

## Estrogen and dendritic cell differentiation

with

Susan Kovats, Ph.D. Arthritis & Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104, U.S.A.

Susan-Kovats@omrf.ouhsc.edu



Clear sex biases in autoimmune disease and immunity against pathogens have been observed in humans and rodent models (1). Studies of immune responses in the normal state, during autoimmunity and after infection or trauma indicate that estrogen receptor (ER) ligands can modu-

late innate and adaptive immunity and hematopoiesis (2-5). Several groups have reported that 17- $\beta$ -estradiol (E2) regulates dendritic cell (DC) differentiation and function *in vivo* and *in vitro* (6), and DC are known to be important in initiation of immunity and tolerance.

### Do immune cells express estrogen receptors?

**Susan Kovats:** ER ligands include endogenous estrogens, and exogenous compounds such as selective ER modulators (SERM), phytoestrogens, and environmental chemicals. Two ERs,  $\alpha$  and  $\beta$ , exist as either homo- or heterodimers. Ligand-bound nuclear ER directly targets estrogen response sequences in regulatory regions of genes or forms a complex with other DNA-binding transcription factors (7). Structurally distinct ligands alter ER conformation, which imparts specificity. This leads to recruitment of cell-type specific coactivators or corepressors into DNA-binding complexes and subsequent increases or decreases in transcription rates (8). Estrogens also induce rapid intracellular signaling events, suggesting that ER is associated with the plasma membrane (9).

ER expression by B and T lymphocytes, NK cells, DC, macrophages, monocytes, mast cells, and bone marrow (BM) hematopoietic progenitors has been reported (1). Although ER $\alpha$  appears to be universally expressed in immune cells, ER $\beta$  expression is more restricted; for example ER $\beta$  is present in murine macrophages but not splenic DC (10). ER $\alpha$  and ER $\beta$  function in immune cells needs further study, although analyses of ER $\alpha$  or ER $\beta$  deficient mice indicate that each contributes distinctly to immune responses, in some cases by expression in non-immune cells. The molecular mechanisms by which ER signaling regulates immune cell fate remain to be discovered.

### What is known about estrogen effects on DC differentiation and function?

**Susan Kovats:** We have identified ERs as critical regulators of DC differentiation. We found that physiological doses of E2 act via ER $\alpha$  on murine BM DC precursors to promote the GM-CSF mediated development of functional DC *ex vivo* (11). Of two types of DC that differentiate in GM-CSF supported cultures, the development of Langerhans (CD11c<sup>+</sup> CD11b<sup>int</sup> Ly6C<sup>+</sup> langerin<sup>+</sup>) DC requires estrogen and ER $\alpha$  function (12). Clinically used SERM

such as tamoxifen were shown to inhibit GM-CSF mediated DC differentiation from murine BM progenitors and human blood monocytes; those DC that did develop were hyporesponsive to activating stimuli (13,14).

DC development from both lymphoid and myeloid BM progenitors is instructed by GM-CSF or Flt3 ligand. We are now testing whether E2 acts directly on defined BM progenitors to regulate DC differentia-

tion that is mediated by these cytokines. Murine Lin<sup>-</sup> c-kit<sup>+</sup> progenitors contain ER $\alpha$  and ER $\beta$  mRNA (15). Early lymphoid progenitors were selectively depleted by *in vivo* estrogen treatment or pregnancy and increased in ovariectomized mice (5), consistent with negative regulation of lymphopoiesis by estrogen. These data imply that DC differentiation from lymphoid progenitors can be negatively regulated by estrogen. In contrast, myeloid progenitors may be spared or increased by systemic estrogen treatment. A recent study of plasmacytoid DC development in estrogen treated mice showed that this subset of DC can develop from estrogen-resistant myeloid progenitors (16).

Our data indicate that E2 promotes GM-CSF mediated DC differentiation. The *in vivo* role of GM-CSF as a regulator of myeloid cell survival, proliferation, activation or differentiation is most prominent during inflammation and autoimmunity, at which time GM-CSF is produced and acts locally (17). We therefore pre-

dict that estrogen regulation of GM-CSF mediated DC differentiation will be most important *in vivo* as GM-CSF levels rise during inflammation due to infection, injury or autoimmunity.

Contrasting results from DC studies in disease models, in which a constant amount of E2 was imposed, suggest that potential difficulties exist in defining a single effect of estrogen on regulation of DC numbers and function *in vivo*. E2 levels higher than those in female estrus led to decreased DC numbers in lymphoid organs during experimental autoimmune encephalomyelitis, and DC showed reduced inflammatory cytokine levels after LPS stimulation. This correlated with a dominant Th2 response and disease amelioration (18). In contrast, similar systemic E2 levels led to increased splenic CD8 $\alpha$ <sup>+</sup> DC numbers in experimental autoimmune myasthenia gravis. These DC showed enhanced IL-12 production after TLR ligand stimulation, correlating with a dominant Th1 response (19).

### If sex hormones modulate immune function, can differences between females and males be observed during infection or autoimmunity?

**Susan Kovats:** Evidence shows that relative to males, females have increased immunity to bacterial and viral pathogens (1,4). Females generate stronger adaptive immune responses, particularly B cell mediated responses, after vaccination or infection. Interestingly, females tend to have more controlled innate immune responses, while males suffer more pathology due to excessive inflammation (4). Females are less prone to bacterial sepsis and complications of traumatic injury, correlating with lower production of proinflammatory mediators and attenuation of IL-6 production by estrogen (3,4). Consistent with more robust immunity, females show a significantly higher incidence of autoimmune disease, and modulation of innate or adaptive immune function by sex hormones has been demonstrated in rodent models of autoimmunity

(20). Although manipulation of E2 levels alters DC function *in vivo* as discussed above, divergent function of DC in males and females during infection or autoimmunity has not been reported.

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