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Sexual dimorphism in immune function: the role of prenatal exposure to androgens and estrogens

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Abstract

Perinatal exposure to androgens permanently transforms certain tissues, e.g., the brain, the genitalia, etc. This process involves both masculinization and defeminization. Immune function also is transformed by early steroid exposure; however, it is not yet known whether the response capabilities of the immunocytes themselves are directly modified or whether they are responding to signals from other masculinized tissues, e.g., the brain. Most evidence points to a direct effect since androgen and estrogen receptors are present in developing immunocytes. Both androgens and estrogens have a role in regulating adult immunity including Th1/Th2 balance. Adult susceptibility to autoimmune and other diseases is also related to steroid exposure. How immune cells respond to gonadal steroids in adulthood may depend on the pattern of androgenic and estrogenic stimulation during early development.

Keywords: Androgen; Th1/Th2; Immune function; Prenatal; Estrogen; Autoimmunity

1. Introduction

In 1974, I had the good fortune to work as a postdoc under the tutelage of David de Wied at the Rudolf Magnus Institute for Pharmacology. I had a background in ethology and was fresh from a dissertation project on behavior development in birds. Although most of the research at the Institute concerned the mechanism of action of neuropeptides on learning and memory, it soon became clear that "the boss" was also interested in behavior development when he introduced me to his student, Hermina Van der Helm-Hylkema. She had just finished her dissertation showing that early postnatal treatment with brain-derived peptides and fragments of pituitary hormones could influence the anatomical and behavioral development of rat pups (Van der Helm-Hylkema, 1973). Prof. de Wied also recognized the possibility that neuropeptides might be involved in regulating other systems. That the immune system might be one such candidate became crystal clear to me the day that Prof. de Wied invited an American fellow named Robert Ader to speak at the institute. How odd it seemed at the time that classical conditioning could be demonstrated in the immune response (Ader, 1974); how odd that principles of central nervous system (CNS) function might be applicable to the immune system.

Thanks to the pioneering vision and effort of David de Wied and others like him, many cell-signaling molecules and peptide messengers have been discovered in the central and peripheral nervous systems and their functions clarified. Many of these substances are also players in immune system function (Payan et al., 1987). Genes for the production of neuropeptides, pituitary hormones, cytokines and their receptors can be expressed constitutively in both systems. At the cellular level, we have come to expect commonality among organ systems rather than difference. Still, there remain many unanswered questions with respect to how analogous the development processes in the nervous system are to those in the immune system. Among the major questions are how sexual dimorphism in immunity arises, i.e., whether the perinatal masculinization and defeminization that alters the brain also transforms the immune system, and whether early sensitive or critical periods of development exist in the immune system as they

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do in the brain. This paper reviews studies about early developmental effects of steroid hormones on adult immune function.

2. Sex steroids

2.1. Sex differences in immune function

Adult female mammals generally have greater humoral and cell-mediated immunity than males. The thymus in female mice is larger, and castration of young males leads to an increase in weight of the thymus and secondary immune organs (Castro, 1976; Nelson and Steinberg, 1987), and to an expansion of bone-marrow B cells (Viselli et al., 1997). In the hamster, sexual dimorphism in the primary and secondary antibody response arises around puberty and is correlated with larger relative spleen weights in females (Blazkovec and Orsini, 1976). Sexual dimorphism can be demonstrated in many aspects of immune function. For example, in the mouse, complement is particularly interesting, because there is not only a quantitative, but also a qualitative difference. Electrophoresis of plasma from the male reveals molecular forms of complement C5 and BF which are absent in females (Roos and Demant, 1982; Baba et al., 1984). In vitro studies of Fisher-344 rat macrophages also show that cells derived from females produce larger amounts of prostaglandin E_2 and thromboxane B_2 than those from males (Du et al., 1984).

Women develop higher titers of antibodies in response to immunization, reject transplanted tissues more quickly, are more susceptible to allergies and live longer than men. They also experience elevated frequencies of many autoimmune diseases with systemic lupus erythematosus (SLE) and Sjogren's syndrome nine times more common in females. A majority of the 40 autoimmune diseases listed by Beeson (1994) is more common in women. Autoimmune nephropathies and Type 1 diabetes, on the other hand, are slightly more common in males. Interestingly, SLE can occur prepubertally but it is not clear if the sex ratio favors girls at this stage; however, some parameters of immune function are sexually dimorphic even in childhood. For example, Butterworth et al. (1967) showed that differences in immunoglobulin-M levels begin in mid-childhood with girls having higher levels. A whole host of sex differences such as these has prompted extensive research on sex-steroid regulation of immune response and autoimmunity in animal models. The earlier studies have been well reviewed by Ahmed et al. (1985) and by Schuurs and Verheul (1990).

