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Estrogen Selectively Promotes the Differentiation of Dendritic Cells with Characteristics of Langerhans Cells

Allen Mao,* Vladislava Paharkova-Vatchkova,* John Hardy, † Marcia M. Miller, † and Susan Kovats2*

The steroid hormone estrogen regulates the differentiation, survival, or function of diverse immune cells. Previously, we found that physiological amounts of 17β-estradiol act via estrogen receptors (ER) to promote the GM-CSF-mediated differentiation of dendritic cells (DC) from murine bone marrow progenitors in ex vivo cultures. Of the two major subsets of CD11c+ DC that develop in these cultures, estrogen is preferentially required for the differentiation of a CD11bintLy6C− population, although it also promotes increased numbers of a CD11bhighLy6C+ population. Although both DC subsets express ERα, only the CD11bintLy6C+ DC express ERβ, perhaps providing a foundation for the differential regulation of these two DC types by estrogen. The two DC populations exhibit distinct phenotypes in terms of capacity for costimulatory molecule and MHC expression, and Ag internalization, which predict functional differences. The CD11bintLy6C− population shows the greatest increase in MHC and CD86 expression after LPS activation. Most notably, the estrogen-dependent CD11bintLy6C− DC express langerin (CD207) and contain Birbeck granules characteristic of Langerhans cells. These data show that estrogen promotes a DC population with the unique features of epidermal Langerhans cells and suggest that differentiation of Langerhans cells in vivo will be dependent upon local estrogen levels and ER-mediated signaling events in skin. The Journal of Immunology, 2005, 175: 5146–5151.

Estrogen is a regulator of growth, differentiation, survival, or function in many cell types, including cells of the immune system (6). In addition to estrogens, ER ligands include selective ER modulators, such as tamoxifen and raloxifene; phytoestrogens; or other environmental endocrine disruptors, such as bisphenol A (7–9). Upon ligand binding, ER modulate gene transcription or mediate rapid nongenomic signaling events. Each structurally distinct ligand imparts a specific conformation to either ERα or ERβ dimers, leading to recruitment of distinct profiles of coactivators or corepressors into multiprotein transcription complexes that bind DNA (10). Thus, ligation of one or both ERα or ERβ may have disparate outcomes in different cell types depending on ligand form and concentration, the relative cellular expression of the two ER, and the availability of coactivators or corepressors (11). Both ERα and ERβ are expressed in lymphoid organs. Although there are some reports of differential ERα and ERβ expression by immune cells, the relative roles of ERα and ERβ in myeloid cell regulation remain poorly understood (12–16).

Langerhans cells (LC)3 are a specialized dendritic cell (DC) subset resident in the epidermis and mucosal tissue (1). In the skin, LC act during initial immune responses to pathogens, neoplasia, or cellular damage resulting from exposure to environmental chemicals or UV light. LC are potent APC capable of internalizing and processing Ags, responding to activating signals via TLRs, and, upon migration to lymph nodes (LN), efficient activation of T cells via MHC and costimulatory molecules (2). Skin immunity is regulated by LC and, therefore, indirectly by factors that modulate LC differentiation, survival, or function. The steroid sex hormone estrogen is one such potential regulator of LC differentiation. Estrogen is known to have effects on estrogen receptor (ER)-expressing DC, macrophages, lymphocytes, or their undifferentiated progenitors as well as on epidermal keratinocytes and dermal fibroblasts (3–5).

