

17 β -Estradiol (E₂) modulates cytokine and chemokine expression in human monocyte-derived dendritic cells

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The effects of estrogen on the immune system are still largely unknown. We have investigated the effect of 17 β -estradiol (E₂) on human monocyte-derived immature dendritic cells (iDCs). Short-term culture in E₂ had no effect on iDC survival or the expression of cell surface markers. However, E₂ treatment significantly increased the secretion of interleukin 6 (IL-6) in iDCs and also increased secretion of osteoprotegerin (OPG) by DCs. Furthermore, E₂ significantly increased secretion of the inflammatory chemo-

kines IL-8 and monocyte chemoattractant protein 1 (MCP-1) by iDCs, but not the production of the constitutive chemokines thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC). However, after E₂ pretreatment the lipopolysaccharide (LPS)-induced production of MCP-1, TARC, and MDC by DCs was clearly enhanced. Moreover, mature DCs pretreated with E₂ stimulated T cells better than control cells. Finally, we found that E₂ provides an essential signal for migration

of mature DCs toward CCL19/macrophage inflammatory protein 3 β (MIP3 β). In summary, E₂ may affect DC regulation of T-cell and B-cell responses, as well as help to sustain inflammatory responses. This may explain, in part, the reason serum levels of estrogen correlate with the severity of certain autoimmune diseases. (Blood. 2004;104:1404-1410)

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Introduction

Dendritic cells (DCs) are key antigen-presenting cells (APCs), which recognize, capture, and process antigens, express costimulatory molecules, and then migrate to secondary lymphoid organs, where they can help initiate immune responses.¹ In addition to stimulating naive T cells and initiating primary immune responses, DCs act as effector cells in innate immunity and also play a role in maintaining peripheral tolerance.² DCs are also involved in T-cell polarization, for example, into T helper 1 (Th1) and Th2 cells.² For example, interleukin 12 (IL-12) secreted by DCs induces the production of interferon- γ (IFN- γ) by CD4⁺ T cells, which promotes Th1 cell differentiation and proliferation.³ Furthermore, IL-6 derived from DCs can drive Th2 differentiation and at the same time inhibit Th1 polarization.⁴⁻⁶ DCs can both produce and respond to chemokines,⁷ which play a crucial role in leukocyte trafficking.⁸ These include inflammatory, for example, IL-8 and monocyte chemoattractant protein 1 (MCP-1), as well as constitutive, eg, thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC).⁷ DC-derived chemokines not only are involved in T-cell priming and Th1/Th2-mediated responses,⁹ but also contribute to the pathology of certain autoimmune conditions.^{10,11}

In many autoimmune diseases women are more likely to be affected than men. For example, in systemic lupus erythematosus (SLE), Sjögren syndrome, autoimmune thyroid disease, and scleroderma, more than 80% of the patients affected are women.¹² Sex hormones, particularly estrogen, may contribute to the pathogenesis of some autoimmune diseases.¹³ Fluctuations in estrogen levels may correlate with disease status; for example, during pregnancy

circulating levels of estrogen increase notably.¹⁴ In both multiple sclerosis and rheumatoid arthritis, disease activity decreases during the third trimester when estrogen levels are highest and flares again when the estrogen levels decrease postpartum.¹³ In SLE, however, the disease status during pregnancy either worsens or is unchanged.¹³ Although these sex-based differences in autoimmune responses are well known, the underlying cellular mechanisms are not well understood.

To control various cellular processes, circulating estrogen interacts with intracellular receptors. 17 β -Estradiol (E₂) is the most common circulating form of estrogen. The receptor-ligand complexes function as transcription factors that regulate the expression of certain genes.¹⁵ Estrogen is very important for the control of bone mass in adults¹⁶ and has been reported to up-regulate the production of osteoprotegerin (OPG) in bone cells.¹⁷ OPG is a member of the tumor necrosis factor receptor (TNFR) superfamily¹⁸ and acts as a decoy receptor, preventing the interaction between receptor activator of nuclear factor- κ B (RANK, expressed by osteoclasts) and RANK ligand (RANKL, expressed by osteoblasts).¹⁹ OPG is produced by osteoblasts and has an important function as a protector of bone,²⁰ as demonstrated by severe osteoporosis in OPG^{-/-} mice.²¹ Our earlier studies on the role of OPG in the immune system²²⁻²⁴ showed that DCs from OPG-deficient mice stimulate allogeneic T cells more efficiently²³ and secrete elevated amounts of inflammatory cytokines when stimulated with bacterial products or soluble RANKL in vitro.²⁴

The effect of estrogen on human DCs is still largely unknown. Here, we show that E₂ has several effects on human DCs in vitro,

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including the modulation of cytokine and chemokine secretion by DCs. The effects of E₂ on DC functions may affect the regulation of T-cell and B-cell responses, as well as on the induction and sustenance of inflammatory responses. This may explain, in part, why women are affected more often by autoimmune diseases than men, and why levels of estrogen often correlate with the severity of autoimmune disease.