2.2. Sexual differentiation

Sexual dimorphism in human immune function arises during childhood, but it is not clear whether the molecular and cellular mechanisms involved are the same as those involved in the sexual differentiation of the brain. In all mammals that have been studied, a female phenotype unfolds during development in the absence of the androgenic products of the testes. During embryogenesis, the testes develops only after transcription of a gene on the Y chromosome, which encodes the testes determining factor. Early androgen secretions by the testes act to masculinize and defeminize the circuitry of the male brain. This organizational action of androgen sets the stage for the postpubertal activational effects of sex steroids. A number of genetic anomalies, e.g., testicular feminization syndrome (Tfm) or environmental perturbations, e.g., maternal stressors, drugs, etc., are known to interrupt the actions of androgens and consequently the process of masculinization of the brain. Sexual dimorphism in the adult rodent or primate brain is evident even in the absence of sex steroids. For example, male rats castrated in adulthood are more sensitive to exogenous androgen as measured by behavioral tests and are less likely to respond with lordosis to estrogen treatment than gonadectomized females. This dimorphism can be abolished if castration occurs prior to the perinatal androgen surge that organizes the CNS, or if females are treated with sex steroids early in development. Therefore, the exposure to secretions from the testes early in life has a permanent effect on the CNS. What evidence is there that a similar organizational action of androgen occurs in the tissues and cells of the developing immune system?

2.3. Evidence for perinatal masculinization / defeminization in immune function

Gonadectomy in adulthood does not abolish the sexual dimorphism in primary antibody response to immunization with a bacterial antigen in adult Fisher-344 rats (Scherl et al., 1997) or (C57Bl/6 × DBA/2)F1 mice (Nelson and Steinberg, 1987). This is similar to the pattern seen in the nervous system, where behavioral sex differences persist after adult gonadectomy, and suggests that like the nervous system, the immune system may be masculinized during early development. Further support for this idea comes from the work of Dorner et al. (1980) who reported that female mice have higher splenic T-cell/B-cell ratios than males and that these ratios can be permanently reduced in females by neonatal androgen treatment or permanently increased by neonatal castration in males. Nelson and Steinberg (1987) studied anti-DNA antibody production in the (NZB × NZW)F1 mouse. Their results also support a perinatal sensitive period for immune system masculinization. They found that females have much higher plasma anti-DNA immunoglobulin (IgG) levels than males, and that these levels can be permanently reduced by early testosterone treatment or elevated in males by early castration. Interestingly, early estrogen treatment of males or

females did not masculinize the antibody response suggesting that this immune response is mediated by the androgen receptor, and not the result of estrogen-receptor stimulation following cytoplasmic aromatization of androgens by the immunocytes.

Another intriguing experiment dealing with perinatal masculinization is that of Konstadoulakis et al. (1995). They treated pregnant rats with 5α-dihydrotestosterone during the last days of pregnancy and examined the offsprings' in vitro cell-mediated immune responses. Their data show that in males, early androgen exposure is required for a normal immune response in the mixed lymphocyte reaction to syngenic antigens (MLR-S). In contrast, their data suggest that neither perinatal androgen nor circulating gonadal androgen is essential for the heterologous mixed lymphocyte reaction (MLR-A). The MLR-A is an in vitro assay which assesses the ability to recognize and respond to non-self antigens. Excessive (supraphysiological) perinatal androgen, however, did elevate this response to above normal in the male offspring. These investigators also examined females which had been androgenized perinatally. Masculinized females responded more strongly in the MLR-S than control females; furthermore, a normal level of response in this test required gonadal steroids, i.e., intact ovaries. In contrast to the male, gonadal steroids were important for a normal response in the MLR-A, but the effect of these gonadal steroids was to suppress the level of immune reaction. Acute estrogen exposure is presumably necessary for these lymphocytes to upregulate functional elements in the MLR-S and to downregulate elements of the MLR-A. These studies strongly support the concept that early androgen exposure permanently masculinizes the immune system of rodents.

