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Growth Factors, Cytokines, Cell Cycle Molecules

Regulation of Monocyte Chemoattractant Protein-1 Expression by Tumor Necrosis Factor-α and Interleukin-1β in First Trimester Human Decidual Cells

Implications for Preeclampsia

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The current study describes a statistically significant increase in macrophages (CD68-positive cells) in the decidua of preeclamptic patients. To elucidate the regulation of this monocyte infiltration, expression of monocyte chemoattractant protein-1 (MCP-1) was assessed in leukocyte-free first trimester decidual cells. Confluent decidual cells were primed for 7 days in either estradiol or estradiol plus medroxyprogesterone acetate to mimic the decidualizing steroidal milieu of the luteal phase and early pregnancy. The medium was exchanged for a serum-free defined medium containing corresponding steroids +/− tumor necrosis factor (TNF)-α or interleukin (IL)-1β. After 24 hours, enzyme-linked immunosorbent assay measurements indicated that the addition of medroxyprogesterone acetate did not affect MCP-1 output, whereas 10 ng/ml of TNF-α or IL-1β increased output by 83.5-fold ± 20.6 and 103.1-fold ± 14.7, respectively (mean ± SEM, $n = 8$, $P < 0.05$). Concentration-response comparisons revealed that even 0.01 ng/ml of TNF-α or IL-1β elevated MCP-1 output by more than 15-fold. Western blotting confirmed the enzyme-linked immunosorbent assay results, and quantitative reverse transcriptase-polymerase chain reaction confirmed corresponding effects on MCP-1 mRNA levels. The current study demonstrates that TNF-α and IL-1β enhance MCP-1 in first trimester decidua. This finding suggests a mechanism by which recruitment of excess macrophages to the decidua impairs endovascular trophoblast invasion, the primary placental defect of preeclampsia. ***(Am J Pathol 2006, 168:445–452; DOI: 10.2353/ajpath.2006.050082)***

Interstitial cytotrophoblasts invade the underlying decidua, surround and penetrate spiral arteries and arterioles, and become endovascular cytotrophoblasts that transform the smooth muscle layer and endothelium of these vessels.[1] This process converts small-bore, high-resistance vessels to large-bore, low-resistance vessels that meet the demands of the growing feto-placental unit by increasing maternal blood flow.[1–3] Impaired endovascular trophoblast invasion is the primary placental defect of preeclampsia and fetal intrauterine growth restriction. It leads to inadequate conversion of the uterine arteries and reduced uteroplacental blood flow.[1,3–5] Affecting 3 to 10% of all pregnancies, preeclampsia is a leading cause of maternal and fetal mortality and morbidity throughout the world.[2]

The predominant cell types of first trimester gestational endometrium include decidualized stromal cells as well

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as bone marrow-derived cells of which 70% are natural killer (NK) cells, 20 to 25% are monocyte/macrophages with some T cells and CD1d-restricted NK cells.[6–8] Monocyte/macrophages are concentrated around the implantation site and near trophoblasts.[6,7,9] At the implantation site, apoptosis plays a crucial role in remodeling the decidua.[9] Macrophages indirectly affect trophoblast invasion by removing apoptotic cells.[9] The decidual vessels of preeclamptic patients frequently exhibit acute atherosis, a vasculopathy characterized by an accumulation of lipid-laden macrophages and a mononuclear perivascular infiltrate.[10] In placental bed biopsies, endovascular trophoblast invasion correlates inversely with macrophage numbers, suggesting that macrophages inhibit this process perhaps by inducing the trophoblast to undergo apoptosis.[11,12]

The mechanisms by which macrophages are activated and recruited to the placental bed in early, uncomplicated pregnancy and in preeclampsia are unclear.[3,6] The presence of monocyte chemoattractant protein-1 (MCP-1) in cycling endometrium and first trimester decidua[13–16] suggests that it is involved in this infiltration. Tumor necrosis factor (TNF)-α is a potent enhancer of MCP-1 expression in several cell types.[17–21] Circulating levels and placental expression of TNF-α are significantly elevated in pregnancies complicated by preeclampsia compared with normal pregnancies.[22,23] Moreover, TNF-α is implicated in the inhibition of endovascular trophoblast invasion[12,24] and in promoting endothelial cell activation,[23,25–27] the characteristic placental and systemic vascular pathology of preeclampsia.[28] Other reports indicate that interleukin (IL)-1β, which also induces MCP-1 expression,[21] is involved in the genesis of preeclampsia as well.[29,30] Thus, TNF-α and IL-1β are potential mediators of physiological and pathological MCP-1-mediated decidual macrophage infiltration.