Materials and methods

Cell isolation and culture

Monocyte-derived immature dendritic cells (iDCs) were generated from human peripheral blood mononuclear cells (PBMCs) obtained from buffy coat preparations (Red Cross Blood Bank, Portland, OR) or from leukapheresis products from healthy donors. The iDCs obtained from buffy coat products were isolated as previously described.²⁵ Monocytes (> 97%-99% CD14⁺) purified from leukapheresis products by CD14⁺ immunomagnetic-positive selection were purchased from the Cellular Therapy Laboratory at the Fred Hutchinson Cancer Research Center (Seattle, WA). Monocytes were either directly used for experiments or frozen in RPMI 1640 (Gibco Invitrogen, Carlsbad, CA), 30% human AB serum (ICN Biomedicals, Aurora, OH), and 10% dimethyl sulfoxide (DMSO; Sigma, St Louis, MO) and stored in liquid nitrogen until use. Freezing had no effect on DC phenotypes or the performance in experiments. CD4⁺ T cells were obtained by isolating CD3⁺ T cells from PBMCs by sheep red blood cell (SRBC; Triple J Farms, Bellingham, WA) agglutination, and then panning with CD8 monoclonal antibody (mAb; G10-1) for 1 hour at 37°C. The CD4⁺ T cells were routinely more than 97% pure.

To obtain iDCs, CD14⁺ cells were seeded at a density of 3×10^6 cells/mL in 6-well plates in 5 mL RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS; BioWhittaker, Walkersville, MD), human granulocyte-macrophage colony-stimulating factor (GM-CSF; 100 ng/mL, Leukine; Amgen, Seattle, WA), and human IL-4 (30 ng/mL; RDI, Flanders, NJ) and cultured for 6 days. Cells were fed at days 2 and 4 by replacing half the medium and adding fresh cytokines. At day 6 the cells exhibited an immature DC phenotype, that is, CD1a^{high} and CD14⁻.

In some experiments E₂ (20 μ g/mL; Sigma) or β -cyclodextrin (BC; 20 μ g/mL; Sigma) was added to the iDC cultures. For maturation of the iDCs the cells were stimulated for 24 or 48 hours with *Escherichia coli* lipopolysaccharide (LPS; 1 μ g/mL; Sigma) or cultured for 48 hours with CD32- or CD40L-transfected mouse fibroblast L cells irradiated for 14 minutes.

Flow cytometry

The following mAbs were used for surface staining: phycoerythrin (PE)-conjugated anti-CD1a (Beckman Coulter, Miami, FL), anti-CD80, and anti-CD86 (BD Pharmingen, San Diego, CA); fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (G19-4), anti-CD14, and anti-CD83 (BD Pharmingen), anti-CD40 (G28-5), anti-CD20/DC-SIGN (UW60.2), and anti-HLA-DR (HB10a). The cells were also stained with corresponding PE- or FITC-conjugated isotype-matched control mAbs. The viability of the iDCs was tested by staining cells with annexin V and propidium iodide (PI; TACS annexin V-FITC; R&D Systems, Minneapolis, MN). The cells were analyzed using a FACScalibur flow cytometer and CELLQuest software (BD Biosciences, San Jose, CA).

Cytokine, chemokine, and OPG ELISA

The levels of cytokines, chemokines, and OPG in cell culture supernatants were analyzed using matched pairs of antibodies in an enzyme-linked immunosorbent assay (ELISA). Briefly, a 96-well plate (Nunc, Rochester, NY) was coated overnight at room temperature with a mouse antihuman capture antibodies to IL-6, TNF- α , and IL-10 (BD Pharmingen) and to IL-12p40, IL-12p70, OPG, IL-8, MCP-1, TARC, and MDC (R&D Systems). After blocking with PBS containing 1% bovine serum

albumin (BSA; Sigma), samples or the standard protein including recombinant human IL-6 (rhIL-6), rhTNF- α , or rhIL-10 (BD Biosciences), rhOPG, rhIL-12p40, rhIL-12p70, rhIL-8, rhMCP-1, rhTARC, or rhMDC (R&D Systems) was added in triplicates and incubated overnight at 4°C. Biotinylated antibodies against IL-6, TNF- α , or IL-10 (BD Pharmingen), OPG, IL-12p40, IL-12p70, IL-8, MCP-1, TARC, or MDC (R&D Systems) were then added, incubated for 2 hours, and visualized and read by standard methods.

