1990) 432–436.
123. Gaillard R. C. and Al-Damluji S.: Stress and the pituitary–adrenal axis. *Baillere's Clin. Endocr. Metab.* **1** (1987) 319–354.
124. Fitzpatrick F., Lepault F., Bach J. F. and Dardenne M.: The influence of castration on the nonobese diabetic mouse. Immunology of diabetes. Presented at the *10th Int. Wkshp*, Jerusalem, Israel (March 1990) (Abstr.).
125. Goldstein D. S.: Stress-induced activation of the sympathetic nervous system. *Baillere's Clin. Endocr. Metab.* **1** (1987) 253–278.
126. Delitala G., Tomasi P. and Virdis R.: Prolactin, growth hormone and thyrotropin-thyroid hormone secretion during stress states in man. *Baillere's Clin. Endocr. Metab.* **1** (1987) 391–414.
127. Hubbard J. W., Kox R. H., Sanders B. J. and Lawler J. E.: Changes in cardiac output and vascular resistance during behavioral stress in the rat. *Am. J. Physiol.* **251** (1986) R82–R90.
128. De Vries G. J.: Sex differences in neurotransmitter systems. *J. Neuroendocr.* **2** (1990) 1–13.
129. Kennett G. A., Chaouloff F., Marcou M. and Curzon G.: Female rats are more vulnerable than males in an animal model of depression: the possible role of serotonin. *Brain Res.* **382** (1986) 416–421.
130. Maes, M., Vandewoude M., Schotte C., Maes L., Martin M. and Blockx P.: Sex-linked differences in cortisol, ACTH and prolactin responses to 5-hydroxytryptophan in healthy controls and minor and major depressed patients. *Acta Psychiat. Scand.* **80** (1989) 584–590.
131. Dunn A. J.: Interleukin 1 as a stimulator of hormone secretion *Prog. Neuroendocrinoimmun.* **3** (1990) 26–34.
132. Munck A., Guyre P. J. and Holbrook N. J.: Physiological functions of glucocorticoid in stress and their relation to pharmacological actions. *Endocrine Rev.* **5** (1984) 25–44.
133. Neeck G., Federlin K., Graef V., Rusch D. and Schmidt K. L.: Adrenal secretion of cortisol in patients with rheumatoid arthritis. *J. Rheum.* **17** (1990) 24–29.
134. Macphee I. A. M., Antoni F. A. and Mason D. W.: Spontaneous recovery of rats from experimental allergic encephalomyelitis is dependent on regulation of the immune system by endogenous adrenal corticosteroids. *J. Exp. Med.* **169** (1989) 431–445.
135. Felten D. L., Felten S. Y., Carlson S. L., Olschowka J. A. and Livnat S.: Noradrenergic and peptidergic innervation of lymphoid tissue. *J. Immun.* **135** (1985) 775s–765s.
136. Bost K. L.: Hormone and neuropeptide receptors on mononuclear leukocytes. *Prog. Allergy* **43** (1988) 68–83.
137. Weigent D. A. and Blalock J. E.: Structural and functional relationship between the immune and neuroendocrine systems. *Bull. Inst. Pasteur* **87** (1989) 61–92.
138. Takasu N., Komiya I., Nagasawa Y., Asawa T. and Yamada T.: Exacerbation of autoimmune thyroid dysfunction after unilateral adrenalectomy in patients with Cushing's syndrome due to an adrenocortical adenoma. *New Engl. J. Med.* **322** (1990) 1708–1712.
139. Cupps T. R. and Fauci A. S.: Corticosteroid-mediated immunoregulation in man. *Immun. Rev.* **65** (1982) 133–155.
140. Homo-Delarche F.: Glucocorticoids, lymphokines and the cell response. In *Progress in Endocrinology* (Edited by H. Imura *et al.*). Elsevier, Amsterdam (1988) pp. 349–354.
