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Mechanisms of lupus: The role of estrogens

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'Anyone who has seen half a dozen examples of common lupus (cutaneous tuberculosis) and of lupus erythematosus is capable with ease to distinguish the one from the other …. but let him wait awhile and see more, and he will find before long that there are examples of mixed forms of disease which it is impossible to denote correctly without employing hybrid names or qualifying adjectives. The reason of this is .... that lupus is not produced by one cause, but is the result of various modifications of vital endowment existing in its subjects.'

Hutchinson, 1880

Introduction

Although the discovery by Hargraves (1) of the 'LE cells' in 1948 has helped clinicians to identify the group of conditions we now call systemic lupus erythematosus (SLE), defining the disease continues to be a problem. Both constitutive and environmental factors are thought to be important in determining the protean manifestation of SLE.

The striking predominance of women with lupus was noted more than a hundred years ago by Erasmus Wilson (1809 - 1884), with the female:male ratio now estimated to be between 9-15: 1 (2). The peak incidence of lupus occurs in the 15- to 45-year old age group (3), during the time in life when estrogen levels are highest in women. In many genetic based murine models of lupus, including the NZB/NZW F1 and MRL/*lpr*/*lpr* mice, the females also develop more severe disease and higher titer of autoantibodies at an earlier age (4, 5).

While the precise factors responsible for this gender difference remain elusive, it is known that normal females are more immunoreactive than males (6, 7). For example, women have higher baseline immunoglobulin levels and produce greater and more prolonged antibody responses following immunization (7, 8). Gender differences in cell-mediated responses, including accelerated allograft rejection, greater proliferative responses and relative resistance to immunotolerance in females, have all been documented (8, 9). It is likely that both hormonal and non-hormonal gender-related factors contribute to the abnormal immune responses seen in lupus patients. In this review, we will examine the evidence for the role of estrogens in the pathogenesis of lupus.

Sex hormones are the obvious candidates to explain the sexual dimorphism in SLE. Although female lupus patients have normal estradiol levels, decreased testosterone metabolism and increased levels of estrogenic metabolites have been noted in male and female lupus patients. For example, plasma levels of 16α-hydroxyesterone and estrone are elevated in both males and females, and estriol levels are elevated in females with lupus (10-12). It is worth pointing out that gonadal and non-gonadal androgenic hormones, which may possess 'anti-estrogen' properties, also have important effects on the immune system (reviewed in 7-9, 13, 14). Lower plasma androgens, including testosterone, dihydrotestosterone, dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS), have been reported in active and inactive lupus (15). Dehydroepiandrosterone has also been shown to enhance IL-2 production by murine (16) and human (17) T cells. Decreased anti-DNA antibody formation and prolonged survival in NZB/NZW mice (18), as well as an improved clinical status in some lupus patients (19-21), have also been associated with DHEA supplementation.

Pregnant lupus patients may also have lower plasma level of testosterone and estradiol than non-lupus pregnant patients and patients with rheumatoid arthritis (22). However, the question of whether pregnancy induces lupus flare, especially when the disease is quiescent at the beginning of the pregnancy, has not been fully answered. Some studies have found increased exacerbations during pregnancy and in the postpartum period (23, 24), while others have found a similar risk regardless of the patients' pregnancy status (25, 26).

Estrogens in murine lupus

The importance of sex hormones in animal models of lupus has been most clearly established in the autoimmune NZB/NZW F1 mouse. Female NZB/NZW F1 mice develop more severe renal disease and die earlier than male mice (4, 5, 27). Although elevated estrogen 2-hydroxy-

lase activity was found, unlike human lupus patients, abnormal formation of 2-hydroxylated and 16α-hydroxylated products have not been demonstrated in these animals (28). In female NZB/NZW F1 mice estrogen supplementation is associated with worsening disease and accelerated mortality. Castration or supplementation with the male sex hormone 5α-dehydrotestosterone has the opposite effects, and causes delayed onset of lupus and prolong survival in these mice (29, 30). Similarly, administration of 19-nortestosterone, which has minimal virilizing effects, decreases anti-DNA antibody production and improves longevity in NZB/NZW/ F1 mice (31). Lastly, male sex hormones have been shown to retard autoimmune processes in a number of hybrid mice with the NZB background (32, 33).

The effect of estrogens is not as strong in other autoimmune mouse strains. The MRL/*lpr*/*lpr* mouse with a defective Fas molecule develops massive lymphoproliferation and late-life lupus. Compared with NZB/NZW F1 mice, gender has a smaller effect on longevity in MRL/*lpr*/*lpr* mice (27). However, castrated male mice supplemented with estradiol developed more severe lymphadenopathy, worse renal disease and increased mortality (34). BXSB mice are unique in that males have more severe autoimmune disease and a much shorter life span than females. The gene responsible for this difference, *Yaa* (Y chromosome autoimmunity accelerator), is located on the Y chromosome. Female BXSB mice develop late-life lupus, suggesting that additional unidentified genes also contribute to disease development. Sex hormones do not appear to be as important in BXSB mice, since neither castration nor testosterone supplementation affect the disease outcome (35).

