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ESTROGEN RECEPTORS IN DISEASES OF THE IMMUNE SYSTEM

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Abstract Estrogen has distinct functions in the immune system where it acts via ERα and HERβ respectively. Surprisingly, the defects in the immune system which develop in the absence of either ERα or ERβ are quite distinct from what develops in the absence of estrogen. We have found by studying mice in which either or both estrogen receptors have been inactivated that the two estrogen receptors play distinct roles in the immune system. Loss of ERβ leads to chronic myeloid leukemia and loss of ERα leads to autoimmune disease. In mice which cannot synthesize estrogen there is also autoimmune disease resembling Sjögren's Syndrome. We plan to study these mice further with the aim of finding the exact sites of action of the two receptors in the immune system and by using specific ligands for ERα or ERβ to examine how specific parts of the immune system can be modified. These studies should lead to improved treatment of patients with diseases of the immune system.

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Estrogen exerts its effects through two distinct receptors, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ)[1,2]. Both receptors are found in nonhematopoietic cells in bone marrow and in B lymphocyte precursors in mouse. ERβ has also been found in human spleen[3-6]. We have used ER$\alpha^{-/-}$ and ER$\beta^{-/-}$ mice to help to elucidate the role of the two receptors in the immune system. Estrogen is a Janus-like molecule with contradictory effects in autoimmune diseases. Although it appears to offer protection against end-stage renal disease, during the development of systemic lupus erythematosus (SLE), estrogen blocks the destruction of immature auto-reactive B cells in the bone marrow of mice and promotes autoimmunity[7]. Furthermore, treatment of lupus-prone mice with estrogen increases the incidence of autoimmune disease[8-10], while tamoxifen, an ERα antagonist, seems to suppress SLE[11]. With the exception of estrogen treatment of neonatal (NZB X NZW F1) mice[12], it is generally widely accepted that estrogen aggravates lupus nephritis. In direct contrast, estrogen can suppress the development of the autoimmune exocrinopathy, Sjögren's Syndrome (SS)[13] and ovariectomy mimics SS in mice of healthy background[14].

The incidence of many autoimmune diseases is higher in women[15]. For this reason it has been speculated for a long time that estrogen plays important roles in the immune system. Loss of estrogen, in ovariectomized mice, results in splenomegaly[16] and increased production of colony-forming units-granulocyte-erythroid-macrophage-megakaryocytes (CFU-GEMM), burst-forming units-erythroid (BFU-E) cells[17-19] and B lymphocytes in mouse bone marrow[20]. Conversely, pregnancy or administration of exogenous estrogen decreases bone marrow B lymphocyte population in mice[21,22]. Recently, it was shown that estrogen represses the differentiation of multipotent hematopoietic stem cells into both lymphoid and myeloid cells [23, 24]. Thus, estrogen is directly implicated in the proliferation and differentiation of various cell

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lineages in normal hematopoietic tissue.

The incidence of end-stage renal disease increases after menopause[25]. Before menopause, diabetic women have a lower risk of developing this disease than age matched male diabetics[25]. These data suggest that estrogen may partially prevent the development or progression of renal diseases. The renal glomerulus has been reported to be a direct target tissue for estrogen[26,27] and there is evidence that an ERα gene polymorphism may be associated with susceptibility to lupus nephritis in males[28]. Human and mouse mesangial cells, which are the cells involved in the pathogenesis of end-stage renal disease, glomerulosclerosis (GS), express both ERα and ERβ[24]. Renal protective effects of estrogen have been demonstrated both in cultured renal proximal tubular cells[29] and in whole animals. In (GS)-prone mice, estrogen treatment results in a reduced prosclerotic response[30] and ovariectomy accelerates its progression[31]. Therefore, it has been suggested that estrogen replacement would help in preventing the progression of end-stage renal disease.

Germinal centres (GC) are sites of immunoglobulin (Ig) class switch, Ig gene V-region somatic hypermutations and B cell tolerization and are thought to be an important site of immune dysregulation in autoimmune disease[32-34]. In NZB X NZW F1 mice[35], a well-characterized model of autoimmunity in mice, GC formation occurs in the absence of antigen challenge[35-37]. There is evidence that murine lymphocytes[38] and lymphocytes in GC of human lymph nodes[39] are ERα positive. But expression and function of ERα in the GC still remains to be investigated by additional methods.

We have found that ER$\alpha^{-/-}$ mice develop signs of autoimmunity. This is spontaneous and involves ERα but not HERβ[40]. Our results indicate that (1) ERα may have an indispensible function during the development of B-lymphocytes in splenic germinal centres and in the kidney; (2) defects in kidney and immune organs due to aberrant signalling of ERα may play central roles in the development of glomerulonephritis; and (3) ERα-selective agonists could have beneficial effects in renal disease.

The most striking phenotype in ER$\beta^{-/-}$ mice is one due to loss of regulation of the immune system[41]. There is myelogenous hyperplasia in bone marrow, an increase in the number of granulocytes and B lymphocytes in blood, lymphadenopathy and infiltration of leukocytes (mainly granulocytes and few B lymphocytes) in the liver and lung. The number of B cells in the bone marrow and spleen is significantly higher in ER$\beta^{-/-}$ mice than in wild-type littermates. As they age, ER$\beta^{-/-}$ mice develop a myeloproliferative disease resembling human chronic myeloid leukemia (CML) with lymphoid blast crisis.

Estrogen has been shown to suppress Sjögren's Syndrome (SS). In mouse models of this disease, ovariectomy (Ovx) for 4 months increases production of B lymphocytes in bone marrow and accelerates destructive autoimmune lesions. In mice made estrogen deficient by inactivation of the aromatase gene, aromatase knock-out (ArKO) mice, there is increase in B lymphopoiesis in bone marrow. We evaluated the immune system of aromatase -/- mice and found that by one year, severe destructive autoimmune lesions developed in the salivary glands in female and male ArKO mice. There was a mild but significant infiltration of B lymphocytes in these glands[42].

We plan to identify the exact site of action of ERα and ERβ in the proliferation and differentiation of lineages of the immune system. For this we will use bone marrow cell cultures to study the effects of selective ERα and ERβ ligands on the growth and differentiation of bone marrow cells.

We will replace estrogen deficient mice with

selective ER α and ER β ligands and examine the proliferation and differentiation of cells in the bone marrow, spleen, thymus and blood. We will use FACS analysis to sort and study changes in cell populations in response to these ligands. We will collect the separated cells and examine them for estrogen receptor expression.

We will use human lymphomas and tonsils to examine the distribution of estrogen receptors in human immune tissues and we will begin our study on the search for mutations in estrogen receptors in patients with leukemia, lymphoma and autoimmune disease.

These studies should lead to improved treatment of patients with diseases of the immune system.

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