Several lines of evidence support an association between excess decidual macrophage infiltration and failure of endovascular trophoblast invasion.[3,11,12] Paradoxically, there are also reports that macrophage numbers in the decidua are unchanged or decreased in preeclampsia.[31,32] Therefore, we first conducted immunohistochemical staining for the presence of macrophages in decidua obtained from preeclamptic versus unaffected patients. After confirming that preeclamptic placental beds contain excess decidual macrophages, we sought to identify regulators of such macrophage infiltration by assessing the effects of TNF-α and IL-1β on MCP-1 expression in leukocyte-free first trimester decidual cells. The integral role played by progesterone in inducing and maintaining decidualization prompted us to determine whether progestin modulates cytokine effects on MCP-1 expression in the cultured decidual cells.

Materials and Methods

Tissues

Seven placentas were obtained from preeclamptic women, defined as having elevated blood pressure (>140/90 mmHg) and proteinuria (>+1) on two occasions 6 hours apart after 20 weeks of gestation. Seven control placentas were obtained after uncomplicated pregnancies. All cases and controls were at term and delivered by cesarean section. The study was approved by the local ethics committee (University of Siena, Siena, Italy), and informed consent was obtained from all women. Placentas were obtained immediately after delivery and several (10 mm $\times$ 10 mm) full-thickness blocks were cut and either fixed in 10% buffered neutral formalin and embedded in paraffin or frozen in Tissue-Tek OCT (Sakura Finetek, Torrance, CA) medium in liquid nitrogen and stored at −80°C. For each specimen, the block most representative of the maternal decidua was selected for immunohistochemistry.

Decidual specimens from elective terminations between 6 and 12 weeks of gestation were obtained with the approval of the Institutional Review Board of New York University Medical Center-Bellevue Hospital, New York, NY. A small portion of each specimen was formalin-fixed and paraffin-embedded then examined histologically for signs of underlying acute and chronic inflammation. The remainder was used for decidual cell isolation.

Immunohistochemistry

Sections (4 μm) of paraffin-embedded placental tissues were cut, deparaffinized, rehydrated, and washed in Tris-buffered saline [20 mmol/L Tris-HCl, 150 mmol/L NaCl (pH 7.6)]. TBS was used for all subsequent washes and for dilution of the antibody. Antigen retrieval was performed by incubating sections in sodium citrate buffer (10 mmol/L, pH 6.0) in a microwave oven at 750 W for 5 minutes. Sections were subsequently rinsed in 3% hydrogen peroxide to block endogenous peroxidase and incubated for 1 hour at room temperature with a monoclonal antibody against the macrophage marker CD68 (Dakopatts, Carpinteria, CA). Immunostaining was visualized using the avidin-biotin peroxidase complex (Vectastain ABC kit; Vector Laboratories, Burlingame, CA) and 3,3′-diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St. Louis, MO) as chromogen substrate. Light hematoxylin stain was used for nuclear counterstaining. Sections of selected specimens were stained for vimentin with a monoclonal antibody (Dakopatts) following the procedure detailed above. Negative controls for each tissue section were prepared by substituting the primary antibody with the corresponding preimmune serum. The CD68-positive cells in the decidua were evaluated by two independent observers using a semiquantitative method in accordance with the following scoring system: 0, absence of positive cells in the decidua; 1, presence of few isolated cells; 2, numerous isolated cells; 3, numerous positive cells, either isolated and/or grouped in clusters.

Laser Capture Microdissection (LCM)

Serial 4-μm cryostat sections of term placentas were mounted on uncoated glass slides, fixed in 70% ethanol,

and stained with the HistoGene LCM frozen section staining kit (Arcturus, Mountain View, CA). Pure decidual cells were isolated using a PixCell II LCM system equipped with an Olympus microscope (Arcturus). Captured microdissected decidual cells from two different slides were pooled for subsequent analyses.