Effects of estrogens on immune cells

The effect of estrogens on T and B cells is complicated and incompletely understood. Earlier studies demonstrated thymic sensitivity to gonadal hormones, with thymic atrophy and lymphocyte depletion in the thymic cortex following estrogen treatment in rodents (36). Thymic CD8+ cells may be preferentially affected by estrogen in both normal and autoimmune prone mice (37). T cell-dependent immune functions such as cutaneous delayed-type hypersensitivity and lymphocyte proliferative responses to concanavalin A in MRL/*lpr*/*lpr* mice are depressed following exposure to estrogen (34). Estrogen supplementation is associated with decreased natural killer (NK) cell cytotoxicity in both non-lupus prone and lupus prone mice. On the other hand, similar hormonal treatments have been reported to cause enhanced polyclonal B cell immunoglobulin (38) and anti-DNA antibody (34) production. *In vitro* suppression of T cell function and exaggerated B cell antibody production following estrogen treatment have been observed in normal (39) and lupus patients (40).

Implanted estradiol also has a stimulatory effect on antibody production against both thymic-dependent and thymic independent antigens in NZB/NZW F1 mice (41). B cells with the CD5 marker spontaneously produce IgM antibodies of low affinity and high cross-reactivity (42, 43). These cells have been implicated in lupus, and are found in higher numbers in NZB mice (44) and lupus patients (45).

Estrogen supplementation has been shown to increase CD5+ B cells in orchidectomized normal (46) and NZB/NZW male mice (47). The precise manner by which estrogen affects T and B cell functions is unclear. Expression of estrogen receptor (ER-α) has recently been demonstrated in T (48-51) and B cells (51). This opens up the possibility that estrogens may have a direct effect on the immune cells. A second estrogen receptor, termed ER-β, has recently been identified (52). Understanding the respective roles of the two receptors in lupus may prove to be important.

Multiple cytokine abnormalities have been described in human and murine lupus. Estrogens may modulate lupus by affecting immune cell cytokine production and function. High levels of serum estrogen have been associated with depressed IL-2 production in NZB/NZW mice (53). Conversely, DHEA may enhance human T cell IL-2 production (17). Estrogen may also be a regulator of a number of inflammatory cytokines implicated in the pathogenesis of lupus. Estradiol treatment was shown to result in increased serum TNF-α and IL-6 levels following LPS challenge in normal and MRL/*lpr*/*lpr* mice. These changes are blocked by the anti-estrogen tamoxifen (54). The estrogen effect on IL-1 production is unclear, with reports of both reduced and increased levels following estrogen treatment (55-57). Enhancement of antigen-stimulated T cell IL-10, TNF-α and β, and IFN-γ production have also been described (58).

Estrogens and drug-induced lupus

One human 'model' of lupus is drug-induced lupus (DIL). Unlike idiopathic lupus, the precise etiological factor inciting the disease is known. Although procainamide and hydralazine are the best known examples, more than 70 agents have been shown to be capable of inducing a lupus-like disease in humans (59).

There are some obvious clinical differences between the idiopathic and the drug-induced forms of the disease. With the exception of anticonvulsant-induced lupus in children, the age of DIL patients is greater than that of idiopathic lupus patients. For example, the average age of onset for procainamide-induced lupus (PIL) is 59 to 68 years, with 35% to 58% being female (59). Hydralazine-induced lupus (HIL) tends to develop in patients younger than those with PIL, presumably because the drug is prescribed to a younger age group. Interestingly, the risk for women of developing HIL is much higher than that of men receiving hydralazine (59). In general, patients with DIL produce fewer anti-DNA antibodies and have less renal and central nervous system involvement (59).

The clinical features of DIL caused by different drugs may also vary. For example, patients with HIL are much more likely to develop renal disease than patients with PIL (59). Sex hormones may play a role in determining the clinical manifestations in some cases of DIL. The milder disease in older men and postmenopausal women with DIL may in part be due to the lower endogenous sex hormone levels in these populations. In support of this, cases of DIL occurring in younger patients, such as that induced by sulfasalazine (59-61) and interferon

(59,62), have a higher incidence of renal disease and are more likely to develop anti-DNA antibodies.

Unfortunately, the rarity and transient nature of DIL make it a difficult disease to study systematically in humans. Genetic factors related to drug metabolism have been shown to be important in some cases. However, the constitutive factor(s) determining which individual will develop DIL is poorly understood. We recently showed that certain lupus-inducing drugs may change T cell gene expression via their effects on DNA methyltransferase (63, 64), the enzyme involved in epigenetic modification of unmethylated DNA sequences. Interestingly, idiopathic lupus patients also have T cell DNA hypomethylation (65, 66), thus suggesting a common mechanism between the two forms of the disease.

