Temporal Changes in Myeloid Cells in the Cervix during Pregnancy and Parturition

Brenda C. Timmons, Anna-Marie Fairhurst and Mala S. Mahendroo

J Immunol 2009; 182:2700-2707; doi: 10.4049/jimmunol.0803138
http://www.jimmunol.org/content/182/5/2700

References
This article cites 50 articles, 13 of which you can access for free at:
http://www.jimmunol.org/content/182/5/2700.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Preterm birth occurs at a rate of 12.7% in the U.S. and is the primary cause of fetal morbidity in the first year of life as well as the cause of later health problems. Elucidation of mechanisms controlling cervical remodeling is critical for development of therapies to reduce the incidence of prematurity. The cervical extracellular matrix must be disorganized during labor to allow birth, followed by a rapid repair postpartum. Leukocytes infiltrate the cervix before and after birth and are proposed to regulate matrix remodeling during cervical ripening via release of proteolytic enzymes. In the current study, flow cytometry and cell sorting were used to determine the role of immune cells in cervical matrix remodeling before, during, and after parturition. Markers of myeloid cell differentiation and activation were assessed to define phenotype and function. Tissue monocytes and eosinophils increased in the cervix before birth in a progesterone-regulated fashion, whereas macrophage numbers were unchanged. Neutrophils increased in the postpartum period. Increased mRNA expression of Csf1r and markers of alternatively activated M2 macrophages during labor or shortly postpartum suggest a function of M2 macrophages in postpartum tissue repair. Changes in cervical myeloid cell numbers are not reflected in the peripheral blood. These data along with our previous studies suggest that myeloid-derived cells do not orchestrate processes required for initiation of cervical ripening before birth. Additionally, macrophages with diverse phenotypes (M1 and M2) are present in the cervix and are most likely involved in the postpartum repair of tissue. The Journal of Immunology, 2009, 182: 2700–2707.
activity of collagenase, a proteolytic enzyme made by leukocytes, is not increased in the term cervix of numerous species (25, 26). Moreover, exogenous collagenase treatment of rat cervices does not replicate the natural remodeling process (27).

To better define the role of myeloid cells at each phase of cervical remodeling before, during, and after birth, we have measured myeloid infiltration and activation in the cervix during softening, ripening, and PP phases. Using multiparameter flow cytometry and cell sorting, the expression of multiple markers was analyzed simultaneously on mouse cervical immune cell populations during pregnancy, parturition, and PP. We demonstrate that eosinophils are increased in numbers at the time of labor. In addition, a progesterone-regulated monocyte infiltration occurs in the cervix during ripening, and these cells are phenotypically distinct from the resident macrophage population. We also provide evidence that macrophages with phenotypes similar to classically activated proinflammatory M1 macrophages and alternatively activated M2 macrophages are both present in the cervix shortly after birth. These studies support a role of eosinophils in the final stages of labor or PP, and they suggest the importance of macrophages in the PP phase of remodeling in clean up of the disorganized matrix and in the suppression of uncontrolled tissue damage, thus promoting rapid and appropriate repair of the cervix back to the nonpregnant state.

Materials and Methods

Mice

Steroid 5α-reductase type 1-deficient mice (Srd5a1−/−) were generated and genotyped, as described previously (28). Timed pregnant NIH Swiss (Harlan) and HSD: ICR (CD-1) (Harlan) were obtained from The Jackson Laboratory. C57BL/6J/129 SveB timed matings were conducted in our colony by housing one male with four females in a cage from 5:00 p.m. to 8:00 a.m. Females were checked at 8:00 a.m. for vaginal plugs. Plug day was determined as day 0, and birth occurred in the early hours on day 19. All studies were conducted in accordance with the standards of humane animal care described in the National Institutes of Health Guide for the Care and Use of Laboratory Animals using protocols approved by an institutional animal care and research advisory committee.

FIGURE 1. Cervical tissue monocytes, but not neutrophils, increase before parturition. Cervical suspensions were stained with anti-CD45, anti-Gr1, and anti-Neu 7/4. A, Leukocytes were gated on CD45 and further analyzed on the Neu 7/4 vs Gr1 dot plot. Monocytes were identified as Neu 7/4−/+ and Gr1 low to intermediate. Neutrophils were defined as Neu 7/4−/−, Gr1−/+. These populations were sorted, cytopsined onto slides, and stained to visualize cell morphology. Bar of measure represents 20 μm. B, Neutrophils are present before cervical ripening, but do not increase significantly until PP. Monocytes significantly increase by late on gestation day 18 and remain high through PP. Data represent mean ± SEM of 6–10 cervices, as indicated. *, p ≤ 0.05 compared with day 15. IL, Indicates in labor samples.
through a 23-gauge needle, filtered with 100 µm Nitex (SEFAR), and pelleted by centrifugation (500 \( g \), for 5 min at 4°C).