First Trimester Decidual Cell Cultures

Tissues were minced and digested with 0.1% collagenase type IV, as well as 0.01% DNase in RPMI containing 20 μg/ml penicillin/streptomycin and 1 μl/ml fungizone (Invitrogen, Grand Island, NY) in a 37°C shaking water bath for 30 minutes. After washing with sterile phosphate-buffered saline (PBS) the digestate was washed three times and subjected to consecutive filtration through 100-μm, 70-μm, and 40-μm Millipore filters (Bedford, MA). Cells were then resuspended in RPMI and seeded on polystyrene tissue culture dishes. Cells were harvested using trypsin/ethylenediaminetetraacetic acid and analyzed by flow cytometric analysis with anti-CD45 and anti-CD14 monoclonal antibodies (mAbs) (BD Pharmingen, San Diego, CA) to monitor the presence of leukocytes after each passage. After three to four passages, cell cultures were found to be leukocyte-free (<1%). Cell aliquots were then frozen in fetal calf serum/dimethyl sulfoxide (9:1) (Sigma-Aldrich) and stored in liquid nitrogen.

Thawed cells were incubated in basal medium, a phenol red-free 1:1 (v:v) mix of Dulbecco's modified Eagle's medium (Invitrogen) and Ham's F-12 (Flow Labs, Rockville, MD), with 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml fungizone supplemented with 10% charcoal-stripped calf serum (BMS). After two more passages, confluent cultures were incubated in parallel in BMS containing either 10^{-8} mol/L estradiol (E_2) or E_2 plus 10^{-7} mol/L medroxyprogesterone acetate (MPA) (Sigma-Aldrich). After 7 days, the cultures were washed twice with Hanks' balanced salt solution to remove residual serum. The cultures were then switched to a defined medium (DM) consisting of basal medium plus ITS+ (Collaborative Research, Waltham, MA), 5 μmol/L $FeSO_4$, 50 μmol/L $ZnSO_4$, 1 nmol/L $CuSO_4$, 20 nmol/L Na_2SeO_3, and trace elements (Invitrogen) as well as 50 μg/ml of ascorbic acid (Sigma-Aldrich) and 50 ng/ml of epidermal growth factor (Becton-Dickinson, Bedford, MA) with either vehicle control (0.1% ethanol) or steroids added +/− IL-1β or TNF-α (R&D Systems, Minneapolis, MN). After the test period, cells were harvested by scraping into ice-cold PBS, pelleted, and extracted in ice-cold lysis buffer. Conditioned medium supernatants and cell lysates were stored at −70°C.

Biochemical Assays

Total cell protein levels were measured by the Bio-Rad assay (Bio-Rad Laboratories, Inc., Hercules, CA). A commercial enzyme-linked immunosorbent assay (ELISA) kit was used to measure immunoreactive levels of MCP-1 in the cell-conditioned medium according to instructions provided by the manufacturer (R&D Systems). The ELISA assay has a sensitivity of 5.0 pg/ml, and intra- and inter-assay coefficients of variation of 5.0% and 5.1%, respectively.

Western blot analysis was conducted on supernatants of conditioned medium concentrated using Microcon filter devices (Millipore) and then diluted 1:1 in reducing sample buffer composed of Laemmli sample buffer and 2-mercaptoethanol (Bio-Rad) and boiled for 3 minutes. The prepared media was subjected to electrophoresis on a 10 to 20% sodium dodecyl sulfate-polyacrylamide linear gradient gel (Bio-Rad). The gel was electroblotted onto a 0.2-μm nitrocellulose membrane (Bio-Rad). After transfer, the membrane was blocked overnight in PBS with 4% bovine serum albumin and then incubated for 2 hours in 1.5 μg/ml of a mouse anti-human MCP-1 monoclonal antibody (R&D Systems) diluted in PBS with 1% casein. Membranes were rinsed in PBS and 0.2% Tween 20 before and after incubation with horseradish peroxidase-conjugated anti-mouse IgG (ICN Biomedicals, Aurora, OH). Chemiluminescence was detected with ECL reagents (Perkin Elmer Life Sciences, Boston, MA) and audioradiography film (Amersham Pharmacia, Buckinghamshire, UK) according to the instructions provided by the manufacturer.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and Real-Time Quantitative RT-PCR

Total RNA of cultured cells was extracted with Tri Reagent (Sigma-Aldrich). Total RNA from the LCM-captured cells was extracted using the RNeasy micro kit (Qiagen Inc., Valencia, CA) according to the manufacturer's recommendation. RNA was subjected to RT-PCR with a kit from Invitrogen (Carlsbad, CA) on an Eppendorf Mastercycler (Eppendorf, Westbury, NY). For each RNA specimen, a negative control was prepared by omitting the reverse transcriptase.