Investigators have studied a mutant male mouse with the inability to respond to perinatal androgen due to a mutation in the gene for the androgen receptor. This Tfm mouse model of androgen insensitivity syndrome would be expected to show little, if any, masculinization. As would be predicted, lymphocytes from male Tfm/y mice are similar to those from females. In the mixed lymphocyte reaction to alloantigens, these males have more active lymphocytes than males of the background strain. Estrogen treatment further increases the MLR-A reaction. Importantly, when Tfm mice were implanted with estrogen capsules, their lymphocytes were correspondingly more reactive than those of estrogen-implanted male controls (Weinstein et al., 1984). This is strong evidence that normal males are defeminized. Tfm males have up to 36 times more thymocytes than control males and, in culture, these cells produce several times more interleukin-2 than controls (Olsen and Kovacs, 1989). Tfm male mice have elevated numbers of B-cell precursors in the bone marrow in comparison to wild-type males; and estrogen treatment reduced this level (Smithson et al., 1998). However, the data do not address the question of whether normal male castrates would show less of a reduction in response to estrogen, which would be expected assuming neonatal defeminization. Taken together, these data agree with the previously mentioned rodent studies supporting the idea that an androgen signal during development is responsible for sexual dimorphism in the immune system.

Can similar conclusions be drawn from primate studies? Mann and Fraser (1996) and Mann et al. (1994, 1999) administered a gonadotrophin-releasing hormone (GnRH) receptor antagonist, which reduces endogenous androgen production, to male rhesus monkeys during the early postnatal period. They found that these animals later displayed abnormal patterns in several immune measures. As juveniles, they showed elevated levels of antibody to tetanus toxoid, elevated proliferative responses to some, but not all, mitogens, decreased CD8 + T cells, and lower white blood cell counts. Most of these values had returned to normal by adulthood, but lower white blood cell counts persisted. In studies using male marmoset twins, neonatal GnRH receptor-antagonist treatment caused an enlargement of the thymus (Lunn et al., 1997) and reduced thymic T-cell and B-cell markers as well as altered splenic morphology (Mann et al., 1999). Both T-cell and B-cell proliferation was found to be elevated in the basal state, but depressed in response to mitogens. The interpretation of these studies with respect to masculinization of the immune system is somewhat complicated for two reasons. First, developing primates have a prenatal androgen surge which is known to masculinize some CNS functions, and they have a second period of hyperactivity in the hypothalamic–pituitary–gonadal axis which occurs postnatally. These animals presumably experienced normal prenatal androgen exposure. Second, the neuropeptide GnRH and its receptor appear to be expressed in lymphocytes; therefore, using a GnRH receptor antagonist to block neuronal control of testosterone secretion may short-circuit an autocrine signaling system potentially important for normal lymphocyte development. Nevertheless, many of the patterns reported after GnRH receptor-antagonist treatment are similar to those in females suggesting a masculinization/defeminization deficit.

Data on humans with early androgen or estrogen anomalies are scarce. Lymphocyte subset distributions and natural killer cell (NK-cell) activity in human males with untreated hypogonadotrophic hypogonadism have been studied (Kiess et al., 1991). Percentages of CD4 + cells were higher and CD16 + cells, lower than normal; but most subsets did not differ from normals and did not change following T treatment. It is not known whether these men experienced reduced perinatal androgen output, but it seems unlikely that the prenatal androgen surge was absent since most such men have normal genitalia at birth. Ho et al. (1991) examined lymphocyte subsets in women with gonadal dysgenesis prior to estrogen replacement. This group of women is likely to have received even less masculinization during fetal development than normal

women since gonadal steroids are absent. Blood CD4 + /CD8 + ratios in these women were lower than controls due to higher CD8 + counts. This would be expected if CD8 + cells are reduced following masculinization; Viselli et al. (1995) have reported that mouse CD8 + thymocytes, but not CD4 + cells have androgen receptors. Alternatively, the presence of estrogen in control women may have depressed the CD8 + count.

Estrogen, rather than androgen, receptors may be an important factor in masculinization because many cell types possess the cytochrome P-450 enzyme machinery to intracellularly aromatize testosterone to estrogen. Greenstein et al. (1988) have shown, using the aromatase inhibitor, androst-1,4,6-triene-3,17-dione (ATD), that the inhibitory actions of testosterone on thymus growth in rats are due in part to its conversion to estradiol. Receptors for estrogens have been demonstrated in fetal guinea pig thymocytes and lymphoblasts (Screpanti et al., 1982; Gulino et al., 1985) and in human osteoblast-like cells (Eriksen et al., 1988). Migliaccio et al. (1992) have found that neonatal treatment of mouse pups with the nonsteroidal estrogen diethylstilbestrol (DES) causes permanent effects on skeletal development in adulthood; DES-treated animals had larger femurs and vertebrae. Since osteoblasts share common lineages with various immune cells, it is reasonable to anticipate a role for estrogens in other hematopoietic cells. Mutations in the aromatase gene in humans are quite rare, but Simpson (1998) and Grumbach and Auchus (1999) recently described several cases which indicated a major role for estrogen in skeletal maturation. Androgen receptors have also been found in thymocytes of very young children (Kovacs and Olsen, 1987), but it is not known at what stage of development they were first expressed. Recently, de Fougerolles Nunn et al. (1999) have found that estrogen receptor concentrations during the neonatal period in the thymus of Wistar rats followed the same developmental pattern as those in the hypothalamus. They also found that these receptors were significantly more numerous in males than in females. From these data, it seems likely that sex-steroid receptors are present in immune cells at the same time that androgen signals are masculinizing the nervous system. Whether fetal or maternal estrogen production counteracts or modifies the action of androgen on immune-cell development would seem to be an area ripe for investigation.