Mixed lymphocyte reaction

iDCs were treated with BC, E₂ (20 μ g/mL), or medium alone for 24 hours. To mature the cells, iDCs were stimulated with LPS (1 μ g/mL) for 24 hours. Different concentrations of iDCs or mature DCs (0-50 000 cells) were cultured in round-bottomed 96-well plates in triplicates with 50 000 allogeneic CD4⁺ T cells for 5 days in RPMI with 10% FCS. T-cell proliferation was assessed after the addition of 1 μ Ci/well (0.037 MBq/well) [³H]-thymidine (Amersham, Arlington Heights, IL) for the final 18 hours. Incorporation of [³H]-thymidine was measured by liquid scintillation counting. All determinants were performed in triplicate and measured as the mean counts per minute (cpm) \pm SD.

Migration assay

The iDCs were cultured for 24 hours in presence or absence of 1 μ g/mL LPS with or without 30 minutes of preincubation with BC (20 μ g/mL) or E₂ (20 μ g/mL). Then migration was measured using the transwell system (24-well plates; 8.0- μ m pore size; Costar, Corning, NY). To the lower chamber 600 μ L RPMI medium containing or not 200 ng/mL of recombinant human CCL19/macrophage inflammatory protein 3 β (MIP3 β ; RDI) was added. Wells containing RPMI medium alone were used as controls for spontaneous migration. Then, 2.5×10^5 cells in a total volume of 100 μ L were added to the upper chamber and incubated at 37°C for 3 hours. Cells that migrated into the lower chambers were harvested, concentrated to a volume of 200 μ L, and counted by flow cytometry using CELLQuest software (BD Biosciences). Each experiment was performed in duplicate. The mean number of spontaneously migrated cells was subtracted from the total number of cells that migrated in response to the chemokine. Values are given as the mean number of migrated cells \pm SEM.

Statistical analysis

Cell medium levels of cytokines, chemokines, and OPG in DC cultures were compared using the nonparametric Wilcoxon matched pairs test. A *P* value of less than .05 was considered statistically significant.

Results

E₂ does not change the viability or phenotype of iDCs

After 6 days of culturing CD14⁺ monocytes with GM-CSF and IL-4, the cells had acquired a typical iDC phenotype, that is, CD1a^{high}, CD80⁺, CD86⁺, CD40⁺, DC-SIGN⁺, HLA-DR⁺, CD83^{low/-}, and CD14⁻ (Figure 1A). To test if E₂ had an effect on the phenotype of iDCs, the cells were treated with medium only, BC (20 μ g/mL), or E₂ (20 μ g/mL) for 24 hours, and thereafter analyzed for changes in the expression of surface markers by flow cytometry. Treatment with E₂ had no effect on the surface expression of any marker tested (Figure 1A).

In some cells estrogen protects from cell death,²⁶⁻²⁹ whereas in others it can induce apoptosis.^{30,31} To test whether E₂ modulated the survival of iDCs, cells were cultured with BC, E₂, or in medium alone for 24 hours and then stained with annexin V and PI and analyzed with flow cytometry. No change in viability was noted in the cells treated with E₂ compared to cells cultured in medium only or with BC (Figure 1B).

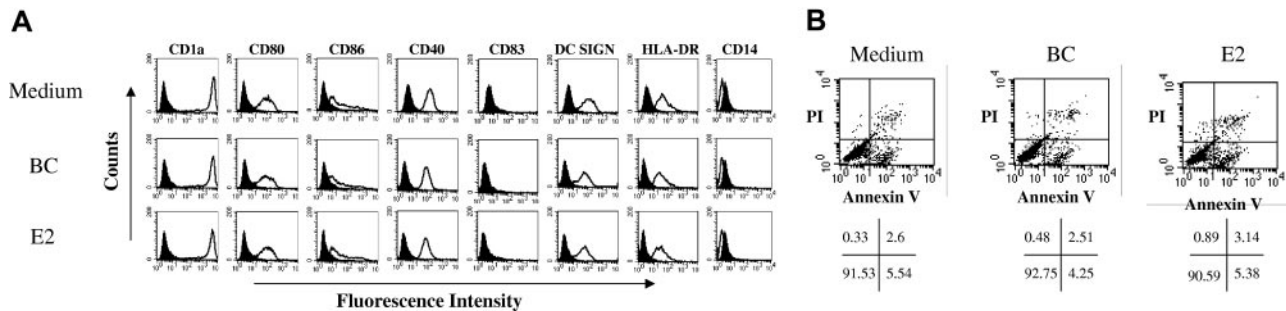


Figure 1. Treatment with E₂ does not change the phenotype of iDCs. Immature DCs, derived from monocytes cultured with GM-CSF and IL-4 for 6 days, were treated with medium only, BC (control, 20 μ g/mL), or E₂ (20 μ g/mL) for 24 hours. The expression of surface markers (open histogram) was detected by flow cytometry using mAbs specific for CD1a, CD80, CD86, CD40, CD83, DC-SIGN, HLA-DR, and CD14 (A). For each mAb, an isotype-specific control antibody was used (black filled histogram). Data are representative of 3 different donors. After treatment with medium alone, BC, or E₂ as described for panel A for 24 hours, the viability of the iDCs was tested by staining the cells with annexin V and PI (B). The cells were analyzed by flow cytometry. The data shown are 1 representative of at least 3 experiments.