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LC are characterized by the presence of unusual cytoplasmic rod-like or tennis-racket-shaped structures termed Birbeck granules (BG), which can be visualized by electron microscopy as two apposed membranes separated by a striated zipper-like lamella (17, 18). LC express langerin (CD207), a type II membrane-associated C-type lectin that binds mannose residues via a single carbohydrate recognition domain (19). Langerin-deficient mice lack BG, and introduction of the langerin gene into fibroblasts induces the formation of BG, suggesting that langerin has a formative role in BG structure (19, 20). In the steady state, langerin is retained primarily in endosomal recycling compartments, and BG form apparently where langerin accumulates in pericentriolar endosomes (21). Upon binding mannose or anti-langerin Abs, cell surface langerin rapidly internalizes preferentially into BG-rich, and not MHC class II (MHCII)-rich, compartments (19, 21). Several studies indicate that langerin functions as an endocytic receptor to target mannosylated ligands to BG, leading to Ag presentation by CD1a (reviewed in Ref. 22).

Upon arrival to LN, epidermal LC may be distinguished from other CD11c+ DC subsets by the CD4+ CD8α−DEC-205high phenotype (23). A recent report of a mouse expressing langerin linked to enhanced GFP showed that langerin is expressed at high levels in LC.
levels by skin-derived MHCII\textsuperscript{high}CD8\textsuperscript{α}−CD205\textsuperscript{high} DC found predominantly in cutaneous LN and at lower levels by MHCII\textsuperscript{low}CD8\textsuperscript{α}−CD205\textsuperscript{mid} DC found in spleen, LN, and thymus (24). In bone marrow (BM) DC cultures, langerin mRNA was most highly expressed by immature MHCII\textsuperscript{low}CD86\textsuperscript{−} DC; langerin protein expression also was reported in BM DC cultures supplemented with TGF-β (25, 26). Of two distinct CD11c\textsuperscript{+} DC populations (CD11b\textsuperscript{int} and CD11b\textsuperscript{hi}) that appear in GM-CSF-supported BM DC cultures, the CD11b\textsuperscript{hi} DC express the characteristic LC molecules E-cadherin and CD205 and are enhanced by the presence of TGF-β (27, 28).

We previously demonstrated that physiological amounts (0.1 nM) of 17β-estradiol (E2) promote the GM-CSF-mediated differentiation of DC from BM progenitors in ex vivo cultures derived from either female or male mice (29). Our experiments with the ER antagonist ICI 182,780, selective ER modulators, and ERα/-/ BM cells indicated that estrogen acts via the ER to promote DC differentiation (29, 30). The CD11c\textsuperscript{+} DC that differentiate in this culture system may be divided into two major subsets based on relative expression of CD11b and Ly6C and dependence upon estrogen. E2 is preferentially required for the differentiation of a CD11b\textsuperscript{hi}Ly6C\textsuperscript{−} population, although it also promotes increased numbers of a CD11b\textsuperscript{int}Ly6C\textsuperscript{+} population. In this report we have further characterized functional features of these two predominant DC populations. The two DC subsets show distinct capacity for Ag internalization when immature and for MHC and costimulatory molecule expression after LPS activation. The estrogen-dependent CD11b\textsuperscript{hi}Ly6C\textsuperscript{−} DC uniquely express langerin and contain BGs characteristic of Langerhans cells. Although both DC populations express ERα only the CD11b\textsuperscript{hi}Ly6C\textsuperscript{−} DC express ERβ, consistent with the differential regulation of these two DC types by E2. Taken together, these data indicate that differentiation of Langerhans cells in vivo may be regulated by ER-mediated signaling events.

Materials and Methods

Cell culture reagents

DC were differentiated in steroid hormone-deficient RPMI 1640 medium containing 10% charcoal-dextran (DEX)-stripped FBS (Omega Scientific) and lacking phenol red. Charcoal-Dex stripping of FBS extracts steroid hormones and reduces their levels below the detection limits of a standard RIA (31). Phenol red was omitted because it can have weak estrogenic activity at the concentration that is present in RPMI 1640 medium (32). The medium was supplemented with 2 mM glutamine, 10 mM HEPES buffer, 1 mM sodium pyruvate, 50 μM 2-ME, 100 U/ml penicillin, and 100 μg/ml streptomycin. Medium conditioned by the GM-CSF-producing cell line, J558L (33), was added to DC culture medium at 5% to promote DC development. J558L cells were provided to us by Dr. R. Steinman (Rockefeller University, New York, NY). E2 (Sigma-Aldrich) was solubilized in 95% ethanol and diluted into cultures at the concentrations indicated from a 1 mM stock solution. An equal amount of ethanol alone was added to cultures as a vehicle control.