To perform quantitative real-time RT-PCR, reverse transcription was initially performed with AMV reverse transcriptase (Invitrogen). A quantitative standard curve was created between 500 pg to 250 ng of cDNA with a Roche Light Cycler (Roche, Indianapolis, IN) by monitoring increasing fluorescence of PCR products during amplification. On establishing the standard curve, quantitation of the unknowns was determined with the Roche Light Cycler and adjusted to the quantitative expression of β-actin from the corresponding unknowns. Melting curve analysis determined the specificity of the amplified products and the absence of primer-dimer formation. All products obtained yielded correct melting temperatures. The following primers were synthesized and gel-purified at the Yale DNA Synthesis Laboratory, Critical Technologies. For MCP-1 mRNA detection in LCM-isolated cells, sense and anti-sense primers were 5′-CCCCAGTCACCTGCTGTTAT-3′ and 5′-TGGAATCCTGAACCCACTTC-3′, respectively. The expected size of the amplified

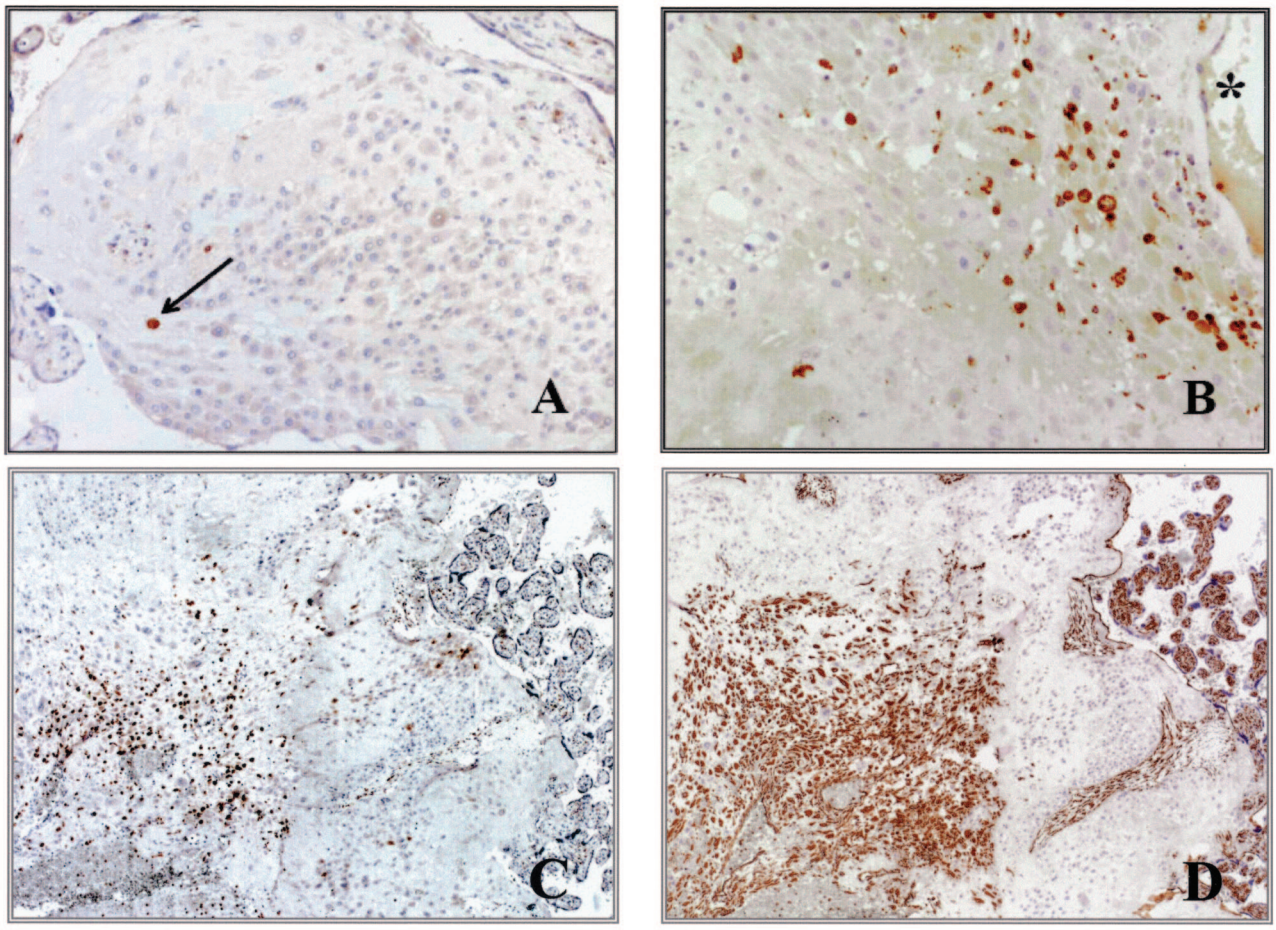

Figure 1. Immunohistochemical analysis of decidual monocyte/macrophage infiltration in preeclampsia. **A:** Control decidua. Isolated CD68$^+$ cell (indicated by the **arrow**). **B:** Preeclamptic decidua. Numerous CD68$^+$ cells are present in this field. The **asterisk** indicates a maternal vessel. Statistical analysis showed that CD68$^+$ score was significantly higher in cases compared to controls ($P = 0.01$). **C** and **D:** Serial sections stained for CD68 (**C**) and vimentin (**D**). Original magnifications: ×200 (**A**, **B**); ×100 (**C**, **D**).

fragment was 171 bp. For MCP-1 mRNA detection in decidual cell cultures the sense primer was 5′-GCT-CAGCCAGATGCAA-3′ and the anti-sense primer was 5′-GTCCAGGTGGTCCATG-3′. The β-actin sense and anti-sense primers were 5′-CGTACCACTGGCATCGT-GAT-3′ and 5′-GTGTTGGCGTACAGGTCTTTG-3′, respectively. The expected sizes of the amplified fragments for MCP-1 and β-actin mRNA were 452 and 459 bp, respectively.

Statistical Analysis

