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Estradiol Acts Directly on Bone Marrow Myeloid Progenitors to Differentially Regulate GM-CSF or Flt3 Ligand-Mediated Dendritic Cell Differentiation

Esther Carreras,* Sean Turner,* Vladislava Paharkova-Vatchkova,²† Allen Mao,† Christopher Dascher,‡ and Susan Kovats³*

Estrogen receptor (ER) ligands modulate hemopoiesis and immunity in the normal state, during autoimmunity, and after infection or trauma. Dendritic cells (DC) are critical for initiation of innate and adaptive immune responses. We demonstrate, using cytokine-driven culture models of DC differentiation, that 17β-estradiol exerts opposing effects on differentiation mediated by GM-CSF and Flt3 ligand, the two cytokines that regulate DC differentiation in vivo. We also show that estradiol acts on the same highly purified Flt3⁺ myeloid progenitors (MP) to differentially regulate the DC differentiation in each model. In GM-CSF-supplemented cultures initiated from MP, physiological amounts of estradiol promoted differentiation of Langerhans-like DC. Conversely, in Flt3 ligand-supplemented cultures initiated from the same MP, estradiol inhibited cell survival in a dose-dependent manner, thereby decreasing the yield of plasmacytoid and conventional myeloid and lymphoid DC. Experiments with bone marrow cells from ER-deficient mice and the ER antagonist ICI182,780 showed that estradiol acted primarily via ERs to regulate DC differentiation. Thus, depending on the cytokine environment, pathways of ER signaling and cytokine receptor signaling can differentially interact in the same Flt3⁺ MP to regulate DC development. Because the Flt3 ligand-mediated differentiation pathway is important during homeostasis, and GM-CSF-mediated pathways are increased by inflammation, our data suggest that endogenous or pharmacological ER ligands may differentially affect DC development during homeostasis and disease, with consequent effects on DC-mediated immunity. The Journal of Immunology, 2008, 180: 727–738.

Antigen-specific T cell responses depend on the function of dendritic cells (DC)¹ (1). DC initiate innate immune responses via their ability to sense invariant pathogen molecules and secrete inflammatory cytokines. After activation and Ag exposure, DC display high levels of MHC-bound Ags and costimulatory molecules and produce cytokines, leading to the activation and differentiation of naïve T cells during adaptive immunity. DC also have a role in immunological tolerance to self-Ags and have been implicated in initiation of autoreactive T cell responses in murine autoimmune disease models and in humans (2–4). Dysregulated cytokine networks involving DC play a role in autoimmunity, particularly in diseases that preferentially affect women such as rheumatoid arthritis and systemic lupus erythematosus (SLE) (4). Thus, it is of clinical interest to identify and understand how sex-based differences, including sex hormones, influence the development and function of DC.

Estrogens are regulators of growth, differentiation, survival, or function in many cell types and act by binding two forms of estrogen receptor (ER), α or β (5, 6). In addition to endogenous estrogens, ER ligands include pharmacological agents such as the selective ER modulators (SERM) tamoxifen and raloxifene used for prevention or treatment of breast cancer and osteoporosis (7), phytoestrogens such as the soy isoflavones (8), and environmental endocrine disruptors such as the industrial chemical bisphenol A used in manufacture of plastics (9). Ligand-bound nuclear ER dimers function as transcription factors (10). Ligation of one or both ER may lead to disparate patterns of gene expression in different cell types, depending on ligand form and concentration, the relative cellular expression of the two ER, the relative levels of transcription coactivators or corepressors, and the nature of ER-dependent promoter regulatory sequences (11, 12). SERM have cell type-specific agonist or antagonist effects, depending on these parameters. ER also mediate rapid signaling events in cooperation with growth factor or cytokine receptors (13). Via these ER-mediated mechanisms, estrogens act to modulate the development or severity of human diseases affecting many organ systems, including the immune system (14).

Sex biases in incidence of autoimmune disease, recovery from sepsis and trauma, and immunity to pathogens have been observed in humans and rodent models (reviewed in Ref. 15). Progenitors and mature cells of the immune system express ER and androgen receptors (16), suggesting that sex hormones can directly modulate

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4 Abbreviations used in this paper: DC, dendritic cell; SLE, systemic lupus erythematosus; ER, estrogen receptor; SERM, selective ER modulator; E2, 17β-estradiol; BM, bone marrow; LC, Langerhans cell; flt3 ligand; MP, myeloid progenitor; CMP, common MP; LP, lymphoid progenitor; CLP, common LP; int, intermediate; SA, streptavidin; FSC, forward scatter; SSC, side scatter; MHCI, MHCI class II; QRT PCR, quantitative real-time PCR; GMP, granulocyte/macrophage progenitor; PDC, plasmacytoid DC; MDC, myeloid DC; LDC, lymphoid DC; ELP, early lymphoid progenitor.

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the development or function of immune cells, although the mecha-
nisms by which this might occur are not completely understood.
Studies of immune responses in the normal state, during autoim-
unity and after infection or trauma, indicate that ER ligands mod-
ulate innate and adaptive immunity and hemopoiesis (9, 17–23).
Hemopoietic progenitor numbers, lymphopoiesis, and plasmacy-
toid DC differentiation were modulated by variation in estrogen
levels in pregnant, ovarectomized or 17-β-estradiol (E2) treated
mice (20, 24–26). Multiple reports show that normal or manip-
ulated systemic estrogen variation alters APC numbers or func-
tion in vivo or in vitro (reviewed in Ref. 27). Our previous work
has shown that E2 acts via ERα on murine bone marrow (BM)
cells to promote the GM-CSF-mediated development of func-
tional myeloid DC with characteristics of Langerhans cells (LC)
(28, 29). The SERM tamoxifen and raloxifene inhibit this DC dif-
ferrntiation (30).

Multiple DC subsets in vivo have been distinguished on the
basis of location, surface markers, function and migratory ca-
pability, and whether they are present in steady-state conditions or de-
develop as a consequence of inflammation or infection (31, 32).
The cytokines Flt3 ligand (FL) and GM-CSF mediate DC differen-
tiation in vitro and in vivo (33–38). FL-mediated differentiation of
the DC subsets in lymphoid organs appears to predominate during
homeostasis. In contrast, GM-CSF-mediated DC differentiation is
likely to be most important in vivo as GM-CSF levels rise during
inflammation resulting from infection, injury, or autoimmunity
(39). Notably, elevated GM-CSF levels have been associated with
the inflammation and DC dysregulation in SLE (4, 40).