141. Nunez E. A.: Modulation of cell-mediated immune response by steroids and free fatty acids in AIDS patients: a critical survey. *Tumor Biol.* **9** (1988) 225–232.
142. Knudsen P. S., Dinarello C. A. and Strom T. B.: Glucocorticoids inhibit transcriptional and posttranscriptional expression of interleukin-1 in U937 cells. *J. Immun.* **139** (1987) 4129–4134.

143. Kern J. A., Lamb R. J., Reed J. C., Daniele R. P. and Nowell P. C.: Dexamethasone inhibition of interleukin-1 beta production by human monocytes. Post-transcriptional mechanisms. *J. Clin. Invest.* **81** (1988) 237–244.
144. Zanker B., Walz G., Wieder K. J. and Strom T. B.: Evidence that glucocorticosteroids block expression of the human interleukin-6 gene by accessory cells. *Transplantation* **49** (1990) 183–185.
145. Kelso A. and Munck A.: Glucocorticoid inhibition of lymphokine secretion by alloreactive T lymphocyte clones. *J. Immun.* **133** (1984) 784–791.
146. Grabstein K., Dower S., Gillis S., Urdal D. and Larssen A.: Expression of interleukin-2, interferon-$\gamma$, and the IL-2 receptor by human peripheral blood lymphocytes. *J. Immun.* **136** (1986) 4503–4508.
147. Jiayi D., Shikun Y. and Renbao X.: The inhibitory effect of hydrocortisone on interferon production by rat spleen cells. *J. Steroid Biochem.* **33** (1989) 1139–1141.
148. Thorens B., Mermod J. M. and Vassalli P.: Phagocytosis and inflammatory stimuli induce GM-CSF mRNA in macrophages through posttranscriptional regulation. *Cell* **48** (1987) 671–679.
149. Kull F. C.: Reduction in tumor necrosis factor receptor affinity and cytotoxicity by glucocorticoids. *Biochem. Biophys. Res. Commun.* **153** (1988) 402–409.
150. Emilie D., Crevon M. C., Auffredou M. T. and Galanaud P.: Glucocorticosteroid-dependent synergy between interleukin-1 and interleukin-6 for human B lymphocyte differentiation. *Eur. J. Immun.* **18** (1988) 2043–2047.
151. Lehmann D. D., Siebold K. and Emmons L. R.: Androgens inhibit proliferation of human peripheral blood lymphocytes *in vitro*. *Clin. Immun. Immunopath.* **46** (1988) 122–128.
152. Ansar Ahmed A., Dauphinee M. J. and Talal N.: Effect of short term administration of sex hormones on normal and autoimmune mice. *J. Immun.* **134** (1985) 204–210.
153. Dunkel L., Taino W. M. and Salvilahti E.: Effect of endogenous androgens on lymphocyte populations. *Lancet* **ii** (1985) 440–441.
154. Ariga H., Edwards J. and Sullivan D. A.: Androgen control of autoimmune expression in lacrimal glands of MRL/Mp-lpr/lpr mice. *Clin. Immun. Immunopath.* **53** (1989) 499–508.
155. Paavonen T., Andersson L. D. and Adlercreutz M.: Sex hormone regulation of *in vitro* immune response: estradiol enhances human B cell maturation via inhibition of suppressor T cells in pokeweed mitogen-stimulated cultures. *J. Exp. Med.* **154** (1981) 1935–1945.
156. Novotny E. A., Raveche E. S., Sharrow S., Ottinger M. and Steinberg A. D.: Analysis of thymocyte subpopulations following treatment with sex hormones. *Clin. Immun. Immunopath.* **28** (1983) 205–217.
157. Screpanti I., Morrone S., Meco O., Santoni A., Gulino A., Paolini R., Crisanti A., Mathieson B. J. and Frati J.: Steroid sensitivity of thymocyte subpopulations during intrathymic differentiation. Effects of 17$\beta$-estradiol and dexamethasone on subsets expressing T cell antigen receptor and IL-2 receptor. *J. Immun.* **142** (1989) 3378–3383.