2.4. Models of masculinization / defeminization of immune function

Three models can be proposed as an explanation for these observations on early androgen exposure and adult immune function. In the first model, *the CNS control model*, the early steroid exposure causes reorganization of the nervous system and all subsequent immune dimorphism can be attributed to signals emanating from the masculinized brain. Considering that most neurons were born prior to the perinatal androgen message, whereas most immune cells are produced more or less on demand from stem cells, this model would seem to be inherently appealing. Modifications of synaptic circuitry would seem like an ideal and efficient way to encode information about early experience or memory of past events, e.g., the early androgen message. Functional changes in immunocytes throughout life could then be controlled by signals from the brain. Although parsimonious, the ability of immune cells to retain dimorphic functionality in culture weigh against such a model.

It is, moreover, clear that the immune system also has the ability to store information about events in ontogeny, e.g., memory cells for specific antigens encountered perinatally. Therefore, a second model, *the immune autonomy model*, posits a direct effect of perinatal steroid exposure on primary and secondary lymphoid organs. The microenvironments in which lymphocytes or other hematopoietic cells develop would be permanently altered by the androgen signal, and immune cells developing there in later life would acquire a different functional response potential, i.e., would be masculinized. The validity of this model may be potentially assessed in the future by adoptive-transfer experiments (Kraal et al., 1979; Taube et al., 1998).

The third model, *the interaction model*, attributes the observed sexual dimorphism in immunity to perinatal androgen action on multiple organ systems, each of which provides a portion of the information or cell signaling that enables functional dimorphism in adulthood. This model envisions that various elements of immunity, e.g., antigen presentation or antibody production, respond to signals originating in sexually dimorphic organs, e.g., gonads, brain, mammary glands, thymus, etc. This model would be rejected if adoptive-transfer experiments could show that the host sex did not influence functional outcomes from an opposite sex donor.

2.5. Adult disease as a function of perinatal masculinization / defeminization

Could differences in perinatal masculinization/defeminization correlate with clinical variation in susceptibility to particular illnesses in adulthood? This is an attractive hypothesis, but one that still has not been rigorously tested. If the degree of perinatal masculinization is an important risk factor in illness, then epidemiological studies of women born with congenital adrenal hyperplasia who have had elevated prenatal androgen exposure should provide some important insights. One might predict a reduced incidence of autoimmune disease in congenital adrenal hyperplasia women. Since mutations in the gene for 21-β-hydroxylase are quite common, with carrier frequencies of ~10% (Witchel et al., 1997), it may be possible to use epidemiological methods to test this hypothesis. Unfortunately, such data are not, to the author's knowledge, yet available.

Correlational evidence does, however, already suggests that perinatal factors are important in adult disease. A study by Sandson et al. (1992) of women with breast cancer serves as one example. These researchers examined computer tomography (CT) scans of 79 breast cancer patients, as well as 97 controls, and located the wider of the two hemispheres at occipital and frontal poles. Women with breast cancer were much more likely to have left-hemisphere dominance in the frontal pole and right dominance in the occipital pole in comparison to the matched control group. Brain asymmetry and lateralization has been associated with elevated prenatal steroid levels (Geschwind and Galaburda, 1985; Stewart and Kolb, 1988; Diamond, 1991; Wisniewski, 1998). This idea that abnormal prenatal hormone levels are a causal factor in developmental brain disorders and adult immune disorders was popularized by Norman Geschwind; but studies relating indices of brain lateralization to immunity have so far produced conflicting results (Bryden et al., 1991; Gilger et al., 1991; Stanton et al., 1991; Wood and Cooper, 1992; McManus et al., 1993). Fortunately, more direct tests of the prenatal steroid–adult disease hypothesis are available in autoimmune animal models.