In vivo, all splenic DC subsets and epidermal LC can arise from
the flt3+ fractions of the common myeloid progenitor (CMP; 
Lin- c-kit+ Sca-1+ CD34+ FcγRIIb-IL-7Rα+) and the common
lymphoid progenitor (CLP; Lin- c-kit+ Sca-1- IL-7Rα+) (41–44). A Lin-
c-kit+ CD11b+ CX3CR1+ clonogenic progenitor specific for macrophages, DC, and monocytes (45) and CD31+ Ly6C+ undif-
ferentiated myeloid blasts (46) also yield myeloid DC in response
to GM-CSF. Culture models in which DC differentiation is driven
by FL or GM-CSF lead to the development of DC subsets similar
to those found in lymphoid organs and skin. BM cells cultured
with FL generate DC that are functionally and phenotypically
equivalent to splenic conventional myeloid (CD11c+ B220- 
CD11b+) lymphoid (CD11c’ B220- CD11b’low), and plasmacy-
toid (CD11c+ B220+) DC (47). In contrast, BM cells cultured
with GM-CSF yield myeloid (CD11c+ CD11b+) DC including a
subset with characteristic features of LC (CD11c+ CD11bhi
Ly6C-) (29, 48).

Because the relative importance of the GM-CSF- and FL-me-
diated DC differentiation pathways may vary in vivo during ho-
meostasis and inflammation, we studied the impact of ER signaling
on these two developmental pathways. To determine a direct effect
of ER ligands on DC differentiation from defined hemopoietic pro-
genitors, we isolated MP from murine BM and incubated them
with agonist or antagonist ER ligands in the cytokine-driven cul-
ture models that support DC differentiation. Physiological amounts of
estradiol acted directly on highly purified flt3+ MP to promote
GM-CSF-mediated differentiation of myeloid DC, which we pre-
viously showed have features of LC (29). In contrast, estradiol
significantly inhibited FL-mediated DC differentiation from flt3+ 
MP by decreasing numbers of viable cells and differentiated DC.
Experiments with the ER antagonist ICI182,780 and BM cells
from ER-deficient mice showed that estradiol mediates its effects
primarily via ERα in these models. Our data show that depending
on the cytokine environment, pathways of ER signaling and cyto-
kine receptor signaling can differentially interact in the same
flt3+ MP to regulate DC development. Thus, by modulating DC differ-
entiation in vivo, endogenous estrogens or systemic SERM expo-
sure are likely to regulate DC-mediated immune responses during
infection, tumor immunity, or autoimmunity.

Materials and Methods

Mice

Female C57BL/6 mice were purchased from the National Cancer Institute
Animal Production Program and were used at 8–12 wk of age. Mice
expressing enhanced GFP at the locus of the CX3CR1 gene (CX3CR1gfp/gfp)
were purchased from The Jackson Laboratory, and female CX3CR1gfp/gfp
mice generated by breeding with C57BL/6 mice. Homozygous ERα- or
ERβ-deficient (B6.129-Esr1m1KsdN10 or B6.129-Esr2m1KsdN9) female
mice were purchased from Taconic Farms. Mice were housed in the patho-
gen-free barrier facility of the Oklahoma Medical Research Foundation
(OMRF) Laboratory Animal Resource Center. The OMRF Institutional
Animal Care and Use Committee approved the studies.

Cell culture reagents

Regular medium was RPMI 1640 with 10% FCS (Omega Scientific), 2 mM
glutamine, 100 U penicillin/0.1 mg streptomycin/ml, 10 mM HEPES
buffered to pH 7.2 with 1 μM sodium pyruvate. Hormone-deficient me-
tium was RPMI 1640 lacking phenol red, with 10% charcoal-dextran-
treated FCS (Omega Scientific) and the other additives listed for regular
medium. Charcoal-dextran stripping of FCS extracts steroid hormones and
reduces their levels below the detection limits of a standard radioimmu-
nosassay (49). Phenol red was omitted because it has weak estrogenic
activity at the concentration that is present in RPMI 1640 medium (50). E2
in ethanol or water soluble cycloestrin encapsulates (E2 Sigma-Aldrich)
was diluted into the hormone-deficient medium cultures at varying con-
fcentrations. IC1182,780 (Tocris Bioscience) in DMSO was diluted into
regular medium cultures to 100 nM. Addition of ethanol or DMSO alone
was always included as a vehicle control and did not change DC differ-
entiation or surface marker expression compared with untreated cultures.

Cell sorting

Allophycocyanin-anti-c-kit/CD117 (2B8), PE-anti-CD135/Flt3 (A2F1.01), and
FITC-anti-Sca-1/IL-7Rα were obtained from BD Biosciences. PE-
anti-CD127/IL-7Rα (A7R34) and PE-Cy5-anti-Sca-1 (D7) were obtained
from eBioscience. BM cells were isolated from the spines of four to eight
female C57BL/6 mice, washed with PBS containing 2% charcoal-dextran-
treated FCS. FeRcs were blocked with anti-Cd16/32 mAb (2.4G2). Cells
bound by biotinylated mAb directed at lineage markers (CD3e, CD11b,
CD45R/B220, Ly-6G/Ly-6C(Gr-1), TER-119) were removed with strepta-
vidin (SA)-conjugated immunomagnetic beads (BD Biosciences). The
Lin- c-kit+ flt3+ population, MP (Lin- c-kit+ Sca-1- IL-7Rα+), MP-flt3+
(Lin- c-kit+ Sca-1- flt3+), and MP-CX3CR1+ (Lin- c-kit- Sca-1- IL-7Rα- 
CX3CR1+gfp+) and LSK (Lin- c-kit+ Sca-1- IL7Rα-) were isolated by
again incubating the enriched Lin+ cells with anti-Cd16/32 and the mix-
ture of biotin-conjugated mAb to lineage markers along with mAb to the
desired progenitor markers; biotin-labeled mAbs were detected with
FITC or SA-allophycocyanin-Cy7. Cells were sorted on a FACSARIA
machine (BD Biosciences). In most experiments, postsort analyses indi-
cated >90% purity.