DC generation from marine BM

DC were generated from murine BM precursors as previously described (34). BM was isolated from 8- to 12-wk-old female 129S6 mice (Taconic Farms), which were housed at the City of Hope animal resource center in compliance with federal and institutional guidelines. BM was flushed from femurs and tibiae with PBS, resuspended in medium containing GM-CSF at 1.65–2 x 10\(^5\) cells/ml, and plated at a cell density of 3.3–3.5 x 10\(^4\) cells/cm\(^2\). Cells were cultured for 7 days in 100-mm Teflon dishes or six-well plate inserts (Savillex) to reduce cell adherence. E2 was added from the first day of culture (day 0), and cells were fed every 3 days with medium containing GM-CSF and E2. On day 3, cells were fed by adding a volume of fresh medium equivalent to that of the culture. For feeding on day 6, one-half of the culture supernatant was first removed and centrifuged to recover any cells before disposal. The cell pellet was then resuspended in an equal volume of fresh medium and added back to the original culture. For DC maturation, cultures were incubated for 12–14 h, spanning days 6–7, with 0.2–2.0 μg/ml LPS from Escherichia coli serotype 055:B5 (Sigma-Aldrich).

Flow cytometry

DC cultures were analyzed on day 7 by four-color flow cytometry. FACS staining buffer (PBS, 5% newborn calf serum, and 0.1% sodium azide) was used for all washes and incubations. After harvest, 2 x 10\(^6\) cells were incubated for 5 min with anti-CD16/32 mAb (2.4G2) to block FeR. Then surface staining was performed by incubating samples for 15 min with combinations of optimally titrated, fluorochrome- or biotin-conjugated mAbs specific for CD11c, CD11b, Ly6C, CD86, CD80, CD83, MHCII (K\(^{\beta}\), MHCII (I-A\(^{\beta}\)), CCR5, CCR7, and TLR4. Subsequently, fluorochrome-conjugated streptavidin (SA) was added for 15 min to detect biotin conjugates. Cells were fixed with 1% paraformaldehyde before analysis. Unlabeled anti-CD16/32 and biotin-conjugated anti-MHCII (mAb Y3P) were produced from hybridomas (American Type Culture Collection) and were purified and labeled in our laboratory. SA-phycoerythrin, SA-PE, and the following mAbs: anti-CD80-biotin (16-10A1), -CD86-PE (GL1), -Ly6C-FFTC (AL-21), -CD11c-alkaliphycocyanin (HL3), -MHC class I-bio (AF6-88.5), and -CD11b-PerCP-Cy5.5 (M1/70), were obtained from BD Pharmingen. Anti-TLR4-PE (M1S510), -CCR5-PE (HM-CCCR5), and -CCR7-PE (4B12) were purchased from BioLegend. Anti-CD83-PE was obtained from eBioscience. Appropriately conjugated, isotype-matched control Abs were used and did not nonspecifically stain cells in BM cultures. Samples were run on a FACS caliber instrument (BD Biosciences) capable of four-color detection and were analyzed with FlowJo software (TreeStar).

Cell sorting

CD11c\textsuperscript{+} DC from day 7 cultures were isolated using anti-CD11c (mAb N418) microbeads and magnetic columns according to the manufacturer’s instructions (Miltenyi Biotech). Subsequently, DC were labeled with fluorochrome-conjugated mAbs specific for CD11c (mAb HL3), Ly6C, CD11b, and sorted into two groups on a Mo-Flo cytometer (DakoCytomation) in the City of Hope analytical cytometry core facility. The purity of the sorted cells was >95%.