2.5.1. Autoimmune animal models

Experimental studies in the NZB/NZW mouse model of SLE suggest an inverse relationship between perinatal androgen exposure and later autoimmunity. This autoimmune-prone F1 hybrid mouse, when compared to a related hybrid, had low testicular testosterone levels during fetal development, but high circulating levels of alpha-fetoprotein and estradiol (Keisler et al., 1995). The high level of alpha-fetoprotein may have bound the estradiol and prevented it from causing masculinization. When dams of the autoimmune strain were treated during pregnancy with T at doses that masculinized the genitalia of female fetuses, the T-treated males lived longer than controls; however, the treatment did not directly alter those SLE markers of renal disease that were measured (Walker et al., 1996).

2.5.2. Studies of diethylstilbestrol exposure

There is epidemiological evidence for a perinatal steroid connection to disease, but the relevant inferences from these studies are quite indirect and circumstantial. Several studies have focused on the sequelae of fetal diethylstilbestrol exposure. Depue et al. (1983) and Swerdlow et al. (1997) report elevated rates of testicular cancer in men exposed to this estrogen during fetal development. The risk of cervical and vaginal cancer is also elevated in exposed women. Ekbom et al. (1992) studied the relation between measures of estrogen level during pregnancy and the risk of later breast cancer. They found that the offspring of mothers who experienced pre-eclampsia or eclampsia, which is associated with low estrogen levels, had a substantially reduced risk of later breast cancer, while women born in pregnancies with probable high fetal-estrogen exposure tended to be at increased risk. Because these studies relate fetal-steroid exposure to tumors of tissues which are also responsive in adulthood to sex hormones, they may have nothing to do with masculinization of immune function per se. Instead, it might be a simple reflection of abnormal steroid metabolism within these tissues. A recent study by Vingerhoets et al. (1998), however, does suggest that the immune system itself is abnormal in diethylstilbestrol-exposed individuals. They found that diethylstilbestrol-exposed women reported a higher frequency of bladder infections and measles. However, a similar study by Baird et al. (1996) failed to find an elevated risk of allergy, infection or autoimmune disease. Whether immune surveillance, e.g., NK-cell activity, is reduced in diethylstilbestrol-exposed women would be useful information for understanding the mechanism leading to higher cancer risk. Perinatal masculinization may well cause a decrement in immune surveillance functions since males are known to have a higher incidence of susceptibility to induced tumors. Our data showing that NK-cell activity is sexually dimorphic in the rat (females > males) are consistent with such a hypothesis (Martin et al., 1998).

2.5.3. Studies of birth order

Masculinization of the fetus may be the causal factor underlying the finding that birth order affects testicular cancer risk (Depue et al., 1983; Westergaard et al., 1998). First-born males are at increased risk of developing testicular cancer; this may be related to possible higher sex-steroid levels during pregnancy in younger or primiparous mothers. Depue et al. (1983) speculate that free estrogen may be higher in first pregnancies due to a deficit in sex-steroid binding globulin production. Parkening et al. (1978) found that young multiparous female mice had higher estrogen and lower progesterone and follicle stimulating hormone levels at the onset of pregnancy than old multiparous females, and in humans, estrogen levels in the luteal phase of the menstrual cycle are known to decline with age (MacNaughton et al., 1992). Since age and parity are positively correlated, first-born males may be exposed to higher estrogen/progesterone ratios than later-born males. This simplistic and somewhat speculative idea is difficult to evaluate because the dynamics of maternal–fetal steroid metabolism in the rodent and primate are quite complex. Albrecht has studied the control of gestational steroid metabolism extensively, and has documented complex feedback regulation of steroid synthesis involving the maternal ovary, the placenta, the fetal gonads and the fetal adrenal (Albrecht, 1984, 1985; Albrecht and Pepe, 1990; Pepe and Albrecht, 1995).

Birth order is also associated with hypersensitivity reactions involving IgE. In a very large study in Italy involving over 11,000 men, Matricardi et al. (1998) have shown that the prevalence of atopy is much higher among first-born

men, and that there is a 3% decrease in prevalence for each additional younger sibling. They argue that cross-infections acquired during infancy in larger sibships prevent development of atopy in adulthood, but the data might also suggest that an increased prenatal masculinization predisposes the first-born offspring toward the Th2-type (see Section 2.6.1 below) immune response.