DC cultures

When cultures were started from isolated hemopoietic progenitors, to
deplete ER-bound E2 acquired in vivo, cells were incubated 1 day without E2
and cytokines in deficient medium before addition of GM-CSF. GM-CSF-
driven DC differentiation pathways were set up with total BM cells, in-
cluding red cells (5 × 10⁷ cells/ml) as described (28, 51). Conditioned
medium (3.3% v/v) from JS58L cells transfected with the murine GM-CSF
gene was used as a source of GM-CSF (52). MP, MP-CX3CR1+, MP-flt3-
were plated at ~10,000, 6,000, and 1,000 cell/ml, respectively. Cells
were harvested on day 7 and analyzed by flow cytometry for DC surface markers.

In the FL-driven culture model, DC were generated from total BM or
isolated MP in cultures supplemented with FL-IgGFc (400 ng/ml) (47).
Flt3L-IgGFc fusion protein was prepared as described (53). In initial ex-
periments, similar results were obtained with rFL (PeproTech). Total BM
cells after red cell blood lysis were cultured at 1 × 10⁸/ml in medium
containing FL-Ig at 400 ng/ml. MP and MP-flt3+ cells were cultured at
~1.8 × 10⁶ and ~1.8 × 10⁶ cells/ml, respectively. Cultures were har-
vstemmed at Day 7, 9, or 11, counted, and analyzed by flow cytom-
cultures supplemented with both GM-CSF (3.3% J558-conditioned medium v/v) and FL-1g (400 ng/ml) for 7 days.

**Flow cytometric analysis of DC**

Cells harvested from cytokine-driven DC differentiation cultures were washed and preincubated with anti-CD16/32, and labeled with optimally titered mAbs in FACS buffer (PBS, 5% newborn calf serum, 0.1% sodium azide). Fluorochrome- or biotin-labeled mAbs specific for CD11c (HL3), CD11b (M1/70), B220 (RA3-6B2), CD24 (M1/69), Ly6C (AL-21) were obtained from BD Biosciences. PE-labeled mAbs specific for CD40 (1C10), CD86 (GL1), and MHC class II (MHCIIC; M5/114.15.2) were obtained from eBioscience. Anti-PDCA-1 (JF05-1C.4.1) was obtained from Miltenyi Biotec. PE- or FITC-conjugated SA was used to detect the biotinylated mAb. Samples were run on a FACSCalibur or LSRII instrument and analyzed with FlowJo software.

**Real-time PCR analysis**

Total RNA was purified using Trizol (Invitrogen Life Technologies) and a RNeasy spin column (Qiagen) and treated with RNase-free DNase (Promega). Samples were converted to cDNA with an anchored oligo dT22 primer and SuperScript II reverse transcriptase (Invitrogen Life Technologies). cDNAs and primers were mixed with RT²Real-Time SYBR Green Master Mix (Superarray Bioscience); PCR was performed using an iCycler (Bio-Rad), and the relative expression of each gene was normalized to the expression of the housekeeping genes CD11b and β-actin. The primer sequences used are as follows:

- CD11b: 5'GGCCCAGAGCAAGAGAGGTA3' (forward), 5'GCGCCCTGGCGGCACCAG3' (reverse)
- CD11c: 5'GTTG GGCTTAA GGTTTACG3' (forward), 5'GCGCCCTGGCGGCACCAG3' (reverse)
- β-actin: 5'GTTG GGCTTAA GGTTTACG3' (forward), 5'GCGCCCTGGCGGCACCAG3' (reverse)

PCR was performed using the following conditions: 15 s at 95°C and 1 min at 60°C with 40 cycles.

**Statistical analyses**

Prism version 4.0 (GraphPad) or Excel (Microsoft) software was used for statistical analyses. Results are expressed as the mean ± SD or mean ± SEM. The significance of the differences between two means was calculated by an unpaired Student’s t test with either equal or unequal variances. Differences were considered significant if *p* < 0.05.

**Results**

**Estradiol decreases FL-mediated DC differentiation**

In vivo and in culture models, DC differentiation is mediated by either FL or GM-CSF. Previously, we showed that E2 promotes GM-CSF-mediated myeloid DC and LC differentiation in cultures initiated from total BM cells; E2 preferentially increased the numbers of functional CD11b<sup>hi</sup>Ly6C<sup>−</sup>CD11b<sup>−</sup>Ly6C<sup>−</sup> DC, although numbers of CD11b<sup>hi</sup>Ly6C<sup>−</sup>CD11b<sup>+</sup> DC also were increased (28, 29). To determine whether E2 also regulates DC differentiation mediated by FL, we used the FL-driven culture model, which leads to plasmacytoid (PDC; CD11c<sup>−</sup>B220<sup>+</sup>), and “conventional” myeloid (MDC; CD11c<sup>−</sup>B220<sup>−</sup>CD11b<sup>+</sup>) and lymphoid (LCD; CD11c<sup>+</sup>B220<sup>−</sup>CD11b<sup>low</sup>) DC subsets that are phenotypically and functionally similar to the major splenic DC subsets (47, 55). Total BM cells (after red cell lysis) were cultured with vehicle (EtOH) or E2 (1 nM) in “hormone-deficient” medium in which the basal concentration of ER ligands is very low due to use of steroid-depleted FCS and RPMI 1640 lacking the weak ER agonist phenol red (50, 56).

We determined the extent of differentiation of each DC subset after 8 days in the absence or presence of E2 based on hemocytometer cell counts and the cellular expression of the surface markers CD11c, CD11b, and B220; in some experiments, expression of MHCIIC, PDCA-1, and CD24 also was determined. The DC that differentiated in these cultures could be divided into PDC, MDC, and LCD as shown in a representative experiment (Fig. 1A). In some experiments, PDC also were identified as PDCA-1<sup>−</sup>, and LCD also were identified as CD24<sup>high</sup>PDCA-1<sup>−</sup> (data not shown). Most notably, addition of E2 significantly reduced the number of viable cells as assessed by the hemocytometer count and on forward scatter (FSC) vs side scatter (SSC) plots (Fig. 1, A and B). Within this viable cell gate, the three DC subsets were present in both cultures, although the relative percentage of DC was greater in the cultures containing E2 (63 vs 49% in this example), and within the conventional DC fraction, the relative percentage of CD11b<sup>−</sup> MDC was greater in the presence of E2 (Fig. 1A). However, the numbers of each DC subset were reduced, with the most profound effect on PDC and LCD (Fig. 1B). The CD11c<sup>−</sup> DC present in FL-driven cultures displayed low levels of surface CD40 and CD86 that are characteristic of immature/resting DC, while MDC displayed higher levels of MHCIIC than PDC or LCD (Fig. 1C and data not shown). DC differentiated without or with E2 were functionally competent because each increased surface CD40, CD86, and MHCIIC in response to the activating TLR ligands unmethylated CpG-A oligodeoxynucleotides and bacterial LPS (Fig. 1C and data not shown). A greater percentage of the conventional DC that differentiated in the presence of E2 achieved the highest level of CD40, CD86, and MHCIIC, while PDC expression of MHCIIC, CD40, and CD86 after activation did not differ significantly in the presence or absence of E2. In sum, these data show that in FL-driven differentiation cultures, E2 profoundly decreases viability of cells, leading to decreased numbers of functional DC and altering the ratio of the two conventional DC subsets.