**Staining procedure**

One hundred microliters of heparinized whole blood was pelleted in 96-well polypropylene plates at 1900 rpm for 2 min at 4°C. Each sample was stained with 100 µl of diluted Abs for 30 min on ice, as described below. Cervical cells were stained in 96-well polypropylene plates (Greiner Bio-One). Dispersed cells from each cervix were pipetted into separate wells, and the plate was centrifuged (600 \( g \) for 1 min at 4°C). Cells and reagents were kept on ice for the remainder of the experiment. Cell suspensions were washed with staining buffer and suspended in Fc block (mAb 24G2; BD Biosciences) together with a combination of up to six directly conjugated fluorescent Abs and one biotinylated Ab, as described previously (29) (neutrophil (Neu) 7/4-PE and Neu 7/4-biotin (Serotec); F4/80-allophycocyanin, CD45-PE-Cy7, CD11b-Pacific Blue, CD4-PE, CD8-PECy5, CD3-Pacific Blue, and/or CD19-biotin (eBiosciences); and Gr-1 (Ly6C/Ly6G)-allophycocyanin-Cy7, Siglec F- PE, and Ly6G-FITC (BD Biosciences)). Cells were incubated for 30 min in the dark on ice. Cells were washed three times in staining buffer, and then

**FIGURE 2.** Eosinophils are increased during labor, whereas macrophage numbers do not significantly change during pregnancy or PP. Cervical suspensions were stained with anti-CD45, F4/80, Siglec-F, Ly6G, Gr1, and Neu7/4. A. Leukocytes were gated through CD45. Macrophages were defined as F4/80\(^{POS}\) and Neu 7/4\(^{LO} \). B. Population defined as F4/80\(^{POS}\), Neu 7/4\(^{LOW} \) cells (green) strongly express the eosinophil-specific marker, Siglec-F. C. Gating on the eosinophil population and comparing Neu 7/4 vs Gr1 reveals that the eosinophils fall on a different location on the dot plot as compared with neutrophils (blue), suggesting they are distinct populations. D. Further confirmation that eosinophil population (Ly6G\(^{POS}\)) is distinct from neutrophil (Ly6G\(^{POS}\)) population. E. Macrophage numbers do not change during gestation. Eosinophils appear to increase significantly from day 15 to in labor (IL). Data represents mean ± SEM of 6–10 cervices, as indicated. *, \( p < 0.05 \) compared with day 15.
incubated in streptavidin QDot655 (Invitrogen) at 1/600 dilution of stock for 30 min in the dark. RBC lysis and cellular fixation was completed using BD FACs lysing solution (BD Biocytex). Cells were washed as above, and samples were maintained in 1% paraformaldehyde, run within 24 h on a BD Biocytex LSRII flow cytometer using BD FACSDiva (BD Biocytex) software, and analyzed with FloJo 7.1 analysis software (Tree Star). Cells were sorted on a Beckman Coulter MoFlow cell sorter (Beckman Coulter), cyto spun onto slides, and stained with a HEMA 3 stain set (Fisher Scientific), according to manufacturer’s protocols.

Quantitative real-time PCR
Total RNA was extracted from frozen mouse tissue using RNA Stat 60 (Tel-Test B). Subsequently, total RNA was treated with DNase I to remove any genomic DNA using DNA-Free (Ambion). cDNA synthesis was performed, per manufacturer’s protocols (TaqMan cDNA synthesis kit; Applied Biosystems). Quantitative real-time PCR was performed using SYBR Green and a PRISM7900HT sequence detection system (Applied Biosystems). Aliquots (20 ng) of cDNA were used for each quantitative PCR, and each reaction was run in triplicate. Each gene was normalized to the expression of the housekeeping gene cyclophilin B, and relative expression was calculated using the average of the day 18.75 cervices as the external calibrator in the ΔΔCt method, as described in User Bulletin No. 2 (Applied Biosystems). Data are presented as the average relative gene expression ± SEM.

ELISA
Frozen tissues (three to six animals for each time point) were homogenized in 0.01 M PBS with 10% protease inhibitor (Sigma-Aldrich). The samples were then sonicated on ice for 2 × 5 s and centrifuged at 15,000 × g, 4°C, for 20 min. IL-10, M-CSF, IL-13 (R&D Systems), and IL-4 (eBiosciences) were analyzed in supernatants by commercially available ELISA kits and used according to manufacturer’s instruction. Absorbance was read at 450 nm using a Safire 2 microplate reader (Tecan). Tissue cytokine levels were expressed relative to total protein, as determined by the bi- chonic acid assay (Thermo Scientific).