Birth-order-dependent variations in prenatal fetal masculinization might also be related to observations on the rate of progression of HIV disease in homosexuals compared to heterosexuals. A meta-analysis of epidemiological studies of patient survival published prior to the advent of anti-viral drugs indicated that human immunodeficiency virus (HIV) disease progressed most rapidly in females, followed by homosexual males and then by heterosexual males (Martin, unpublished data). This pattern would be expected if HIV disease has autoimmune elements and if immune function in homosexual males is less masculinized. Numerous comparative studies support the possibility that homosexual males experience a deficit in early androgen exposure relative to heterosexual males (Adkins-Regan, 1988), and several investigators have explicated the autoimmune character of HIV disease (Krieg and Steinberg, 1990; Dalgleish, 1993; Hoffmann, 1995; Root-Bernstein, 1995). Moreover, numerous studies have established that male homosexuals are more likely to be born late in the birth order (Blanchard and Bogaert, 1996; Chazan, 1962; Martin and Gugelchuk, 1997) suggesting less masculinization.

2.6. Mechanisms of sex-steroid action in the adult

It is abundantly clear that there are activational effects of sex steroids on immune function both pharmacologically and physiologically. Measures of both humoral and cellular immunity shift during estrus or menstrual cycles and vary in women pre and post menopause. Clearly, autoimmunity is related to sex-steroid balance with plasma androgens often reducing and estrogens potentiating disease (Grossman et al., 1991). The literature on the mechanisms of sexual dimorphism in immunity has been reviewed by Grossman (1985, 1989). Many, but not all, differences between the sexes in adult immune parameters may be attributable to the direct action of steroids on immune cells and to the fact that males and females have different circulating steroid profiles. Danel et al. (1983) showed that there are specific estrogen binding sites in human peripheral blood-mononuclear, splenic and thymic cells. Stimson (1987) has reported specific androgen binding from rat spleen and from both rat and human thymus. Kovacs and Olsen (1987) have localized the binding to thymocytes, but receptors appear to be absent from peripheral blood-mononuclear cells. Enzymes for synthesizing steroids may also be expressed in immune cells. Transcripts for the human genes encoding 5-alpha reductase and 17-β hydroxysteroid dehydrogenase, enzymes which synthesize dihydrotestosterone and T, were found in human B-cell lymphoblasts and in peripheral T lymphocytes, but transcripts of the aromatase gene were not found (Zhou et al., 1998). This raises the question of whether aromatase is present in human thymocytes as it appears to be in the rat. It also raises the even bigger question of what role the production of intracellular steroids by immunocytes might have in regulating cell function.

2.6.1. Regulation of Th1 / Th2 balance by sex steroids

Mosmann and Coffman (1989) proposed that cell-mediated and humoral immunity are under the control of mutually antithetical regulatory processes involving a series of cytokine products from two different subsets of lymphocytes referred to as Th1 or Th2 subsets. The utility of this concept for human infectious, neoplastic and inflammatory diseases has been reviewed by Lucey et al. (1996). Stam et al. (1993) have reviewed the earlier pharmacological studies of steroid effects and second-messenger effects on cytokine production.

That sex steroids are potentially capable of driving the balance toward cell-mediated Th1 (with interleukin-2, interferon-γ, tumor necrosis factor (TNF)-β, interleukin-12 and lymphotoxin) or humoral Th2 (with interleukin-4, interleukin-5, interleukin-6, TGF-β, interleukin-10, interleukin-13) is inferred from studies of pregnancy (Raghupathy, 1997). Pregnant women have an increased vulnerability to infections which would normally require cell-mediated immune attack (Harris, 1966). During pregnancy, maternal Th1 responses are suppressed in order to protect the conceptus, and this may be partly due to the rise in progesterone level which occurs during pregnancy (Marzi et al., 1996; Ostensen, 1999). Certain autoimmune diseases tend to get worse during pregnancy, whereas others seem to improve. Rheumatoid arthritis and multiple sclerosis improve, whereas SLE may be exacerbated. This is thought to be due to a shift away from a Th1 immune profile. Whitacre et al. (1999) argue that females, in general, are more likely than males to develop a Th1 profile when challenged with an infectious agent. They also argue that an imbalance in favor of Th1 is fostered by low estrogen levels and prolactin, whereas high estrogen or testosterone or progesterone favors a Th2 state. However, not all the studies reviewed below readily fit this pattern.

Experimental studies show that estrogen levels similar to those during pregnancy can stimulate production of the Th2 cytokine, interleukin-4, from cultured human peripheral blood-mononuclear cells (Hamano et al., 1998). Although there may be a propensity for human females to respond to immune challenge with a Th1 response, the opposite appears to be true in the female BALB/c mouse. In this animal, males tend to be at greater risk of autoimmune disease, specifically myocarditis subsequent to coxsackievirus infection. Booth and Atkinson (1997) have shown that females produce much more interleukin-4 in response to immune challenge with staphylococcal entero-

toxin B. This propensity for Th2 response by females may explain why they are protected from myocarditis. Huber et al. (1999) have shown that testosterone promotes interferon-γ production from CD4 + cells whereas estrogen promotes interleukin-4 production; and they also showed that treatment of females with testosterone or males with estradiol alters subsequent in vitro differentiation of CD4 + subsets (Huber and Pfaeffle, 1994). However, they treated these animals with milligram quantities of these steroids, thus obscuring the physiological significance of the results. A major question arising from these reports is why human immune cells and BALB/c mouse immune cells appear to respond quite differently to the same sex steroids.