**Estradiol differentially modulates FL- and GM-CSF-mediated DC differentiation at the same threshold concentration**

The ER<sub>K<sub>p</sub></sub> for E2 is 10<sup>−10</sup> to 10<sup>−11</sup> M, which coincides with reported values of E2 in female mouse serum during the adult estrus cycle (57, 58). To determine whether E2 acted at the same threshold concentration in the FL- and GM-CSF-driven models, we performed a broad dose titration (10<sup>−13</sup> to 10<sup>−7</sup> M) using a water-soluble (cycloexdrin-encapsulated) form of E2 in triplicate cultures of total BM cells in hormone-deficient medium and quantified the total number of live cells and DC after 8 days (Fig. 2). According to predetermined optimal cell concentrations, in GM-CSF cultures, ∼1 × 10<sup>5</sup> leukocytes in 1 ml were plated, while in FL cultures, ∼1.5 × 10<sup>5</sup> leukocytes in 1 ml were plated. In FL cultures, the number of live cells and DC decreased significantly upon addition of 5 × 10<sup>−11</sup> to 10<sup>−10</sup> M E2, and after 10<sup>−7</sup> M, greater concentrations of E2 had no additional effect (Fig. 2A). These data indicate that E2 acts to decrease FL-mediated DC differentiation at physiological concentrations characteristic of adult females and at the ER<sub>K<sub>p</sub></sub> for E2. However, in cultures containing 10<sup>−10</sup> M, nearly all of the viable cells were DC (Fig. 2A), and despite the dramatic reduction in cell viability, the percentage of DC increased with increasing concentrations of E2 until 10<sup>−6</sup> M (Fig. 2B). Thus, in FL-driven cultures, E2 may act in two independent ways. E2 may decrease the viability of a majority of DC progenitors, yet increase the DC differentiation from the remaining DC progenitors that are spared by E2.

In GM-CSF-supplemented hormone-deficient medium, E2 increased the percentage of DC in a dose-dependent manner starting at 10<sup>−11</sup> up to 10<sup>−9</sup> M E2 (Fig. 2D). The total number of cells in the culture remained approximately constant over the E2 dose range, while the number of DC increased significantly in the range from 10<sup>−11</sup> to 10<sup>−9</sup> M (Fig. 2C). These data show that at the same threshold concentration of 10<sup>−10</sup> M, at the ER<sub>K<sub>p</sub></sub> E2 leads to opposing outcomes during DC differentiation mediated by FL and GM-CSF.

**Estradiol acts primarily via ER<sub>α</sub> to regulate viable cell numbers and DC differentiation in FL- and GM-CSF-driven BM cultures**

ER<sub>α</sub> and ER<sub>β</sub> have distinct and common target genes, and in some cell types, ER<sub>β</sub> modulates gene expression networks regulated by ER<sub>α</sub> (59). Thus, we determined whether the differential effects of E2 on FL- and GM-CSF-mediated DC differentiation were due to differential dependence on ER<sub>α</sub> and ER<sub>β</sub>, using ER-deficient mice. Initially, we confirmed the report (60) that the number of MP, lymphoid
(CLP), and primitive (LSK) progenitors in the BM of ERα−/− or ERβ−/− mice did not differ significantly from C57BL/6 mice (EC, data not shown). We previously published that GM-CSF-mediated DC differentiation from ERα−/− BM cells was significantly impaired, indicating a primary role for ERα (28).

To determine which ER was involved in FL-driven DC differentiation, total BM cells from ERα−/−, ERβ−/−, and C57BL/6 control mice were cultured in FL-supplemented hormone-deficient medium with or without E2 (10−9 M), and the total numbers of viable cells and DC determined on day 8 (Fig. 3A). In contrast to the marked ~5- to 10-fold reduction in cell numbers upon addition of E2 to C57BL/6 BM cells, the addition of E2 to ERα−/− BM cells did not lead to a reduction in the number of viable cells and DC, indicating that ERα was required for this action of E2. Addition of E2 to cultures of ERβ−/− BM cells also resulted in a decrease in the number of live cells and DC, although not as marked as with C57BL/6 BM.

To confirm these results, we placed BM cells from ERα−/−, ERβ−/−, and C57BL/6 mice in FL-supplemented “regular” medium, containing hormone-replete FCS and phenol red, with or
Isolated BM DC progenitors express ERα and express ERα.

Because our initial studies used total BM cells to define the effect of E2 in the differentiation cultures, we sought to determine whether E2 acts directly on DC progenitors, or alternatively, if E2 acts on mature cells in BM to elicit their production of factors important for DC differentiation. To determine whether DC progenitors express ERα, distinct populations of DC progenitors were isolated from C57BL/6 BM and assessed for expression of ERα and ERβ by quantitative real-time (qRT) PCR. Progenitors of lymphoid and myeloid cells are contained within a lineage marker-negative (Lin−) c-Kit+ fraction of BM cells. We used a combination of magnetic bead depletion and cell sorting to isolate 1) the LSK (Lin−Sca-1−c-Kit+IL-7Rα−) population, which is enriched in multipotent progenitors of both lymphoid and myeloid cells, 2) committed MP defined as Lin−c-Kit+IL-7RαSca-1−, and 3) Lin−c-Kit+flt3− cells, because DC progenitors are contained within the fraction of Lin−c-Kit+ cells expressing flt3 (42) (Fig. 4). qRT PCR with ERα- and ERβ-specific primers was used to determine expression of ER in Lin−c-Kit+flt3−, LSK, and MP populations, in comparison to ovary tissue (Fig. 4C). Each progenitor population expressed ERα mRNA. ERβ mRNA was not detected in these progenitors, although the primer set worked well with ovary RNA. Because there are splice variants of ERβ mRNA, we tested multiple primer sets (62), but we did not detect any ERβ transcripts (S. Turner, data not shown). These data show that defined DC progenitor populations in BM express ERα but not ERβ, in agreement with our finding that E2 acts primarily via ERα to regulate DC differentiation (Fig. 3A).