Ag internalization and processing

Chicken OVA labeled with Alexa 488 (OVA\textsubscript{488}) and Dextran (Dex 10,000 m.w.)-labeled fluorescein (DexFITC) were obtained from Molecular Probes. Human serum albumin (HSA\textsubscript{488}) was labeled with Alexa 488 using an mAb labeling kit (Molecular Probes). Cells from day 7 BM DC cultures (2 x 10\(^5\)) were resuspended in fresh medium at a concentration of 1.5 x 10\(^6\) cells/ml and were incubated with 10 μg/ml OVA\textsubscript{488}, 50 μg/ml HSA\textsubscript{488}, or 100 μg/ml DexFITC for 30 min at 37°C. Ag uptake controls were conducted at 0°C with all other conditions being equal. The samples were then washed twice in FACS staining buffer and surface stained for CD11c, CD11b, and Ly6C as described above. No E2 or ethanol was included in the medium during the 30-min Ag uptake.

Detection of ER and langerin mRNA by RT-PCR

Total RNA was extracted from cell pellets using a RNeasy Micro Kit (Qiagen) and was treated with a DNase kit (Promega) before being converted to cDNA using a SuperScript kit (Applied Biosystems) according to the manufacturer’s instructions. To detect langerin cDNA (25) or ERα, ERβ, and β-actin cDNA (16), gene-specific primers were used as previously described. PCR products were resolved on agarose gels and visualized with SYBR Green stain and a TYPHOON gel imager (Amersham Biosciences). No bands were observed in control samples of RNA not treated with reverse transcriptase.

Electron microscopy

CD11c\textsuperscript{hi} DC were sorted into CD11b\textsuperscript{hi}Ly6C\textsuperscript{−} and CD11b\textsuperscript{hi}Ly6C\textsuperscript{+} populations, and pellets containing 1 x 10\(^6\) cells were prepared for microscopy as described previously (21). Briefly, cells were fixed overnight in 3% glutaraldehyde/2% sucrose in 0.1 M sodium cacodylate buffer, pH 7.3. After washing three times in the buffer, the pellet was postfixed with 1% osmium tetroxide at 4°C for 1 h, washed three times in sodium cacodylate buffer, and dehydrated in sequential 10-min steps through ethanol (60, 70, 80, 95, and 100%). The cell pellet then was incubated sequentially in 100% propylene oxide, propylene oxide/epoxy (1/1, v/v), and 100% epoxy at room temperature before polymerization at 70°C for 72 h. Thin sections were cut on the Leica Ultracut UCT at 50 nm with a diamond knife, picked up on the shiny side of 300-mesh pore size, uncoated copper grids, and polymerized at room temperature before polymerization at 70°C for 72 h. Thin sections were cut on the Leica Ultracut UCT at 50 nm with a diamond knife, picked up on the shiny side of 300-mesh pore size, uncoated copper grids, and polymerized at 70°C for 72 h.
G2 transmission electron microscope in the City of Hope electron microscope core facility.

Results

Total BM cells from female 129S6 mice were cultured in GM-CSF-supplemented, steroid hormone-deficient medium with or without the addition of 0.1 nM E2 for 7 days. DC that had differentiated under these conditions were identified by expression of the integrins CD11b and CD11c using flow cytometry and could be divided into three subsets of CD11c⁺ DC: CD11bhigh, CD11binferm, and CD11blow (Fig. 1). The presence of E2 markedly promoted the differentiation of CD11bhigh DC and, to a lesser degree, enhanced the differentiation of the CD11binferm subset (Fig. 1, A and B). To more accurately define these DC subsets, CD11c⁺ DC (Fig. 1, C and D) were assessed for relative expression of CD11b and Ly6C, a GPI-anchored membrane glycoprotein variably expressed on hemopoietic cells. Using this gating scheme, CD11c⁺ DC were divided into three subsets: CD11bhighLy6C⁺, CD11binfermLy6C⁺, and CD11blowLy6C⁻ (Fig. 1, E and F). Remarkably, the CD11bhighLy6C⁺ subset was observed only in the presence of E2. The total number of each CD11c⁺ DC subset (defined in Fig. 1, A and B) that differentiated in a 5-ml culture in the absence or the presence of E2 is graphed in Fig. 1G. Although the number of CD11blowLy6C⁻ DC did not change, the numbers of CD11bhighLy6C⁺ and CD11binfermLy6C⁻ DC were increased significantly after culture with E2.