Comparisons of control and the various treatment groups were performed using the Kruskal-Wallis analysis of variance on ranks test followed by the Student-Newman-Keuls post hoc test with P value <0.05 representing statistical significance. For immunohistochemistry, the intensity of staining in control versus preeclampsia cases was compared using the χ^2 test, with significance set at a probability value of <0.05.

Results

Presence of CD68$^+$ Cells and MCP-1 mRNA in the Decidua

An increase in the numbers of CD68$^+$ cells was observed in the decidua of preeclamptic mothers, compared to that of normal term pregnancies (Figure 1, A and B). Specifically, four control samples showed a complete absence of CD68$^+$ cells in the decidua, two displayed sparsely isolated cells and only one specimen exhibiting numerous CD68$^+$ cells. By contrast, each specimen of decidua from preeclamptic placentas displayed numerous immunoreactive cells. In three of the cases, the CD68$^+$ cells were focally clustered. Statistical analysis showed that there was a difference in the distribution of case and control specimens such that a higher proportion of decidua from preeclamptic placentas were present in categories with a higher number of CD68$^+$ cells ($\chi^2 = 10.8$; df = 3; $P = 0.01$). The localization of CD68$^+$ cells in decidua was confirmed by staining serial sections for CD68 and the decidual cell marker vimentin (Figure 1, C

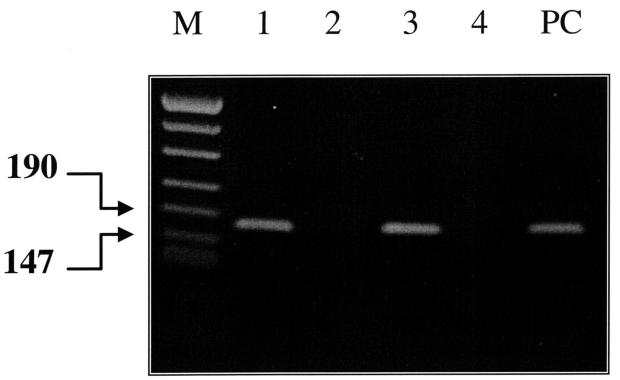

Figure 2. Reverse transcriptase-PCR analysis of MCP-1 mRNA levels in microdissected decidua tissues. Total RNA of two decidua specimens (**lanes 1** and **3**) was reverse-transcribed and amplified in the presence of MCP-1 primers. For each specimen a negative control lacking the reverse transcriptase was amplified and loaded onto the gel (**lanes 2** and **4**). Placental RNA was used as a positive control (PC). Forty-five cycles were run for each PCR. The size of the molecular weight makers (**lane M**; bp) is indicated.

and D). To demonstrate the expression of MCP-1 *in vivo*, RT-PCR analysis was performed on LCM-isolated decidual cells. As shown in Figure 2, a band corresponding in size to the MCP-1 mRNA product was obtained from the cDNA of the two specimens tested.