Estradiol acts on the same highly purified MP to regulate FL- and GM-CSF-mediated DC differentiation

We hypothesized that E2 might mediate differential effects during FL- and GM-CSF-mediated DC differentiation by acting on distinct DC progenitor populations in BM. Thus, we determined the effect of ER ligands on DC differentiation from defined DC progenitors in the GM-CSF- and FL-driven culture models. Our initial experiments with Lin−c-Kit+flt3− cells showed that DC differentiation from this undifferentiated population with DC potential was

FIGURE 2. Estradiol differentially modulates FL- and GM-CSF-mediated DC differentiation at a threshold concentration of 10^{-10} M. A and B, Water-soluble cyclodextrin-encapsulated E2 was titrated into triplicate cultures (1.5 × 10^6 cells in 1 ml each) of total (after red cell lysis) C57BL/6 BM cells in FL-supplemented hormone-deficient medium. A, The numbers of live cells and DC were determined on day 8 of culture. Data are shown as the mean and SD of triplicate cultures for each E2 dose. The numbers of live cells and DC were significantly lower beginning at 5 × 10^{-11} M E2, in comparison to 0 M E2. B, The percentage of CD11c+ DC in the FL-supplemented BM cultures increased with increasing concentration of E2. Data are shown as the mean and SD of triplicate cultures for each E2 dose. C and D, Cyclodextrin-encapsulated E2 was titrated into triplicate cultures (1 × 10^6 leukocytes in 1 ml each) of total C57BL/6 BM cells in GM-CSF-supplemented hormone-deficient medium. After 7 days, cells were assessed for the expression of CD11c, CD11b, Ly6C, and MHCII by flow cytometry and cell counts were determined by hemocytometer counting. C, The numbers of live cells and CD11c+ MHCII+ DC in triplicate cultures at each E2 concentration is shown (mean ± SD). The number of DC increased significantly in the range from 10^{-11} to 10^{-8} M E2. The percentage of CD11c+ DC in the GM-CSF-supplemented BM cultures increased in a dose-dependent manner starting at 10^{-11} M E2. Data are shown as the mean and SD of triplicate cultures for each E2 dose. Individual data points were determined to be significantly different from 0 M E2 in an unpaired t test, * p < 0.05; ** p < 0.01; *** p < 0.001. Data in A–D are representative of two independent experiments.

without the ER antagonist ICI182,780 (100 nM). This steroidal antagonist binds and induces degradation of both ER, and inhibits the effect of agonist ER ligands present in regular medium (28, 61). Addition of ICI182,780 to C57BL/6 BM cells increased the number of viable cells and differentiated DC (Fig. 3B), indicating that blockade of ER responses effectively promotes DC differentiation, yielding a similar number of DC as in cultures of hormone-deficient medium. As a result, blockage of ER responses effectively promotes DC differentiation, yielding a similar number of DC as in cultures of hormone-deficient medium. Thus, E2 is acting primarily via ERα, and ERβ is enriched in multipotent progenitors of both lymphoid and myeloid cells, in agreement with our finding that E2 acts primarily via ERα to regulate DC differentiation (Fig. 3A).
regulated by ER ligands (Fig. 5C and data not shown). Because MP efficiently yield all DC subsets in vivo, highly purified MP were isolated by cell sorting from the pooled BM of eight C57BL6 mice according to two different definitions: Lin−c-kithighIL-7RαhighSca-1high (termed MP) or Lin−c-kithighSca-1−flt3+ (termed MP-flt3+). Isolated MP or MP-flt3+ were plated into four parallel cultures with GM-CSF or FL: hormone-deficient medium with EtOH vehicle or 10−9 M E2. After 9 days, the total cell number and CD11c+ DC number in the E2 and EtOH vehicle-treated cultures was calculated for each strain on the basis of hemocytometer counts and flow cytometry analyses. Shown are the mean ± SEM for each parameter, n = 2 mice of each strain. B, BM cells from C57BL6, ERα+/−, and ERβ+/− mice were incubated in regular medium −/+ the ER antagonist ICI182,780 (100 nM). After 9 days, the total cell number and CD11c+ DC number in the ICI182,780 and DMSO vehicle-treated cultures was calculated for each strain (mean ± SEM, n = 2). Note that the y-axis is a log scale.

FIGURE 3. Estradiol acts primarily via ERα to regulate viable cell numbers and DC differentiation in FL-driven BM cultures. A, Total BM cells (∼8 × 106 leukocytes in 4 ml) from C57BL6, ERα+/−, and ERβ+/− mice were incubated in hormone-deficient medium with FL −/+ 10−9 M E2. After 9 days, the total cell number and CD11c+ DC number in the E2 and EtOH vehicle-treated cultures was calculated for each strain based on the numbers of DC were the same in the absence or presence of E2 (e.g., deficient medium with 10−9 M E2 or regular medium) increased the percentage and numbers of DC (Figs. 5C and 7; Table I).

In FL-driven cultures, the addition of E2 to both MP and MP-flt3+ populations in hormone-deficient medium decreased the number of live cells and CD11c+ DC 2- to 5-fold (Fig. 7; Table I). Addition of the ER antagonist ICI182,780 to MP and MP-flt3+ populations in regular medium increased the number of live cells and differentiated CD11c+ DC 2- to 4-fold (Fig. 7; Table I). Each of the two MP populations was isolated and tested in two independent experiments. Fig. 6 shows representative flow cytometry profiles of cells in FL-driven cultures initiated from MP or MP-flt3+. The DC subsets that were generated from MP after 7 days of culture with FL are comparable in cell surface phenotype and percentage to the DC subsets that are found in unfractionated BM cell cultures containing hormone-deficient or regular medium. However, unlike the experiments initiated with total BM cells, the addition of E2 to MP did not result in a shift in the ratio of CD11b− myeloid and CD11bhigh lymphoid DC subsets (compare Fig. 1A with Fig. 6). These data show that during FL-driven DC differentiation, E2 acts directly on MP to ultimately decrease viable cell numbers with a consequent reduction in differentiated DC.