158. Ansar Ahmed S., Dauphinee M. J., Montoya A. I. and Talal N.: Estrogen induces normal murine CD5$^+$B cells to produce autoantibodies. *J. Immun.* **142** (1989) 2647–2653.
159. Polan M. L., Daniele A. and Kuo A.: Gonadal steroids modulate human monocyte interleukin-1 activity. *Fert. Steril.* **49** (1988) 964–968.
160. Hu S. K., Mitcho Y. L. and Rath N. C.: Effect of estradiol on interleukin-1 synthesis by macrophages. *Int. J. Immunopharmac.* **10** (1988) 247–252.
161. Gasc J. M., Sar M. and Stumpf W.: Androgen target cells in the bursa of Fabricius of the chick embryo: autoradiographic localization. *Proc. Soc. Exp. Biol. Med.* **160** (1979) 55–58.
162. Raveche E. S., Vigersky R. A., Rice M. K. and Steinberg A. D.: Murine androgen receptors. *J. Immunopharmac.* **2** (1980) 425–434.
163. McCruden A. B. and Stimson W. H.: Androgen binding cytosol receptors in the rat thymus: physicochemical properties, specificity and localization. *Thymus* **3** (1981) 105–107.
164. Pearce P., Khalid B. A. K. and Funder J. W.: Androgens and the thymus. *Endocrinology* **109** (1981) 1073–1077.
165. Weaker F. J. and Sheridan P. J.: Autoradiographic localization of sex steroid hormones in the lymphatic organs of baboons. *Cell Tiss. Res.* **231** (1983) 593–601.
166. McCruden A. B. and Stimson W. M.: Androgen receptor in the human thymus. *Immun. Lett.* **8** (1984) 49–53.
167. Reichman M. E. and Villee C. A.: Estradiol binding by rat thymus cytosol. *J. Steroid Biochem.* **9** (1978) 637–641.
168. Danel L., Souweine G., Monier J. C. and Saez S.: Specific estrogen binding sites in human lymphoid cells and thymic cells. *J. Steroid Biochem.* **18** (1983) 559–563.
169. Cohen J. H. M., Danel L., Cordier G., Saez S. and Revillard J. P.: Sex receptors in peripheral T cells: absence of androgen receptors and restriction of estrogen receptors to OKT8-positive cells. *J. Immun.* **131** (1983) 2767–2769.
170. Gulshan S., McCruden A. B. and Stimson W. H.: Oestrogen receptors in macrophages. *Scand. J. Immun.* **31** (1990) 691–697.
171. Pearce P. and Funder J. W.: Cytosol and nuclear levels of thymic progesterone receptors in pregnant, pseudopregnant and steroid-treated rats. *J. Steroid Biochem.* **25** (1986) 65–69.
172. Sakabe K., Seiki K. and Fujii-Hanamoto H.: Histochemical localization of progestin receptors in cells of the rat thymus. *Thymus* **8** (1986) 97–107.
173. Szekeres-Bartho J., Weill B. J., Mike G., Houssin D. and Chaouat G.: Progesterone receptors in lymphocytes of liver-transplanted and transfused patients. *Immun. Lett.* **22** (1989) 259–262.
174. Miller R. E.: Pancreatic neuroendocrinology: peripheral neural mechanisms in the regulation of the islets of Langerhans. *Endocrine Rev.* **2** (1981) 471–494.
175. Ahren B. and Lundquist I.: Adrenalectomy and chemical sympathectomy by 6-hydroxydopamine. Effects on basal and stimulated insulin secretion. *Pflügers Archs Ges. Physiol.* **390** (1981) 17–21.
176. Smythe G. A., Pascoe W. S. and Storlien L. H.: Hypothalamic noradrenergic and sympathoadrenal control of glycemia after stress. *Am. J. Physiol.* **256** (1989) E231–E235.