Regulation of MCP-1 Protein Expression in Decidual Cells

Because circulating levels of both E_2 and progesterone rise during the first trimester, E_2 was used as the control incubation for evaluating the effects of the progestin MPA. Figure 3 indicates that in cultures maintained in E_2 alone, 10 ng/ml of TNF-α and IL-1β increased net immunoreactive MCP-1 outputs by 70.4-fold ± 16.4 and 149.6-fold ± 50.3, respectively (mean ± SEM, $n = 7$, $P < 0.05$). Specifically, incubation with TNF-α and IL-1β elevated MCP-1 output from 7.3 ± 2.4 pg/ml/μg protein in control cultures to 449.5 ± 174.6 and 631.3 ± 180.5 pg/ml/μg protein, respectively ($P < 0.05$). Similarly, in E_2 plus MPA-treated cultures MCP-1 output was increased by 83.5-fold ± 20.6 and 103.1-fold ± 14.7, respectively with values increasing from basal levels of 6.1 ± 1.6 pg/ml/μg protein to 510.1 ± 172.4 and 576.0 ± 141.1 pg/ml/μg protein by TNF-α and IL-1β, respectively ($P < 0.05$). In contrast to the marked elevation of MCP-1 output elicited by the cytokines, the addition of MPA with E_2 did not alter levels compared to cultures treated with E_2 alone and did not alter the response to the cytokines.

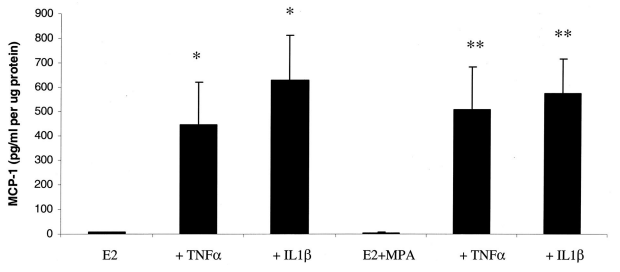

Figure 3. Effects of E_2, MPA, TNF-α, or IL-1β on MCP-1 output by first trimester decidual cell monolayers. Confluent passaged, leukocyte-free decidual cells were incubated for 7 days in 10^{-8} mol/L E_2 or E_2 plus 10^{-7} mol/L MPA then switched to DM with corresponding steroid(s) +/− 10 ng/ml of TNF-α or IL-1β for 24 hours. MCP-1 levels were measured by ELISA in conditioned DM and normalized to cell protein (details in Materials and Methods; $n = 7$, mean ± SEM). *Versus E_2 ($P < 0.05$); **versus E_2 plus MPA ($P < 0.05$).

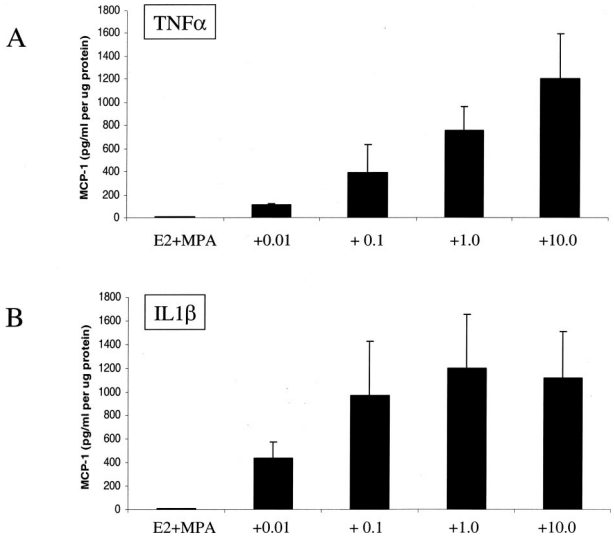

Figure 4. Concentration-response effects of TNF-α or IL-1β on MCP-1 output by E_2 plus MPA-treated first trimester decidual cell monolayers. Confluent passaged, leukocyte-free decidual cells were incubated for 7 days in 10^{-8} mol/L E_2 plus 10^{-7} mol/L MPA, and then switched to DM with E_2 plus 10^{-7} mol/L MPA alone or with a range of concentrations (ng/ml) of TNF-α (**A**) or IL-1β (**B**) for 24 hours. MCP-1 levels were measured by ELISA in conditioned DM and normalized to cell protein (mean ± SD, $n = 2$).