E₂ enhances the secretion of IL-6 in iDCs

DCs can direct different types of T-cell responses, for example, Th1/Th2, by producing different types of cytokines.³² An imbalance in Th1/Th2 may contribute to many autoimmune disease states.³³ To study the effect of E₂ on DC cytokine production, iDCs were cultured with medium alone, BC, or E₂, and after 24 hours supernatants were analyzed by ELISA for the presence of IL-6, IL-10, TNF- α , IL-12p40, and IL-12p70 (Figure 2). E₂ significantly enhanced the production of IL-6 by iDCs ($P < .05$; Figure 2C) and in a dose-dependent manner (Figure 2D). In contrast to IL-6, E₂ had no effect on the levels of IL-10 and TNF- α detected in culture supernatants (Figure 2A-B). Interestingly, E₂ also did not modulate the secretion of the Th1-polarizing cytokine IL-12 (IL-12p40 and IL-12p70), which was produced by iDCs at very low or undetectable levels with or without E₂ or BC (data not shown).

LPS stimulates the production of cytokines by DCs.^{1,34} To investigate if E₂ could modulate LPS-induced secretion of cytokines by iDCs, iDCs were cultured for 24 hours with E₂, BC, or medium alone, followed by stimulation with LPS (1

μ g/mL) for 24 hours. The secretion of IL-6, TNF- α , IL-10, and IL-12p40 by iDCs was markedly increased by LPS. However, E₂ did not alter the LPS-induced cytokine production significantly (data not shown).

E₂ modulates the production of the DC-derived chemokines IL-8, MCP-1, MDC, and TARC

DCs not only respond to chemokines, but also secrete them.^{7,8} We tested whether E₂ affected the production of chemokines by iDCs, which were cultured with BC, E₂, or medium only for 24 hours. Then supernatants were analyzed for MCP-1, IL-8, TARC, and

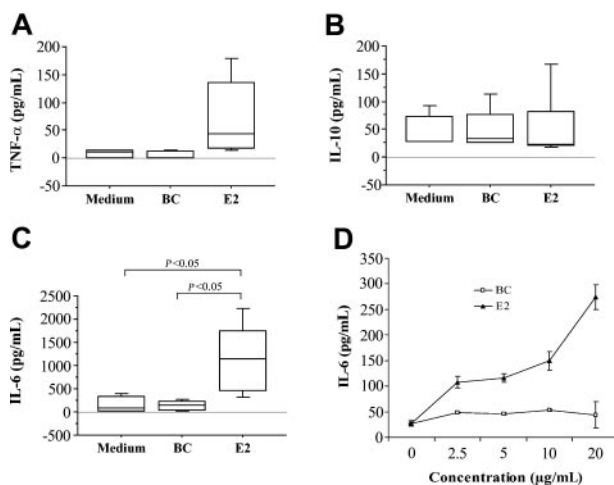


Figure 2. E₂ enhances the secretion of IL-6 in iDCs. CD1a⁺ CD14⁺ iDCs from 5 different donors were cultured with medium alone, BC (20 μ g/mL), or E₂ (20 μ g/mL). After 24 hours the supernatants were collected and analyzed by ELISA for the presence of TNF- α (A), IL-10 (B), and IL-6 (C-D). The secretion of IL-6 was significantly enhanced by E₂ ($P < .05$, $n = 5$). The boxes show the 10th, 25th, 50th (median, central line), 75th, and 90th percentiles of the variable (pg/mL IL-6, IL-10, or TNF- α). Statistics were calculated using the Wilcoxon matched pairs test. When DCs were treated with graded doses of E₂ (\blacktriangle), IL-6 was enhanced in a dose-dependent manner (D). Data shown in panel D are the mean \pm SD of triplicate wells.