The C57Bl/6 mouse strain has also been used to study steroid regulation of cytokine production. Spleen cells from females of this strain stimulated with the T-cell mitogen, concanavalin A, varied in their cytokine profiles as a function of parity and sex (Barrat et al., 1997). A complex pattern emerged in which animals with earlier pregnancies in comparison to virgin females had increased interleukin-4 to interferon-γ ratios, i.e., a shift towards the Th2 state. Males also had lower secretion of interferon-γ than females indicating a bias towards Th2. Apparently, contradictory results were obtained in a study by Olsen et al. (1991) which indicated that spleen and thymus cells from males produce primarily interleukin-2 rather than interleukin-4. They found that cells from the Tfm/y mouse which is derived from C57Bl/6 had elevated interleukin-4 production.

Araneo et al. (1991) studied the regulation of cytokine production by T-cells in a hybrid C3H/HeN mouse population. Their study, conducted with very small sample sizes of three or four animals, suggests that spleen cells from males of this strain are similar to those of the C57Bl/6 animals in that they may produce less interferon-γ. Female T-cells also produced more interleukin-4 than male cells after stimulation in culture with anti-CD3. She and her colleagues also showed in this study that treatment of males with a 5α-reductase inhibitor which blocks the conversion of testosterone to 5α-dihydrotestosterone increased the male spleen-cell cytokine production to the level of the cells from females. Moreover, they found that in vitro exposure of T-cells to dihydrotestosterone reduced the amount of interleukin-4 and interferon-γ produced. This suggests that the androgen receptor in these cells may downregulate cytokine production. Interleukin-2 levels did not differ between males and females. Because both interleukin-4 and interferon-γ are produced in larger amounts by female cells, it is unclear how the Th1/Th2 balance is affected by sex steroids in this strain.

The SJL mouse strain is used as a model of multiple sclerosis. SJL females are more susceptible to viral-induced disease (Hill et al., 1998). Bebo et al. (1998) found that inflammatory infiltration of the nervous system by CD4 + cells in males, following immunization, to induce experimental autoimmune encephalomyelitis, did not occur until after the animals were castrated, suggesting that androgens suppress Th1 responses. They also showed that adoptive transfer of female T-cells caused more severe experimental autoimmune encephalomyelitis than cells from male animals (Bebo et al., 1999). More remarkably, female T-cell clones cultured with androgen produced less severe disease than clones cultured without androgen. Cells exposed to androgen early in their differentiation maintained their cytokine profile even in the absence of the hormone. This suggests that these cells store information about their earlier hormonal environment, and utilize this information in determining how to respond to challenge.

Results from the studies discussed above leave considerable confusion about how sex steroids regulate cytokine release and Th1/Th2 balance. From an evolutionary perspective, it seems highly unlikely that separate mouse strains will have evolved diametrically opposite mechanisms for immune regulation. Part of this confusion is probably due to investigators not controlling the early endocrine environment of their animals. Often, the studies utilize intact, rather than gonadectomized, animals to examine exogenous steroid effects, often without specifying estrus phase, etc. Endogenous hormones may interact with exogenous treatments. Since cells may respond to stimuli differently depending on the steroid environment they encountered during differentiation, the failure to control these variables may lead to inconsistencies. Another factor which may contribute to the confusion is the failure to define the temporal parameters of cytokine production after the stimulation of cells. The cross-regulatory nature of the Th1/Th2 balance assures that immunocytes as a population, or as individual cells, will change their secretory pattern over time, and specific steroids may produce quite different modulatory effects depending on the secretory profile of the cells at the time the hormone-receptor complex is formed. Even within the body at any one time, immunocytes may differ in their cytokine profile depending on their location. For example, there is evidence from studies of rheumatoid arthritis that peripheral blood lymphocytes may have a Th2 profile while lymphocytes near the inflamed joint may have a Th1 cytokine profile (Miossec and van den Berg, 1997). Wira and colleagues have shown that changes can occur quite rapidly in steroid–immune relationships in the rodent and the human reproductive tract (Givan et al., 1997; Kaushic et al., 1998). Before effective therapeutic strategies can be developed for using gonadal steroids or their analogues to treat autoimmune diseases (Schuurs et al., 1985; van Vollenhoven and McGuire, 1994), we must define more clearly the nature of the relationship between these steroids and the immune cell response.