177. Ohneda A., Kobayashi T., Nihei J., Tochino Y., Kanaya H. and Makino S.: Insulin and glucagon in spontaneously diabetic nonobese mice. *Diabetologia* **27** (1984) 460–463.
178. Timmers K., Coleman D. L., Voyles N. R., Powell A. M., Rokaeus A. and Recant L.: Neuropeptide content in pancreas and pituitary of obese and diabetes mutant mice: strain and sex differences. *Metabolism* **39** (1990) 378–383.
179. Lenzen S. and Bailey C.J.: Thyroid hormones, gonadal and adrenocortical steroids and the function of the islets of Langerhans *Endocrine Rev.* **5** (1984) 411–434.
180. Malaisse W. J., Malaisse-Lagae F., McCraw E. F. and Weight P. H.: Insulin secretion *in vitro* by pancreatic tissue from normal, adrenalectomized and cortisol-

treated rats. *Proc. Soc. Exp. Biol. Med.* **124** (1967) 924–928.
181. Curry D. L. and Bennett L. L.: Dynamics of insulin release by perfused rat pancreases: effects of hypophysectomy, growth hormone, adrenocorticotropic and hydrocortisone. *Endocrinology* **93** (1973) 602–609.
182. Kawai A. and Kuzuya N.: On the role of glucocorticoid in glucose-induced insulin secretion. *Horm. Metab. Res.* **9** (1977) 361–365.
183. Fiedorek F. T. and Permutt M. A.: Proinsulin mRNA levels in fasting and fed ADX rats: evidence for an indirect effect of glucocorticoids. *Am. J. Physiol.* **256** (1989) E303–E308.
184. Barseghian G. and Levine R.: Effect of corticosterone on insulin and glucagon secretion by the isolated perfused rat pancreas. *Endocrinology* **106** (1980) 547–552
185. Billaudel B., Mathias P. C. F., Sutter B. C. J. and Malaisse W. J.: Inhibition by corticosterone of calcium inflow and insulin release in rat pancreatic islets. *J. Endocr.* **100** 227–233.
186. Houssay B. A.: Action of sex hormones on experimental diabetes. *Br. Med. J.* **1** (1951) 505–510.
187. Bailey C. J. and Ahmed-Sorour H.: Role of ovarian hormones in the long-term control of glucose homeostasis: effects on insulin secretion. *Diabetologia* **19** (1980) 475–481.
188. Faure A., Aerts L., Aouari M. H., Sutter B. C. J. and Van Assche F. A.: Direct and indirect influences of a 14 days estradiol 17-$\beta$ treatment on the endocrine pancreas of the female rat. *Diabete Metab. (Paris)* **12** (1986) 137–142.
189. Malaisse W. J., Hubinont C. J. and Marynissen G.: Pancreatic islet cell function in oral contraception, pregnancy and lactation: a review. *Archs Gynec. Obst.* **246** (1989) 125–132.
190. Faure A., Billaudel B. and Sutter B. C. J.: Adrenal interference of insulin secretion after 14 days estradiol treatment in female rats. *Diabetologia* **26** (1984) 76–80.
191. Faure A., Sutter-Dub M. T., Sutter B. C. J. and Assan R.: Ovarian adrenal interaction in regulation of endocrine pancreatic function in the rat. *Diabetologia* **24** (1983) 122–127.
192. Kitay J. I.: Sex differences in adrenal cortical secretion in the rat. *Endocrinology* **68** (1961) 818–824.
193. Kitay J. I.: Pituitary adrenal function in the rat after gonadectomy and gonadal hormone replacement. *Endocrinology* **73** (1963) 253–260.
194. Ottenweller J. E., Meier A. H., Russo A. C. and Frenzke M. E.: Circadian rhythms of plasma corticosterone binding activity in the rat and the mouse. *Acta Endocr.* **91** (1979) 150–157.