To determine whether the two predominant DC subsets express ER, CD11c⁺ DC from day 7 BM cultures supplemented with E2 were sorted into CD11bhighLy6C⁺ and CD11binfermLy6C⁻ subsets on the basis of expression of CD11b and Ly6C and were assessed for the presence of ERα and ERβ mRNA using RT-PCR (Fig. 2). Both DC subsets as well as the CD11c⁻ cells in day 7 cultures contained mRNA for ERα. In contrast, ERβ mRNA was expressed exclusively by CD11bhighLy6C⁺ DC. These data indicate that the two DC populations will be subject to differential regulation by E2 or other ER-binding compounds.

To identify potential functional differences in these two major DC subsets that differ in their dependence on E2, DC were analyzed using four-parameter flow cytometry with a panel of mAbs specific for MHC (class I or II), costimulatory molecules (CD86, CD80, and CD83), and chemokine receptors (CCR5 and CCR7) before and after activation by bacterial LPS (Fig. 3). The two DC subsets, identified by relative expression of CD11c, CD11b, and Ly6C, displayed distinct profiles of most of these cell surface molecules. Before LPS activation, CD11bhighLy6C⁻ DC displayed a higher intrinsic level of CD86 and MHCII. After LPS activation, the expressions of MHCI Kb, MHCII I-Ab, and the costimulatory molecules CD80, CD83, and CD11bhighLy6C⁺ DC were significantly increased (Fig. 3). In contrast, the CD11binfermLy6C⁻ DC either lacked or showed more moderate increases in these MHC and costimulatory molecules upon LPS activation. These data suggest that the CD11bhighLy6C⁻ DC will be superior in stimulation of T cell responses. FACS analyses with anti-CCR5 or -CCR7 did not show staining of these mAbs above the level of matched isotype control mAbs (data not shown). TLR4 is the TLR expressed by DC subsets show differential expression of ERα, ERβ, and CD11bhighLy6C⁺ DC were increased significantly after culture with E2.

The abilities of the two DC subsets to internalize native Ags were assessed. FITC- or Alexa 488-labeled OVA, Dex, or HSA

![Figure 1](http://www.jimmunol.org/)  
Estrogen most profoundly promotes the differentiation of CD11bhighLy6C⁻ DC. Female 129S6 BM cells were cultured in GM-CSF-supplemented, steroid hormone-deficient medium in the absence (left panels) or the presence (right panels) of 0.1 nM E2. After 7 days, cells were assessed for the relative expression of CD11c, CD11b, and Ly6C by flow cytometry. A and B, Analyses of CD11b and CD11c expression show three DC populations differing in the level of CD11b, and that E2 preferentially promotes the CD11binferm subset. In an alternate gating scheme, CD11c⁺ cells were gated (indicated by the horizontal bar; C and D) and those gated CD11c⁺ cells (E and F) were divided into three subsets based on the relative expression of CD11b and Ly6C: CD11bhighLy6C⁺, CD11binfermLy6C⁺, and CD11blowLy6C⁻. The E2-dependent subset is defined as CD11bhighLy6C⁻ by this scheme. G, The total number of DC of each indicated phenotype (based on boxed populations in A and B) is shown. Data are representative of >10 independent experiments.