2.6.2. The role of adrenal androgens

Many investigators as well as a number of lay authors have entertained the idea that the adrenal androgen, dehydroepiandrosterone, is an immunomodulator. In some

countries, this hormone is available commercially as a "nutritional supplement". Despite the lack of conclusive evidence of its efficacy or safety, many people are self-medicating with this hormone. Scholars agree that production of this steroid in humans declines with age in both sexes, and many argue that replacement therapy is desirable (Nippoldt and Nair, 1998). Few seem to have considered the possibility that this age-related decline might be a useful physiological adaptation, e.g., to reduce the growth-promoting environment for androgen-sensitive cells that have undergone age-related oncogenic changes.

Dehydroepiandrosterone is secreted in large quantities by the primate adrenal; however, it is not normally secreted by the rat testis or adrenal, although it may be synthesized in the rodent brain (Robel and Baulieu, 1997). Apparently, only one study has been reported on the effects of perinatal dehydroepiandrosterone treatment. Shelat et al. (1997) treated pregnant female rats during the last two-thirds of gestation with different doses of this steroid hormone. They found that offspring thymic weights were elevated, suggesting reduced masculinization and, in contrast, that males had lower proliferative responses to mitogen and lower interleukin-2 mRNA levels in adulthood, suggesting that treated males were masculinized more than the control males. It is unclear why the dehydroepiandrosterone-treated females did not also have an altered response to mitogen, but it may be that the enzyme, 3β-hydroxysteroid dehydrogenase, needed to convert this steroid to androstenedione or 17α-hydroxylase needed to produce testosterone, is not expressed in females. Hobe et al. (1994) have reported sex differences in the metabolism of this steroid in the rat.

Although space considerations and the large number of studies of dehydroepiandrosterone in immunity preclude a thorough review here, much of the literature is related to the ability of this hormone to block chemically-induced carcinogenesis in rodent models (Shibata, 1995; Simile et al., 1995; McCormick et al., 1996). Some studies have found that dehydroepiandrosterone itself can be carcinogenic (Hayashi et al., 1994). Whether these effects are due to actions on the immune system per se is unclear. We have found that dehydroepiandrosterone does not elevate NK-cell activity in the Fischer-344 rat, and moreover, we found in a rat model of tumor metastasis, that dehydroepiandrosterone increased the neutrophil fraction of peripheral blood leukocytes and that the percentage of neutrophils was positively correlated with tumor load (Smart and Martin, 1997) suggesting that the hormone is promoting metastasis or tumor growth. Casson et al. (1993) examined the effect of dehydroepiandrosterone supplementation on cellular immune responses in a small study of 11 postmenopausal women. They found that treatment with this steroid increased NK-cell activity, decreased T-cell mitogenesis and reduced the CD4 + fraction. In contrast, plasma dehydroepiandrosterone was positively correlated with CD4 + counts, and levels were elevated in the early stages of HIV disease (Christeff et al., 1996). Since this steroid is produced in such large amounts by the human adrenal (ca. 20 mg/day), it may well be a major regulator of the immune function. Future studies may focus more on its role as a prohormone and how it can be converted in situ into more active metabolites. Rodents may not be the best models for studying such questions because they lack circulating dehydroepiandrosterone, and thus the local enzymatic machinery to process this hormone may not have evolved.

3. Concluding remarks

I have considered in this review whether basic principles of sexual differentiation gleaned from studies of the nervous system are also applicable to the immune system. One of these basic principles is that perinatal steroid exposure causes permanent shifts in adult function by acting at critical periods of development. Another is that early exposure to sex steroids acts both to masculinize and to defeminize the underlying female phenotype. Because effective immunity is essential for both sexes, one should not expect to find fundamental differences in immune system development between males and females. Nevertheless, there does appear to be an organizational effect of early steroid exposure on various elements of immune function, which partially accounts for the sexual dimorphism in adult mammals. Direct activational effects of gonadal steroids in adulthood also contribute to this dimorphism. Three models are proposed to guide further experimentation on how sexual dimorphism in immunity develops. The relative importance of sex steroids as physiological regulators of immune function compared to other classes of signaling molecules, e.g., glucocorticoids, neuropeptides, etc., is still unclear. The use of sex-steroid analogues to influence immunity may ultimately come to be a valuable clinical option for treating hypofunctional or hyperactive immune states, e.g., immune deficiency diseases, autoimmunity, or for boosting immunity in the elderly.

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