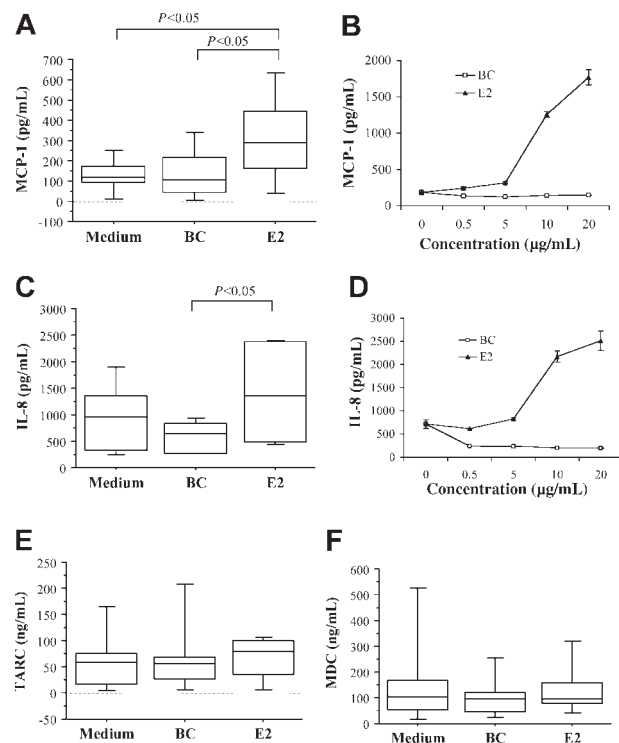


Figure 3. E₂ modulates the secretion of DC-derived chemokines. CD1a⁺ CD14⁺ iDCs from at least 5 different donors were cultured with medium only, BC (20 μ g/mL), or E₂ (20 μ g/mL). After 24 hours the supernatants were collected and analyzed by ELISA for the presence of MCP-1 (A-B), IL-8 (C-D), TARC (E), and MDC (F). The boxes show the 10th, 25th, 50th (median, central line), 75th, and 90th percentiles of the variable (pg/mL MCP-1, IL-8, or ng/mL TARC and MDC). Statistics were calculated using the Wilcoxon matched pairs test. When DCs were treated with graded doses of E₂ (\blacktriangle) or BC (\square), MCP-1 and IL-8 was enhanced in a dose-dependent manner (B-D). Data shown in panels B and D are the mean \pm SD of triplicate wells.

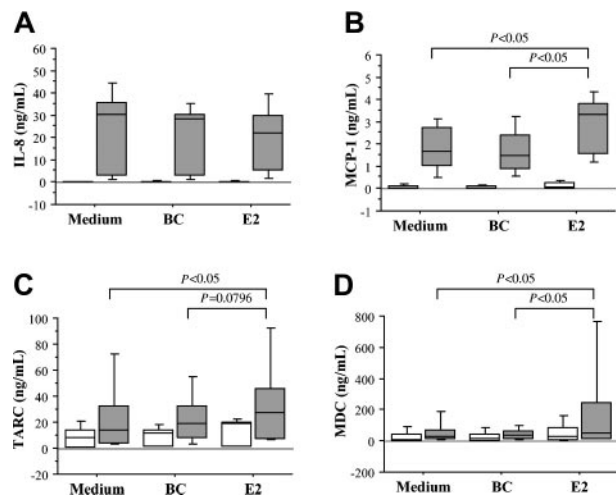


Figure 4. E₂ pretreatment enhances the LPS-induced production of MCP-1, TARC, and MDC by DCs. Immature DCs from 5 different donors were cultured with BC, E₂ (20 μ g/mL), or medium alone for 24 hours, followed by stimulation with (■) or without (□) LPS (1 μ g/mL) for 24 hours. Supernatants were collected and analyzed by ELISA for the presence of IL-8 (A), MCP-1 (B), TARC (C), and MDC (D). The boxes show the 10th, 25th, 50th (median, central line), 75th, and 90th percentiles of the variable (ng/mL IL-8, MCP-1, TARC, and MDC). Statistics were calculated using the Wilcoxon matched pairs test.

MDC by ELISA. E₂ up-regulated the inflammatory chemokines MCP-1 and IL-8 ($P < .05$; Figure 3A,C), and in a dose-dependent manner (Figure 3B,D). However, E₂ treatment had no significant effect on either TARC or MDC produced by iDCs (Figure 3E-F). Both inflammatory and constitutive chemokines are up-regulated in response to bacterial stimuli, such as LPS.⁷ E₂ did not alter LPS-induced IL-8 production (Figure 4A); however, LPS-induced secretion of MCP-1, MDC, and TARC was clearly enhanced (Figure 4B-D).

E₂ enhances the T-cell stimulatory capacity of mature DCs

Next, iDCs were pretreated with E₂, BC, or medium only. To mature the cells, the pretreated iDCs were then stimulated with LPS. Immature or mature DCs were cocultured with allogeneic T cells in a mixed lymphocyte reaction and compared for their ability to stimulate T-cell proliferation. The mature DCs, as expected, had an enhanced ability to stimulate T cells compared to iDCs (Figure 5A-B). Furthermore, mature DCs pretreated with E₂ had an enhanced ability to stimulate T cells to proliferate compared to control mature DCs (Figure 5B). However, E₂ pretreatment did not affect the ability of iDCs to stimulate T-cell proliferation (Figure 5A).

Figure 5. E₂ pretreatment enhance the T-cell stimulatory capacity of mature DCs. Immature DCs were treated with BC, E₂ (20 μ g/mL), or medium only for 24 hours. To mature the cells, the pretreated iDCs were then stimulated with LPS (1 μ g/mL) for 24 hours. Different concentrations of immature (A) or mature (B) DCs (0–50 000) were cultured in round-bottomed 96-well plates in triplicates with 50 000 allogeneic CD4⁺ T cells for 5 days in RPMI with 10% FCS. T-cell proliferation was assessed. All determinants were performed in triplicate and measured as the mean cpm \pm SD. The figure shows 1 representative experiment of 4.