195. Allen-Rowlands C. F., Allen J. P., Greer M. A. and Wilson M.: Circadian rhythmicity of ACTH and corticosterone in the rat. *J. Endocr. Invest.* **4** (1980) 371–377.
196. Gala R. and Westphal U.: Further studies on the corticosteroid-binding globulin in the rat: proposed endocrine control. *Endocrinology* **79** (1966) 67–76.
197. Critchlow V., Liebelt R. A., Bar-Sela M., Moutcastle W. and Lipscomb H. S.: Sex difference in resting pituitary-adrenal function in the rat. *Am. J. Physiol.* **205** (1963) 807–815.
198. Baron S. and Brush F. R.: Effects of acute and chronic restraint and estrus cycle on pituitary adrenal function in the rat. *Horm. Behav.* **12** (1979) 218–224.
199. Nichols D. J. and Chevins P. F. D.: Plasma corticosterone fluctuations during the oestrous cycle of the house mouse *Experientia* **37** (1981) 319–320.
200. Turner B. B.: Sex difference in glucocorticoid binding in rat pituitary is estrogen dependent. *Life Sci.* **46** (1990) 1399–1406.
201. Endres D. B., Milholland R. J. and Rosen F.: Sex-differences in the concentrations of glucocorticoid receptors in rat liver and thymus. *J. Endocr.* **80** (1979) 21–26.
202. Harkonen M., Naveri H., Kuoppasalami K. and Huhtaniemi I.: Pituitary and gonadal function during physical exercise in the male rat. *J. Steroid Biochem.* **35** (1990) 127–132.
203. Aakvaag A., Bentdal O., Quigstad K., Walstad P., Ronningen M. and Fonnum F.: Testosterone and testosterone binding globulin (TeBG) in young men during prolonged stress. *Int. J. Androl.* **1** (1978) 22–31.
204. Woolf P. D., Hamill R. W., McDonald J. V., Lee L. A. and Kelly M: Transient hypogonadotropic hypogonadism caused by critical illness. *J. Clin. Endocr. Metab.* **60** (1985) 444–450.
205. Klaiber E. L., Broverman D. M., Haffajee C. I., Hochman J. S., Sacks G. M. and Dalen J. E.: Serum estrogen levels in men with acute myocardial infarction. *Am. J. Med.* **73** (1982) 872–881.
206. Christeff N., Benassayag C., Carli-Vielle C., Carli A. and Nunez E. A.: Elevated estrogen and reduced testosterone in the serum of male septic shock patients. *J. Steroid Biochem.* **29** (1988) 435–440.
207. Charpenet G., Tache Y., Forest M. G., Haour F., Saez J. M., Bernier M., Ducharme J. R. and Collu R.: Effects of chronic intermittent immobilization stress on rat testicular androgenic function. *Endocrinology* **109** (1981) 1254–1258.
208. Armario A. and Castellanos J. M.: A comparison of corticoadrenal and gonadal responses to acute immobilization stress in rats and mice. *Physiol. Behav.* **32** (1984) 517–519.
209. Sapolsky R. M.: Stress-induced suppression of testicular function in the wild baboon: role of glucocorticoids. *Endocrinology* **116** (1985) 2273–2278.
210. Mann D. R. and Orr T. E.: Effect of restraint stress on gonadal proopiomelanocortin peptides and the pituitary-testicular axis in rats. *Life Sci.* **46** (1990) 1601–1609.
211. Christeff N., Auclair M. C., Benassayag C., Carli A. and Nunez E. A.: Endotoxin-induced changes in sex steroid hormone levels in male rats. *J. Steroid Biochem.* **26** (1987) 67–71.
212. Berkovitz G. O., Bisat T. and Carter K. M.: Aromatase activity in microsomal preparations of human genital skin fibroblasts: influence of glucocorticoids. *J. Steroid Biochem.* **33** (1989) 341–347.