![Figure 2](http://www.jimmunol.org/)  
CD11c⁺ DC subsets show differential expression of ERα and ERβ. Cells in day 7 BM DC cultures containing E2 were divided into CD11c⁺ and CD11c⁻ populations using anti-CD11c-conjugated magnetic beads. Subsequently, CD11c⁺ cells were sorted into CD11bhighLy6C⁺ and CD11binfermLy6C⁻ subsets by FACS. Each population was assessed for the presence of ERα, ERβ, or β-actin mRNA using RT-PCR and specific primers. ERβ mRNA was uniquely present in CD11bhighLy6C⁺ DC. Data are representative of two independent experiments.
were incubated with cells from E2-supplemented day 7 BM cultures for 30 min at 37°C. Subsequently, cells were washed and incubated with mAbs specific for CD11c, CD11b, and Ly6C. The ability of the CD11b\(^{\text{high}}\) DC to internalize Ags was superior to that of the CD11b\(^{\text{int}}\) DC (Fig. 4).

Previous reports demonstrated the expression of langerin mRNA by DC in GM-CSF-supported BM cultures (25, 26). To determine whether E2 promoted the differentiation of DC with features of Langerhans cells, CD11c\(^{+}\) DC were sorted into CD11b\(^{\text{high}}\)Ly6C\(^{-}\) and CD11b\(^{\text{int}}\)Ly6C\(^{-}\) subsets (as depicted in Fig. 1F), and the two types of DC were assessed for the presence of langerin mRNA (Fig. 5A). CD11b\(^{\text{int}}\)Ly6C\(^{-}\) DC exclusively expressed langerin mRNA. Because langerin is an integral component of BG structure, the two DC subsets were assessed for the presence of BG using electron microscopy. BG are typically located in the pericentriolar region (17, 21); thus, 31 electron micrographs containing centrioles were collected from each of the two DC subsets. At lower magnifications, the distinct cell morphologies of the two DC subsets were apparent. Although cells in both DC subsets exhibited surface protrusions, the CD11b\(^{\text{int}}\)Ly6C\(^{-}\) DC had more extensive filopodia (Fig. 5, B and C). At higher resolution, BG were readily apparent in 65% of the CD11b\(^{\text{int}}\)Ly6C\(^{-}\) DC micrographs (20 of 31 images), but none was found in CD11b\(^{\text{high}}\)Ly6C\(^{+}\) DC micrographs (zero of 31 images; Fig. 5, D and E). These data suggest that estrogen-dependent DC in BM cultures are the correlate of epidermal Langerhans cells in vivo.

**FIGURE 3.** CD11b\(^{\text{int}}\)Ly6C\(^{-}\) DC show more significant increases in cell surface MHC and costimulatory molecules after LPS activation. Cells in E2-supplemented day 7 BM cultures were divided into CD11b\(^{\text{high}}\)Ly6C\(^{-}\) (left panels) or CD11b\(^{\text{int}}\)Ly6C\(^{-}\) (right panels) CD11c\(^{+}\) DC subsets and assessed for the expression of MHC-I, MHC-II, CD80, CD83, and CD86 by flow cytometry before (□) or after (■) activation with bacterial LPS for 14 h. Data are representative of five independent experiments.

**FIGURE 4.** CD11c\(^{+}\) DC subsets show differential ability to internalize native Ags. Cells in E2-supplemented day 7 BM cultures were incubated for 30 min with FITC- or Alexa488-labeled dextran, HSA, or OVA at 4°C (left panels) or 37°C (right panels). Subsequently, cells were washed and incubated with mAbs specific for CD11c, CD11b, and Ly6C. The ability of the CD11b\(^{\text{high}}\) DC to internalize Ags was superior to that of the CD11b\(^{\text{int}}\) DC (Fig. 4).