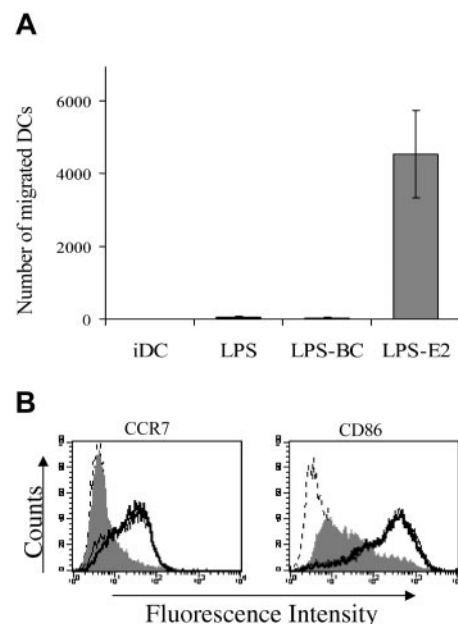
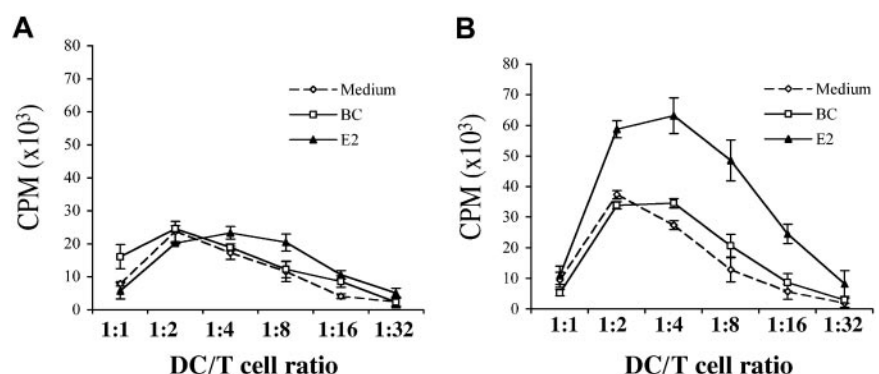


Figure 6. The addition of E₂ is essential for migration of mature DCs toward the lymph node-derived chemokine CCL19/MIP3 β . iDCs were stimulated for 24 hours with 1 μ g/mL LPS in the absence or presence of 20 μ g/mL BC or 20 μ g/mL E₂. Unstimulated and stimulated DCs were tested for their chemotactic response to CCL19/MIP3 β . Migration was measured using a transwell system. Values are given as the mean number of migrated cells \pm SEM and are from 1 representative experiment of 3 performed with different donors. The mean number of spontaneously migrated cells was subtracted from the total number of cells that migrated in response to the chemokine (A). Unstimulated iDCs (filled gray histogram) and iDCs stimulated with LPS alone (bold line) or in combination with E₂ (thin line) were analyzed for CCR7 and CD86 surface expression by flow cytometry. The dashed line represents the staining with an isotype control antibody. Data are from 1 of 3 experiments with different donors that gave similar results (B).

E₂ provides a signal essential for migration of mature DCs toward CCL19/MIP3 β

Because E₂ enhanced the ability of mature DCs to stimulate CD4⁺ T-cell proliferation, we tested if E₂ treatment could also affect another important function of mature DCs, that is, the ability to migrate toward the lymph node-derived chemokine CCL19/MIP3 β . iDCs and DCs stimulated for 24 hours with LPS with or without E₂ or BC were tested for their chemotactic response to CCL19 (Figure 6A). As expected, iDCs, which do not express the receptor for CCL19, CCR7 (Figure 6B), did not migrate in response to CCL19. Interestingly, even though they expressed CCR7, neither LPS-treated nor BC plus LPS-treated DCs migrated efficiently toward CCL19. However, in the presence of E₂ the migratory response of LPS-treated DCs was substantially increased

(> 120-fold; Figure 6A). The induction of migration toward CCL19 in presence of E_2 did not simply reflect an enhanced expression of CCR7, which was induced to the same extent in all LPS-treated samples (Figure 6B). Also the up-regulation of CD86 and CD83 induced by LPS was not significantly affected by either BC or E_2 treatment (Figure 6B and data not shown). A similar dissociation between CCR7 expression on mature DCs and the ability to effectively migrate in response to CCL19 has been reported previously.^{35,36}