In sum, our data show that E2 acts via ERα to differentially regulate FL- and GM-CSF-mediated DC differentiation from the highly purified MP, suggesting differential interaction of ER signaling with signals downstream of the receptors for FL and GM-CSF.

Estradiol acts on MP to regulate DC differentiation mediated by the combination of FL and GM-CSF

We next sought to determine how ER signaling in MP might regulate DC differentiation mediated by the combination of FL and GM-CSF. We isolated MP, MP-flt3+, and LSK populations from C57BL6 BM and placed them with the combination of GM-CSF and FL into the four parallel cultures with variable ER ligands. Previous reports using unfractionated BM showed that GM-CSF inhibited the ability of FL to promote PDC differentiation (55). We obtained a similar result when MP were incubated with both cytokines, as we did not find PDC and the majority of DC were CD11c+CD11b+ similar to when MP or unfractionated BM cells were incubated with GM-CSF alone (Fig. 8A). However, the surface expression of CD11b on CD11c+ DC could be divided into three distinct levels, with the CD11bhigh and CD11bmiddle levels characteristic of DC in a GM-CSF culture and the CD11blow level characteristic of LDC in a FL culture (Fig. 8A).

When DC differentiation from MP was mediated by the combination of GM-CSF and FL, the presence of E2 (e.g., deficient medium with 10−9 M E2 or regular medium) increased the percentage of CD11c+ DC after 7 days of culture, with ∼2- to 3-fold increases in the percentage and numbers of CD11bhigh DC, and a ∼2-fold decrease in numbers of CD11bmiddle DC (Fig. 8A). Although the numbers of DC were the same in the absence or presence of E2, the total number of viable cells at the end of the culture was ∼20–40% lower in the cultures that contained E2. We saw a
similar effect of E2 when the differentiation of total BM, MP-flt3+ or LSK cells was mediated by the combination of GM-CSF and FL. The presence of E2 in either hormone-deficient or regular medium led to a higher percentage of DC and a decrease in the total cell number at the end of the culture, while the absence of E2 or ER blockade by ICI182,780 had a complementary effect (Fig. 8B; Table I). These data show that when DC differentiation from MP is mediated by the combination of GM-CSF and FL, ER signaling appears to differentially regulate GM-CSF- and FL-mediated signaling pathways such that E2 promotes DC differentiation as in GM-CSF cultures, but also reduces cell viability as in FL cultures. Second, these data show that ER signaling also modulates DC differentiation initiated from the more primitive BM LSK fraction, which contains multipotent precursors of committed MP and LP.

Discussion
Because DC differentiation is mediated by FL or GM-CSF in vivo, it is important to identify factors that regulate these two cytokine-driven differentiation pathways. Our previous studies identified estradiol acting via ERα as a potent positive regulator of GM-CSF-mediated Langerhans DC differentiation from BM cells (28, 29). We now show that ER signaling has differential effects on GM-CSF- and FL-driven differentiation in cultures initiated from highly purified ERα+ flt3+ MP. Remarkably, while E2 acted on MP to increase GM-CSF-mediated DC differentiation, E2 acted on MP to significantly decrease the number of viable cells and differentiated DC during FL-mediated DC differentiation. In cultures containing both GM-CSF and FL, E2 appeared to mediate both positive and negative effects on MP, by increasing the percentage of MDC as in GM-CSF cultures, and decreasing total cell numbers as in FL cultures. Our results are based upon imposition of physiological E2 levels found in males and adult cycling females and at the ER Kd of 10−9 M. We validated a role for ER signaling in a complementary model in which the ER antagonist ICI182,780 was added to hormone-replete medium; in all cases, blockade of ER led to the opposite outcome to E2 addition.

In the GM-CSF model, GR ligands acted to promote DC differentiation from MP defined in three different ways based upon subsets of the Lin− c-kit+Sca-1+ population we isolated is primarily the CMP, and two more restricted myeloid lineage progenitors, the granulocyte/monocyte progenitor (GMP) and megakaryocyte/erythrocyte progenitor (63); DC potential is contained within the CMP and GMP. Because the megakaryocyte/erythrocyte progenitor lacks flt3 and the GMP was reported to express little or no flt3 (41, 42), the MP-flt3+ population we isolated is primarily the flt3+ CMP, indicating that ER signaling regulates DC differentiation from the earliest progenitor committed to the myeloid lineage. In addition, E2 promoted DC differentiation from a dedicated Lin− c-kit+Sca-1−CXCR1− precursor of DC and macrophages that is most similar to the GMP (45). A population of undifferentiated CD31+Ly6C+ myeloid blasts (46) also was directly responsive to E2 during GM-CSF-mediated differentiation (V. Paharkova-Vatchkova and A. Mao, unpublished observations). Blockade of ER by addition of ICI182,780 at selected times after culture initiation from Lin− BM