Our laboratory recently established an *in vitro* and *in vivo* murine model to study DIL. We found that the adoptive transfer of syngeneic CD4+ T cells which had been made autoreactive by treatment with DNA hypomethylating drugs, such as procainamide or hydralazine, was sufficient to induce a lupus-like disease in AKR and DBA/2 mice (67-70). Similar to the NZB/NZW and MRL/*lpr*/*lpr* models, the female mice developed a more severe disease than males, and the castration of female mice resulted in milder autoimmune disease (71). What is surprising, though, is the finding that the *in vivo* homing pattern of normal and autoreactive T cells was gender-dependent, with an approximately 7-fold increase in splenic homing in female mice compared with male mice. The effect of male and female castration on splenic homing was also examined. Castration produced a small, but statistically insignificant, increase in male splenic homing. However, splenic homing in oophorectomized females was significantly ($P < 0.001$) less than that in males, suggesting that female gonadal hormones may influence T cell splenic homing. Significantly, splenectomized female mice receiving autoreactive T cells did not develop the expected features of autoimmunity (71). The results of these experiments suggest that gender-specific T cell trafficking differences may contribute to the pathogenesis of lupus disease.

Estrogen, adhesion molecules and inflammation

How then can estrogens affect lymphocyte homing and the development of lupus? Leukocyte trafficking is determined by the expression and interaction of adhesion molecules on leukocytes and vascular endothelial cells. This is an important initial step in both immune and inflammatory responses. However, the effect of estrogen on vascular endothelial cell adhesion molecule expression is unclear.

One group of researchers reported that 17β-estradiol increased the surface expression of E-selectin and VCAM-1, and increased the mRNA expression of E-selectin, VCAM-1 and ICAM-1 on TNF-α stimulated human umbilical vein endothelial cells (HUVECs) (72). 17β-estradiol treatment also enhanced neutrophil binding to TNF-α stimulated HUVECs. Conversely, estrogen increased the lymphocyte binding to IFN-γ and PMA-activated, but not TNF-α, IL-1 or IL-4-activated HUVECs. Another group of researchers reported that 17β-estradiol increased the expression of ICAM-1 on HUVECs stimulated with IL-1 or TNF-α. Increased E-selectin was also demonstrated on TNF-α stimulated HUVECs (73). Caulin-Glaser *et al.*, on the other hand, found that high-dose 17β-estradiol decreased the IL-1-induced E-selectin, VCAM-1 and ICAM-1 expression on HUVECs (74).

The reason for the different results reported in the literature may in part be due to the characteristics of the endothelial cells used, and to the different levels of estrogens to which the cells were exposed in the experiments. HUVECs are easy to grow but are difficult to standardize. There is evidence that vascular endothelial cells from different sources may be physiologically different (75). When we standardized our experiment by using primary microvascular endothelial cells isolated from 4 weeks old female AKR mice, we found that exposure to physiologic doses of 17β-estradiol increased VCAM-1 and ICAM-1, but not E-selectin, expression on TNF-α stimulated endothelial cells.

Interestingly, the effects of 17β-estradiol on endothelial cell adhesion molecule expression may be biphasic. Treatment with high (supraphysiological) levels of 17β-estradiol result in decreased endothelial cell VCAM-1 and I-CAM-1 expression (76). This may in part explain the cardiovascular protective effect of post-menopausal estrogen supplementation and the paradoxical deleterious effects of endogenous estrogen in young lupus patients.

Exogenous estrogens: Hormone replacement therapy, oral contraceptives and lupus

The role of exogenous estrogen supplementation in postmenopausal lupus patient remains controversial. One prospective study of 34 postmenopausal lupus patients found hormone replacement therapy (HRT) to be safe and well tolerated over a median follow-up period of 35 months (77). A case-control study from Canada failed to find any difference in the rate of disease flares over 12 months of HRT use (78). However, another study from Boston suggested that long term estrogen use without progesterone is associated with a small increase in the risk of developing lupus (79). One large prospective study, the Nurses' Health Study, found that postmenopausal estrogen replacement therapy may increase the risk of developing lupus (80).

Whether patients with lupus should take HRT, given the known beneficial effects of such therapy in coronary artery disease and osteoporosis, remains unclear. The effects of another form of estrogen supplementation, oral contraceptives, in lupus are also unclear (81). The use of oral contraceptive therapy has been linked to the development of ANA (82) and clinical lupus (83-85). One large prospective study found the past use of oral contraceptives to be associated with a slightly increased risk of developing SLE (86). One retrospective study indicated that even low dose oral contraceptives may be associated with an exacerbation of lupus activity (87). Other investigators, however, failed to find any significant difference in the rate of flares in lupus patients on oral contraceptive pills (88, 89).

Definitive recommendations for estrogen use in female lupus patients may not be possible until the results of large

multi-center placebo-controlled studies, such as the Safety of Estrogen in Lupus Erythematosus-National Assessment (SELENA) trial, become available.

Conclusions

Sex hormones are probably the most important identifiable constitutive factors in the development of lupus. Estrogens have been shown to affect multiple components of the immune system. Physiological and pharmacological (supraphysiological) levels of estrogen may have different effects on the immune system. Male sex hormones and other hormones of the hypothalamic-pituitary-gonadol axis are also likely to be important in the pathogenesis of SLE. An improved understanding of their individual and cooperative roles in immune and inflammatory responses will bring important new insights into the pathogenesis of the many autoimmune diseases that preferentially affect women.

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