DC-derived OPG is enhanced by E_2 , LPS, and CD40 ligation

OPG mRNA is up-regulated by CD40L in DCs.²² OPG protein is secreted constitutively at relatively low levels and significantly increased when the cells are stimulated with maturation stimuli, such as LPS or CD40 ligation (Figure 7A-B). Because E_2 can up-regulate OPG in other cells,¹⁷ we tested if E_2 could alter OPG expression in iDCs. OPG levels increased after treatment with E_2 compared to the BC control (a median value of 233 versus 311 pg/mL; Figure 7C), and in a dose-dependent manner (Figure 7D). However, E_2 was not as effective as CD40 ligation (Figure 7B) in inducing OPG secretion. Next, iDCs were pretreated for 24 hours with E_2 , BC, or medium alone, then stimulated with LPS for 24 hours. Treatment with E_2 did not alter LPS-induced OPG production (data not shown). DCs express RANK^{19,24,37} and T cells express RANKL.^{24,38,39} Thus, OPG released by DCs might be one of the factors that regulate the survival of mature DCs by blocking RANK-RANKL interactions. Estrogen and CD40L may, in turn, modulate this regulation.

Discussion

Estrogen apparently plays a role in certain autoimmune diseases.^{13,40} However, the underlying cellular mechanisms explaining how estrogen affects these diseases are poorly understood. In this study we show that estrogen affects human DC functions by modulating the secretion of cytokines (Figure 2), chemokines

(Figures 3-4), and OPG (Figure 7) produced by DCs. We also show that estrogen pretreatment enhances the T-cell stimulatory capability of mature DCs but not iDCs (Figure 5). Finally, our results show that E_2 provides a signal essential for migration of mature DCs toward CCL19/MIP3 β (Figure 6).

DCs are remarkable activators of naive T cells. After encounter with pathogens in the periphery, DCs migrate to T-cell-rich areas within secondary lymphoid organs, where they interact with T cells; by providing them with different cytokines, they help direct Th1 versus Th2 responses.⁴¹ An imbalance in Th1/Th2 immune responses can be a factor in the progression or remission of certain autoimmune diseases.³³ We found that E_2 significantly up-regulates IL-6 in iDCs, but not the production of IL-10, TNF- α , IL-12p40, or IL-12p70 (Figure 2 and data not shown). The proinflammatory cytokine IL-6 has been implicated in several disease states.⁴² For example, increased IL-6 levels have been observed in autoimmune diseases such as rheumatoid arthritis and systemic-onset juvenile chronic arthritis, and in experimentally induced autoimmune diseases such as antigen-induced arthritis and experimental allergic encephalomyelitis (EAE).⁴² Modulation of IL-6 secretion by DCs (Figure 2C-D) might explain the effect of estrogen on disease severity in some autoimmune diseases. IL-6 is involved in polyclonal B-cell activation and autoantibody production.⁴² Increased B-cell activity may be a cause of the flare-ups in SLE during pregnancy when estrogen levels are high.⁴³ IL-6 can also promote Th2 differentiation and at the same time inhibit Th1 polarization.^{4,5} The skewing toward Th2 responses could help keep in check the chronic Th1 responses associated with inflammatory tissue injury.

Recently, Liu et al investigated the effects of E_2 on DCs in 2 mouse models of EAE. They found that E_2 decreased production of TNF- α , IFN- γ , and IL-12 in mature DCs.⁴⁴ Moreover, E_2 -treated mature DCs cocultured with MBP-specific T cells, induced a shift toward the production of Th2 cytokines and a decrease of Th1 cytokines.⁴⁴

At different stages of maturation DCs produce different chemokines, which clearly influence various aspects of DC function, such as antigen uptake, migration, and the priming of naive T cells.^{7,9} We found that E_2 induces the secretion of the inflammatory chemokines MCP-1 and IL-8 by iDCs, but does not significantly alter the constitutive chemokines TARC and MDC (Figure 3). However, E_2 pretreatment enhanced the secretion of MDC, TARC, and MCP-1 by LPS-stimulated DCs (Figure 4). The production of inflammatory chemokines by DCs in the peripheral tissues recruits iDCs, macrophages, granulocytes, and T cells.⁹ Multiple chemokines are produced in inflamed tissues, for example, in rheumatoid joints and in lesions of multiple sclerosis and SLE patients.¹⁰ The increased expression of IL-8 and MCP-1 in response to E_2 suggests that elevated estrogen levels may induce and sustain inflammatory responses. Interestingly, Th2 cells express CCR4, the receptor for TARC.^{45,46} Also MDC may play a role in the selective migration of Th2 cells and amplification of Th2 responses.⁴⁷ TARC and MDC, both produced at later time points after DC maturation, may help recruit Th2 cells.⁷ The enhanced LPS-induced production of TARC, MDC, and MCP-1 by E_2 suggests that E_2 can increase the responsiveness of cells to LPS. Similarly, E_2 -pretreated mature DCs were better than control DCs at stimulating allogeneic T cells (Figure 5B). The enhanced ability of estrogen-treated DCs to induce T-cell proliferation could facilitate activation of pathogenic T cells in autoimmune disease.