Given the absence of a steroid effect, further evaluation of the effects of TNF-α and IL-1β on MCP-1 expression were exclusively performed on decidual cells primed with E_2 plus MPA. The dose responses of MCP-1 to the cytokines at concentrations of 0.01 to 10 ng/ml are shown in Figure 4. Figure 4A indicates that the effects of TNF-α on MCP-1 output rose throughout the entire concentration range whereas Figure 4B indicates that MCP-1 output for IL-1β appeared to peak at 1.0 ng/ml.

The Western blot depicted in Figure 5 indicates that conditioned media contained a doublet at the molecular weight of MCP-1 (9 kd for the monomeric band and a band corresponding to slightly higher molecular weight of the glycosylated form). Figure 5 also confirms the ELISA results showing that MCP-1 was markedly enhanced by TNF-α and IL-1β but unaffected by the presence of E_2 plus MPA compared with E_2 alone. Figure 6

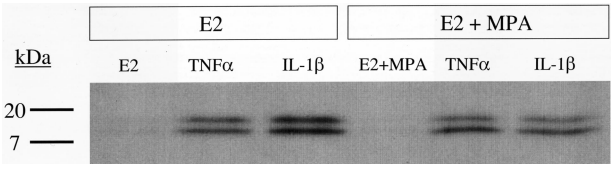

Figure 5. Western blot of effects of TNF-α or IL-1β on MCP-1 output by E_2 and E_2 plus MPA-treated decidual cell monolayers. Confluent, passaged, leukocyte-free decidual cells were incubated for 7 days in 10^{-8} mol/L E_2 or 10^{-8} mol/L E_2 plus 10^{-7} mol/L MPA. The medium was exchanged for DM with the corresponding E_2 or E_2 plus MPA alone or with 1 ng/nl of TNF-α or IL-1β. After 24 hours of incubation, concentrated conditioned DM was subjected to Western blotting.

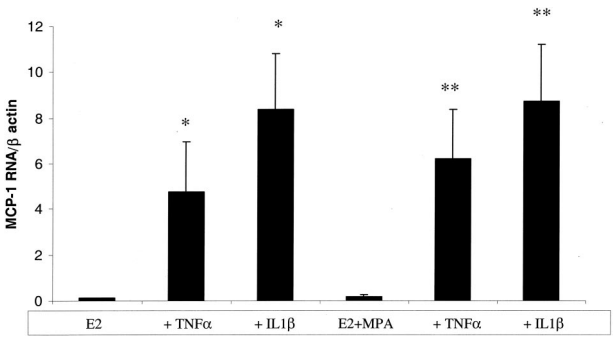

Figure 6. Quantitative RT-PCR of effects of TNF-α or IL-1β on MCP-1 mRNA levels by E_2 and E_2 plus MPA-treated decidual cell monolayers. Confluent passaged, leukocyte-free decidual cells were incubated for 7 days in 10^{-8} mol/L E_2 or 10^{-8} mol/L E_2 plus 10^{-7} mol/L MPA, and then switched to DM with E_2 or E_2 plus MPA alone or with 1 ng/nl of TNF-α or IL-1β for 5 hours. Ordinate: MCP-1 mRNA/β-actin mRNA (details in Materials and Methods; $n = 4$, mean ± SEM). *Versus E_2 ($P < 0.05$); **versus E_2 plus MPA ($P < 0.05$).

indicates that changes in the pattern of MCP-1 mRNA corresponded to that of the MCP-1 protein. Thus, TNF-α and IL-1β also induced multifold increases in MCP-1 mRNA levels whether added with E_2 or with E_2 plus MPA. In contrast, MCP-1 mRNA levels were refractory to MPA.