**FIGURE 5.** CD11c\(^{+}\)CD11b\(^{\text{int}}\)Ly6C\(^{-}\) DC uniquely express langerin mRNA and contain BGs visualized by electron microscopy. CD11c\(^{+}\) DC from E2-supplemented day 7 BM cultures were sorted into CD11b\(^{\text{high}}\)Ly6C\(^{-}\) and CD11b\(^{\text{int}}\)Ly6C\(^{-}\) DC subsets, as defined in Fig. 1F. A, The two DC subsets and CD11c\(^{+}\) cells were assessed for the presence of langerin or β-actin mRNA using RT-PCR and specific primers. CD11b\(^{\text{int}}\)Ly6C\(^{-}\) DC selectively express langerin mRNA. Data are representative of two independent experiments. B and C, Sorted DC subsets were prepared for electron microscopy. At lower magnification, cells representative of the two DC subsets show that CD11b\(^{\text{int}}\)Ly6C\(^{-}\) DC generally had more extensive filopodia. D and E, At higher magnification, 31 cells of each DC subset were examined in the pericentriolar region for the presence of BG. Only CD11b\(^{\text{int}}\)Ly6C\(^{-}\) DC contained BG. Centrioles are indicated by asterisks, and BG are indicated by arrowheads.
Discussion

The steroid sex hormones are involved in the differentiation, growth, and survival of many cell types, including cells of the immune system. We have studied the role of E2 in the GM-CSF-mediated ex vivo differentiation of murine ER\(^+\) DC. In steroid hormone-deficient BM cultures, we monitored the effect of E2 on the differentiation of two distinct CD11c\(^+\) DC populations distinguished by the relative expression of CD11b and Ly6C. The appearance of the CD11b\(^{hi}\)Ly6C\(^{-}\) DC population was dependent on the presence of physiological amounts (0.1 nM) of E2 in the culture medium. This E2-dependent DC population exhibited features of immature epidermal LC, with expression of langerin and the presence of BG. Relative to CD11b\(^{hi}\)Ly6C\(^{hi}\) DC in the cultures, E2-dependent DC displayed higher levels of MHCI, MHCIi, and the costimulatory molecules CD80, CD83, and CD86 after LPS activation, suggesting that CD11b\(^{hi}\)Ly6C\(^{-}\) DC are in a later stage of a developmental pathway. These data indicate that estrogen promotes the differentiation of a DC population with the unique features of LC and suggest that differentiation of Langerhans cells in vivo will be modulated by estrogen levels and ER-mediated signaling events in the skin.

Our definition of two major DC subsets in BM DC cultures is consistent with previous reports. The differentiation of two phenotypically distinct CD11c\(^+\) DC populations from lineage \(c-kit^+\) progenitors in the presence of GM-CSF, stem cell factor, and TNF-\(\alpha\) was observed (28). The CD11b\(^{hi}\)Ly6C\(^{G_{low}}\) Fms-1^- population retained pluri-potent myeloid progenitor potential, because it was capable of differentiating to macrophages in the presence of M-CSF or to mature DC in the presence of GM-CSF and TNF-\(\alpha\). In contrast, the CD11b\(^{hi}\)Ly6C\(^{G_{low}}\) Fms-1^- population remained DC under all conditions and exhibited features of LC, with expression of E-cadherin and CD205; these DC were less capable of internalizing DexFITC and displayed greater levels of MHCIi and allostimulatory capability. A second study extended these observations and divided immature MHCIi\(^{hi}\) BM DC into two groups based on the ability to internalize DexFITC (27). DexFITC\(^+\) DC were CD11b\(^{hi}\)Ly6C\(^{G_{low}}\)E-cadherin^-, whereas DexFITC\(^-\) DC were CD11b\(^{hi}\)Ly6C\(^{G_{low}}\)E-cadherin^-. As we also report in this study, the latter CD11b\(^{hi}\) population matured more readily upon stimulation with LPS. These authors hypothesize that CD11b\(^{hi}\) DC correspond to myeloid monocyte-derived DC, whereas CD11b\(^{hi}\) DC correspond to LC (although significant langerin expression or BG was not found).