The ability of E_2 to facilitate the chemotactic response of mature DCs toward CCL19 (Figure 6A) is in agreement with 2 recent

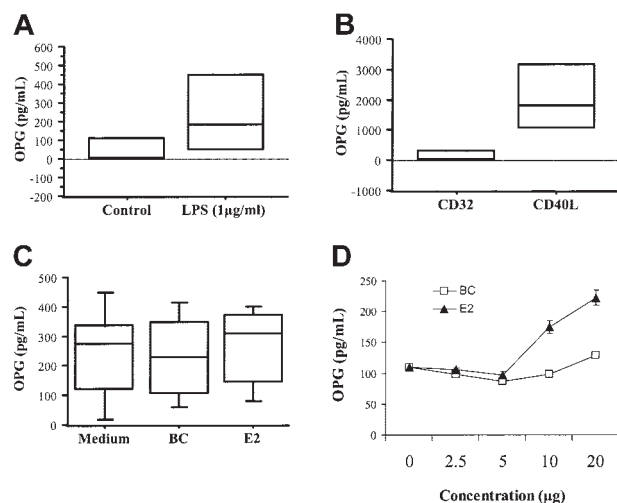


Figure 7. OPG secretion by DCs is enhanced by E_2 , LPS, and CD40 ligation. iDCs were stimulated with or without LPS (1 µg/mL; A) or with CD32- or CD40L-transfected L cells (B) for 48 hours ($n = 4$), or recultured with medium only, BC, or E_2 (20 µg/mL) for 24 hours ($n = 7$; C). OPG was detected in culture supernatants by ELISA. The boxes show the 10th, 25th, 50th (median, central line), 75th, and 90th percentiles of the variable (pg/mL OPG). When iDCs were treated with graded doses of E_2 (▲) or BC (□) for 48 hours, OPG was enhanced in a dose-dependent manner (D). Data shown in panel D are the mean \pm SD of triplicate wells.

studies showing that prostaglandin E₂ (PGE₂) affects DC migration.^{35,36} Both these reports showed that DCs matured with different stimuli, even though they up-regulated CCR7, did not migrate toward CCR7 ligands. However, PGE₂ costimulation provided a signal enabling efficient migration. Thus, PGE₂ and E₂ might exert a similar effect on the migratory behavior of DCs through the activation of a common signal transduction pathway. Both PGE₂ and E₂ can activate the cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) signaling pathway, as well as pathways using calcium as a second messenger⁴⁸; moreover, both these pathways have been implicated in the effect of PGE₂ on DC migration.^{36,49} Our results are the first to show that estrogen affects DC migration and that migration of a myeloid cell type is improved by estrogen. Previous studies reported an inhibitory effect of E₂ on migration of monocytes and macrophages, mainly through the down-regulation of inflammatory chemokines.⁵⁰⁻⁵³ Our findings suggest that E₂ has a different effect on migration and chemokine expression of DCs compared to other myeloid cells. The combined finding that E₂-treated DCs more effectively promote T-cell proliferation and that E₂ facilitates DC migration toward a chemokine, which functions in T cell-rich areas of lymph nodes, suggests that E₂ plays an important role in regulating DC during acquired immune responses.

Using OPG-deficient mice, we found that OPG^{-/-} DCs are more effective at stimulating T cells than OPG^{+/-} DCs.²³ RANK-RANKL interactions increase the ability of DCs to stimulate T cells,³⁸ suggesting that OPG may normally function as a decoy receptor, blocking the interaction between RANK, expressed by DCs, and RANKL, expressed by T cells.²⁴ On the basis of this model we investigated the role of OPG in human DCs. We found

that human DCs can secrete OPG and that this secretion is up-regulated by maturation stimuli, such as LPS and CD40 ligation (Figure 7). Estrogen up-regulates the production of OPG by bone cells,¹⁷ so we tested if E₂ affects OPG secretion by DCs. We found that E₂ does increase OPG secretion by iDCs (Figure 7C-D), but E₂ did not alter LPS-induced increases of OPG. The induction of OPG by LPS and CD40L may influence mature DC survival by blocking the interaction between RANK and RANKL or TNF-related apoptosis-inducing ligand (TRAIL) and its receptors. OPG could have different effects on DCs depending on stage of differentiation/maturation. Thus, it is difficult to designate OPG as being solely proinflammatory or anti-inflammatory.

In conclusion, E₂ affects human DCs in several ways, including promoting the expression of the proinflammatory cytokine IL-6 and the inflammatory chemokines IL-8 and MCP-1. Our results suggest that E₂ may play a role in the induction and sustenance of inflammatory responses. This may explain why in some autoimmune diseases, such as SLE, disease severity can worsen when estrogen levels are high. Furthermore, the enhanced ability of E₂-treated DCs to migrate, induce T-cell proliferation, and induce the modulation of DC-derived Th1/Th2-polarizing cytokines and chemokines suggests that estrogen may regulate the activation and polarization of T-cell responses that could be critical in the progression or remission of autoimmune diseases.

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