Discussion

The documented presence of MCP-1, the primary monocyte/macrophage chemoattractant, in early pregnant decidua[15,16] suggests its likely involvement in monocyte and macrophage recruitment. The current study found that TNF-α and IL-1β markedly elevated MCP-1 mRNA and protein levels in decidual cell monolayers and that this up-regulation was unaffected by MPA. This latter observation contrasts with that of Kelly and colleagues[33] who reported that expression of MCP-1 was under progestin inhibition in an, albeit, mixed population of choriodecidual cells. Previously, we demonstrated that MPA markedly elevated type-1 plasminogen activator inhibitor (PAI-1) and inhibited stromelysin-1 expression in first trimester decidual cells.[34,35] Despite retention of progestin responsiveness, MPA did not affect MCP-1 expression in decidual cell cultures in our present study.

We hypothesize that increased MCP-1 expression in decidualized stromal cells in response to IL-1β may be involved in recruiting macrophages into the postimplantational decidua. Follicular and peri-ovulatory human endometria contain an IL-1β receptor antagonist (IL-1ra) that binds to the IL-1 receptor with high affinity to prevent IL-1 binding and is present at levels that are 10- to 30-fold higher than IL-1β.[6,36] A decline in glandular levels of IL-1ra during the luteal phase coincides with the "window of implantation."[37] Thus, although there is conflicting information as to whether the IL-receptor plays a crucial role in implantation in the mouse,[38,39] appropriate IL-1β levels may promote human implantation. Extrapolation of our *in vitro* results to the decidua *in vivo* suggests that the induction of MCP-1 expression by IL-1β may mediate a physiological influx of monocytes during early gestation that results in their proximity to trophoblast at the implantation site.[6,7,9] This IL-1β-mediated action on MCP-1 expression may be transient because of trophoblast release of IL-1ra.[6,40]

The current study establishes that preeclampsia is associated with a statistically significant increase of decidual $CD68^+$ cells, thereby supporting the hypothesis that macrophages are involved in the pathogenesis of this condition. Moreover, our observations suggest pivotal roles for TNF-α-induced MCP-1 by the decidua in the persistence of macrophages at the implantation site and in impaired endovascular trophoblast invasion during preeclampsia. *In vitro* studies revealed that apoptosis of extravillous trophoblasts was induced by macrophage-associated TNF-α and blocked by an antibody against the TNF-α receptor.[12] TNF-α also directly inhibits trophoblast migration on collagen I and invasion of Matrigel.[24] This process may be mediated through enhanced PAI-1 expression because function-perturbing PAI-1 antibodies restored trophoblast migration and invasion.[24]

The source of excess decidual TNF-α in preeclampsia remains unclear. Despite elevated plasma levels of TNF-α, there is conflicting evidence whether TNF-α expression is also increased in preeclamptic placentas.[22,40] The demonstration that hypoxia-reoxygenation, a likely consequence of impaired endovascular invasion, enhances output of TNF-α in human placental tissues[23] may provide insight into this question. Decidual monocytes and macrophages are also a rich source of TNF-α.[41] Finally, increased expression of TNF-α mRNA was observed in circulating leukocytes derived from preeclamptic compared with normal pregnant women.[42,43]

Flow cytometry demonstrated elevated ratios of Th1 (proinflammatory):Th2 (anti-inflammatory) immune cells as well as persistence of the Th1 immune response in preeclamptic patients.[44–46] The association of this response with excess systemic and local TNF-α and IL-1β expression is consistent with the following model of the early pathogenesis of preeclampsia. Thus, excess decidual production of IL-1β overwhelms trophoblast IL-1ra levels. This leads to enhanced decidual MCP-1 production and the consequent influx and activation of macrophages. The release of TNF-α by the latter would recruit additional macrophages and direct toxic effects at endovascular trophoblast invasion. The resultant hypoxia would sustain local overexpression of TNF-α contributing to maternal systemic endothelial cell activation. The recent demonstration by Heine and colleagues[47] of a specific association between *Chlamydia pneumoniae* infection and the onset of preeclampsia suggests that underlying infection may be a stimulus for supraphysiological IL-1β levels leading to preeclampsia. Subsets of women may develop preeclampsia related to other, perhaps subclinical, infections that are reflected in elevated IL-1β expression. Finally, a growing body of evidence extends the potential between underlying infection and impaired trophoblast invasion to include common viral infections of cyto- and extravillous trophoblasts.[48]

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