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**FIGURE 4.** Isolation of hemopoietic progenitors from bone marrow. BM cells were enriched for lineage marker-negative (Lin−) cells by incubation with Abs to cell surface lineage markers followed by negative selection using magnetic beads. The enriched Lin− cells were stained again with mAbs to the lineage marker mixture, c-kit, Sca-1 and IL-7Rα or CD135/flt3 before isolation of specific progenitors by cell sorting. A, Scheme for isolation of MP and LSK by cell sorting. Lin− cells were divided into MP (Lin− IL-7Rα c-kitSca-1−) and LSK (Lin− IL-7Rα c-kitSca-1−) populations. B, Scheme for isolation of the MP-flt3+ population (Lin− Sca-1− c-kit−Sca-1−). C, RNA was isolated from the indicated BM progenitors and ovary tissue and converted to cDNA. The β-actin, Esr1 (ERα), and Esr2 (ERβ) genes were amplified using specific primers and QRT PCR for 40 cycles. The number of cycles needed to reach a threshold indicating logarithmic amplification was determined. Data are normalized to cycle thresholds (CT) obtained for ovary cDNA, CT = 26 for Esr1, CT = 26 for Esr2. The CT for β-actin varied between 18 and 23 for all samples; nd, not detected.
FIGURE 5. Estradiol acts on highly purified MP to regulate GM-CSF-mediated DC differentiation. Sorted MP (Lin⁻/IL-7Rα⁻/c-kitlow/Sca-1⁻/flt3⁻) or MP-flt3⁺ (Lin⁻/c-kitlow/Sca-1⁻/flt3⁺) cells were incubated 1 day in hormone-deficient medium without GM-CSF and E2 to deplete ER-bound E2 acquired in vivo. Subsequently, MP were plated at 2 × 10⁶ cell/ml (3 × 10⁵ cells in 1.5 ml) (A) and MP-flt3⁺ were plated at 10⁵ cell/ml (1.5 × 10⁵ cells in 1.5 ml) (B) in four parallel GM-CSF-supplemented cultures which contained either hormone-deficient medium −/+ 10⁻⁸ M E2 or regular medium −/+ 100 nM ICI182,780. After 7 days, cells were harvested, counted, and assessed for cell surface expression of CD11c and CD11b by flow cytometry. The percentage of each DC subpopulation (CD11c⁺CD11bhigh and CD11c⁺CD11bint) is indicated in each plot. CD11c⁺CD11bhigh DC were Ly6C⁻ and CD11c⁺CD11bint DC were Ly6C⁺. The data are representative of two independent experiments made with each MP or MP-flt3⁺ population. C, This graph summarizes the experiments with sorted hemopoietic progenitor populations in the GM-CSF-driven culture model. Lin⁻/c-kit⁻/flt3⁻, MP, MP-flt3⁺, and MP-CX₃CR₁⁺ were incubated in GM-CSF-supplemented hormone-deficient medium −/+ E2 or in regular medium −/+ ICI182,780. After 7 days, cells were harvested, counted, and assessed for cell surface expression of CD11c and CD11b as in A. Shown are the percentages of CD11c⁺ DC present in the cultures after 7 days (mean ± SD of two independent experiments for each progenitor population). When data from the six independent experiments with the three distinctly defined MP were pooled and subjected to a t test with unequal variance, the difference in the percentage of differentiated DC in the absence or presence of E2 was highly significant, p = 0.00006.

cells showed that ER signaling begins to promote DC differentiation in the first 24 h of culture; however, cumulative effects may be observed throughout the 7-day culture period, suggesting that E2 acts on MP as well as later developmental intermediates (E. Carreras, unpublished observations).

During FL-mediated DC differentiation, E2 acted on total BM cells to greatly diminish the number of viable cells and consequently the number of differentiated plasmacytoid and conventional DC. Our data suggest that E2 may act in two independent ways during FL-mediated DC differentiation. E2 may decrease the survival of a majority of DC progenitors, yet promote DC differentiation from the remaining DC progenitors that are spared by E2.

LP were selectively depleted by in vivo treatment with supra-physiological levels of E2 (20). This depletion was alleviated in Bcl-2-transgenic mice, suggesting that E2 altered LP survival (20). Thus, initially we hypothesized that within total BM, E2 would decrease differentiation from LP while increasing DC differentiation from MP. However, physiological levels of E2 also negatively regulated numbers of viable cells and differentiated DC in FL-driven cultures initiated from MP, although the E2 impact on MP was less marked than with total BM. It is possible that in addition to MP, ER signaling could regulate DC differentiation from other flt3⁺ progenitor populations. We could not reach a conclusion regarding E2 effects on DC differentiation from the CLP, because these culture systems did not support GM-CSF- or FL-mediated differentiation of CLP as reported (64). E2 also might act on mature cells to either enhance or decrease secretion of factors that modulate DC differentiation in cultures initiated with unfractionated BM cells.

PDC were reported to arise with differential efficiency from the flt3⁺ CMP and LP, including the early lymphoid progenitor (ELP) (Lin⁻/c-kitlow/Sca-1⁻/IL-7Rα⁻/RAG-1⁻/⁻) and CLP (25, 26, 42, 64–66). Relevant to our study, the sensitivity of PDC differentiation to the imposition of constant supraphysiological levels of E2 to ovary intact mice has been used to determine the nature of PDC progenitors in vivo. Imposition of E2 for 1–2 wk decreased numbers of BM ELP and CLP, while sparing myelopoiesis and reducing BM PDC numbers (20, 25). In another study, in vivo E2 treatment significantly depleted the ELP and CLP, yet also reduced numbers of CMP by ~30%, indicating that albeit less profound, E2 negatively regulates numbers of MP in vivo (26). Despite this reduction in numbers of MP and LP, PDC numbers in BM and spleen were unaffected by E2 in the study (26), indicating that most PDC arise from the more estrogen-resistant MP.
These studies of ovary intact mice subjected to constant supra-physiological levels of E2 suggest differential sensitivity of LP and MP to negative regulation by ER agonists (20, 25, 26). In our dose titration in culture, the most profound decrease in viable cell and DC numbers occurred between 10^{-11} and 10^{-10} M, a level comparable to the plasma E2 levels in males (5 x 10^{-11} M) and ovary intact females (10^{-10} to 10^{-9} M), suggesting that the E2-mediated decrease in steady-state BM MP might occur normally and imposition of a greater constant amount of E2 to an ovary-bearing mouse has a more minor effect on MP than LP numbers. Thus, our data and the data of others show that in the presence of physiological levels of E2, ER signaling regulates DC progenitor homeostasis, serving to limit the number of MP and LP in the steady state.

ER signaling by agonists may synergize with cytokine receptor signaling to modulate the survival or proliferation of MP or their descendents, or regulate genes that specify DC fate. The cytokines that instruct DC differentiation from progenitors induce the rapid activation of STAT transcription factors and act to suppress apoptosis (67). GM-CSF activates STAT5 and regulates survival, proliferation, and differentiation of MP (39, 68).

FL activates STAT3, leading to an increase in hemopoietic progenitor survival and initiation of a DC differentiation program, but is a poor mitogen (69–71). Interestingly, in endothelial or epithelial cells, ER ligands were reported to regulate the phosphorylation, nuclear translocation, and transcriptional activity of STAT3 and STAT5, indicating a role for early ER-signaling events (72–76). ER agonist signaling may synergize with GM-CSFR/STAT5 signaling to promote cell survival and proliferation, because E2-ER signaling is known to directly regulate survival and proliferation of breast cancer cells by up-regulating Bcl-2 and cyclin D1 (12). Conversely, ER agonist signaling may negatively regulate Flt3/STAT3 signaling, leading to a decrease in cell survival and STAT3-mediated induction of other genes involved in DC development (70). Future experiments will clarify the multiple roles that E2 might play in survival, proliferation, or cell fate induction in BM progenitors under the influence of distinct growth factors.