The precise developmental relationship between the CD11b\(^{hi}\)Ly6C\(^{hi}\) and CD11b\(^{hi}\)Ly6C\(^{hi}\) DC populations that differentiate in GM-CSF-supported BM cultures remains unclear, although the above-mentioned studies suggest that the CD11b\(^{hi}\) DC may arise via multiple pathways (27, 28). We do not yet have strong evidence that one DC population arises from the other, or that the two DC populations arise from distinct undifferentiated myeloid progenitors. BM progenitors of distinct DC populations have been identified in several studies (35–37), although it is unclear how these previously defined progenitors relate to our E2-responsive populations. The differential E2 requirement of the two DC populations may help us to dissect their origin from myeloid progenitors. Recently, we determined that E2 acts on undifferentiated CD31^hiLy6C^hi myeloid blasts present in murine BM (36) to promote differentiation of the CD11b\(^{hi}\)Ly6C\(^{hi}\) subset (V. Paharkova-Vatchkova, A. Mao, and S. Kovats, manuscript in preparation).

It is difficult to directly correlate the BM DC in this ex vivo culture system with DC in secondary lymphoid organs, because we did not observe the expression of CD4 or CD8a by BM DC. However, analyses of splenic DC in mice treated with Flt3 ligand, which expands the naturally occurring DC populations, identified two populations of CD11c\(^+\) DC that correspond to the subsets defined in BM DC cultures (38). In the spleen, CD11b\(^{hi}\)DC are Ly6C\(^{G_{low}}\)CD8a DC, more phagocytic, and located in the marginal zone. In contrast, CD11b\(^{hi}\)Ly6C\(^{-}\) DC are less phagocytic and located in the T cell areas of the white pulp. Interestingly, a study of the langerin-enhanced GFP-knockin mice showed that, in addition to LC, langerin is expressed in CD8a\(^{hi}\) CD205\(^{low}\) DC in spleen and LN (24). These observations suggest that the estrogen-dependent DC in BM DC cultures may correspond to in vivo populations of both LC and CD8a\(^{hi}\) lymphoid DC.

Differentiation of CD1a\(^+\) LC from human CD34\(^+\) cells or monocytes requires TGF-\(\beta\), which has been shown to promote the survival of DC progenitors by protecting them from apoptosis (39, 40). Mice deficient in TGF-\(\beta\) lack epidermal DC, indicating that TGF-\(\beta\) is required for LC differentiation and migration to skin (41, 42). Interestingly, estrogen induces the expression of TGF-\(\beta\) in multiple cell types (43–47). Thus, we currently are testing the hypothesis that E2 promotes the differentiation of LC-like DC by increasing the level of TGF-\(\beta\) in BM cultures.

Recent studies have suggested that human and murine LC renew from skin-resident proliferating precursors in the steady state (48, 49). The estrogen necessary to support LC differentiation in skin may be produced remotely in gonads or locally in tissue. Skin fibroblasts and adipose tissue are sites of extragonadal estrogen synthesis by aromatase, the P450 enzyme that catalyzes the conversion of C (19) steroid precursors to estrogens (50). Estrogen synthesized in extragonadal tissue acts via paracrine mechanisms, and it is likely that the concentration of E2 in the skin will be sufficient to mediate cellular ER signaling (51). In addition to potential direct effects of estrogen on gene expression patterns in LC progenitors, estrogen increases GM-CSF production by keratinocytes and TGF-\(\beta\) production by fibroblasts (52). Taken together, these observations are consistent with the hypothesis that the paracrine action of locally produced estrogen in the skin, leading to enhanced production of TGF-\(\beta\) and GM-CSF, will promote LC differentiation in vivo.

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Disclosures

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References


23. Takahashi, T., B. E.为客户 on August 31, 2017 http://www.jimmunol.org/ Downloaded from

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