A current paradigm is that ER ligands have agonist or antagonist activity in a particular cell type due to the relative expression of
Deficient medium is indicated for the MP and MP-flt3 progenitor population (mean SEM, n = 2). The fold change in live cell numbers due to the addition of E2 to hormone-deficient medium, relative to addition of the DMSO vehicle, is indicated for each progenitor population (mean SEM, n = 2). The fold change in live cell numbers due to the addition of E2 to hormone-deficient medium, relative to addition of the ER antagonist ICI182,780 to regular (hormone-replete) medium, relative to addition of the DMSO vehicle, is indicated for each progenitor population (mean SEM, n = 2). In the graph, fold decreases are represented as a negative fold change. B, The fold change in live cell numbers due to the addition of E2 to hormone-deficient medium is indicated for the MP and MP-flt3 progenitor population (mean SEM, n = 2). The fold change in live cell numbers due to the addition of E2 to hormone-deficient medium, relative to addition of the DMSO vehicle, is indicated for each progenitor population (mean SEM, n = 2).

The fold change in live cell numbers due to the addition of E2 to hormone-deficient medium is indicated for the MP and MP-flt3 progenitor population (mean SEM, n = 2). The fold change in live cell numbers due to the addition of E2 to hormone-deficient medium, relative to addition of the DMSO vehicle, is indicated for each progenitor population (mean SEM, n = 2).

The fold change in live cell numbers due to the addition of E2 to hormone-deficient medium, relative to addition of the DMSO vehicle, is indicated for each progenitor population (mean SEM, n = 2).

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\text{Fold change in live cell numbers due to the addition of E2 to hormone-deficient medium, relative to addition of the DMSO vehicle, is indicated for each progenitor population (mean SEM, n = 2).}
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**Figure 7.** Estradiol acts directly on MP to differentially regulate numbers of DC in FL- and GM-CSF-driven cultures. A, E2 acted on MP and MP-flt3 to increase the numbers of differentiated DC 2- to 3-fold in GM-CSF cultures containing hormone-deficient medium, and to decrease the numbers of differentiated DC 2- to 5-fold in FL cultures. ICI182,780 to regular (hormone-replete) medium is indicated for each progenitor population (mean SEM, n = 2). The fold change in live cell numbers due to the addition of E2 to hormone-deficient medium, relative to addition of the DMSO vehicle, is indicated for each progenitor population (mean SEM, n = 2).

**Table 1.** ER ligands regulate DC differentiation from isolated progenitors

<table>
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<th>Cytokine</th>
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<th>Input Cell Number(^b)</th>
<th>Live Cell Number(^b)</th>
<th>DC Number</th>
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<td></td>
<td></td>
<td>Live Cell Number(^b)</td>
<td>DC Number</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Deficient medium</td>
<td>Regular medium</td>
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<td></td>
<td></td>
<td></td>
<td>-E2 +E2</td>
<td>-ICI +ICI</td>
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<tr>
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<td>2.6 × 10(^3) 6 × 10(^3)</td>
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<td>6.3 × 10(^3) 7.1 × 10(^3)</td>
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<tr>
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<td>3.4 × 10(^3) 7.2 × 10(^3)</td>
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\(^a\) Numbers shown are from one representative experiment with each progenitor in each culture model.

\(^b\) Number of live cells or DC in one well at the end of the culture period.

\(^c\) Number of sorted progenitors plated in one well.
interest to determine whether the synergy between ER and cytokine receptor signaling leads to altered expression of coactivators or corepressors and consequent changes in gene expression. It is possible that changes in chromatin configuration mediated by cytokine receptor signaling lead to altered accessibility of ER-regulated promoter sequences, thus changing the potency of ER-ligand transcription complexes.

Our data support the hypothesis that ER ligands in combination with cytokines present in the local extracellular environment will modulate pathways of DC differentiation in vivo and ultimately regulate numbers of DC in tissues. The finding that ER signaling differentially regulates GM-CSF- and FL-mediated DC differentiation raises the important question of how and when ER ligands might modulate these two pathways to regulate numbers of DC in vivo. Studies have shown that both GM-CSF and FL contribute to steady-state DC differentiation in vivo (33–38). FL deficiency most significantly reduced all DC populations in lymphoid organs, and decreased numbers of BM MP and LP (33). GM-CSF- and GM-CSFR/βc-deficient mice have a minor decrease in numbers of DC populations, with the most significant impact on the steady-state development of CD11bhighCD8+ DC, and have deficient T cell function mediated by DC (77). Studies of LC development in GM-CSFR/βc-deficient, GM-CSF-transgenic, and FL-injected mice provided evidence that the development of epidermal LC is dependent upon GM-CSF, while independent of FL (78).

The role of GM-CSF as a regulator of myeloid cell survival, proliferation, activation, or differentiation in vivo is most prominent during inflammation and autoimmunity, during which the production and action of GM-CSF occurs locally (39). Thus, while GM-CSF has a minor but normal role in DC (including LC) differentiation in the steady-state, GM-CSF-mediated DC differentiation is likely to be most important in vivo as GM-CSF levels rise during inflammation resulting from infection, injury, or autoimmunity. In contrast, FL-mediated differentiation of the DC subsets found in lymphoid organs appears to predominate in the steady state. Thus, we hypothesize that agonist ER ligands will regulate FL-fTβ3 signaling to decrease DC progenitor numbers and DC differentiation during homeostasis. Second, we hypothesize that agonist ER ligands in synergy with GM-CSF receptor signaling will have the most impact on LC differentiation and on new DC differentiation that might occur during systemic or local inflammation. Indeed, ~80% of autoimmune diseases occur in women, and increases in DC numbers or function in some autoimmune diseases such as SLE correlate with increased GM-CSF production during inflammation (4, 40).

In sum, our data suggest that due to the action of ER ligands on MP, DC differentiation in vivo will be responsive to physiological, pharmacological, and environmental ER ligands present in the individual. These effects of ER ligands are likely to be dependent on the cytokine pathways that might be operative in the steady state or during inflammation and disease. In addition, it is possible that SERM with tissue-specific antagonist or partial agonist activity, including drugs developed for treatment or prevention of breast cancer and osteoporosis (79), or pregnancy doses of estradiol used to treat multiple sclerosis (21), will modulate DC differentiation, and thus immunity, in vivo. By affecting pathways of DC differentiation, endogenous or pharmacological ER ligands might regulate pathogen and tumor immunity, as well as aberrant immune responses during autoimmunity.

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Disclosures
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