Protein blotting
Mouse cervical extracts were prepared by homogenization in 0.01 M PBS containing 3% protease inhibitor (catalog no. 2714; Sigma-Aldrich). Forty micrograms of protein was dissolved in 5× Laemmli buffer (Bio-Rad), boiled for 5 min, and run on a 10% reducing Tris-HCl gel (Bio-Rad) along with protein size standards (Precision Plus Protein Kaleidoscope; Bio-Rad). Proteins were transferred to nitrocellulose membrane (Pall). Nonspecific Ab binding was blocked by an overnight incubation with TBST (10 mm Tris/HCl, pH 7.5, 150 mM NaCl, and 0.05% Tween 20) containing 3% nonfat dry milk. Blots were then incubated for 2 h with the primary Ab, YM1 (1:1000; StemCell Technologies) in blocking solution, washed in TBST, incubated with HRP-labeled anti-rabbit IgG (1:10,000; Jackson ImmunoResearch Laboratories) for 45 min, and washed again in TBST. Chemiluminescence detection was performed using the ECL Western Blotting Analysis System (GE Healthcare). A rabbit polyclonal anti-calnexin Ab (1:1000; Santa Cruz Biotechnology) was used as a loading control. This experiment was conducted using protein extracts from three to nine animals per time point.

Statistics
Data were analyzed using one-way ANOVA with pairwise multiple comparisons performed with Tukey test for data normally distributed. Nonparametric methods were used for nonnormal data. This included the Kruskal-Wallis test for one-way ANOVA with multiple comparisons using Dunn’s method. Values of p < 0.05 are considered statistically significant. Data are displayed as mean plus SEM. Data analyses were performed using Sigma Stat V2.03 (SPSS).

Results

Monocyte and eosinophil, but not neutrophil or macrophage numbers are increased before parturition
Approximately 10–20% of cervical cells expressed the pan leukocyte marker, CD45 (data not shown). The population of cervical cells that expressed CD45 was assessed for Gr1, Ly6G, Neu 7/4, F4/80, CD11b, CD11c, and Siglec-F expression to distinguish tissue monocytes, neutrophils, macrophages, eosinophils, and dendritic cells. The identities of some populations were further confirmed by visualization of cell morphology after cell sorting and staining with a modified Wright’s stain. Neutrophils, defined as Gr1<sup>+</sup>, Neu 7/4<sup>+</sup>, were observed in the cervix before cervical ripening (gestation day 15) and did not significantly increase until 2–4 h PP (Fig. 1) (29–31). Monocytes, defined as Gr1<sup>−/−</sup> and Neu 7/4<sup>+</sup>, significantly increased in numbers between day 15 and late on gestation day 18 (30, 31). The high numbers of monocytes remained throughout labor and PP (Fig. 1). Macrophages, defined as F4/80<sup>+</sup>, Neu 7/4<sup>−</sup> were present in the cervix before (day 15) and during (day 18.75) cervical ripening and 2–4 h PP, with no significant change in numbers during this period (Fig. 2) (30, 31). Cell morphology of all three myeloid cell types was consistent with phenotypes defined using expression markers in flow cytometry (Figs. 1 and 2). In addition to neutrophils, monocytes, and macrophages, another distinct myeloid population was identified as Neu 7/4<sup>low</sup>, F4/80<sup>low</sup> (shown in Fig. 2A). Based on cell morphology and Siglec-F<sup>+</sup> expression (Fig. 2, A and B), the cells were identified as eosinophils (32–34). These cells were recruited to the cervix by day 18.75 with a statistically significant increase in labor (IL) (Fig. 2E). The eosinophil subset was further shown to be distinct from neutrophils based on both low Neu 7/4<sup>+</sup> and Gr1<sup>+</sup>. The eosinophil subset was further shown to be distinct from neutrophils based on both low Neu 7/4<sup>+</sup> and Gr1<sup>+</sup>. The eosinophil subset was further shown to be distinct from neutrophils based on both low Neu 7/4<sup>+</sup> and Gr1<sup>+</sup>. The eosinophil subset was further shown to be distinct from neutrophils based on both low Neu 7/4<sup>+</sup> and Gr1<sup>+</sup>. The eosinophil subset was further shown to be distinct from neutrophils based on both low Neu 7/4<sup>+</sup> and Gr1<sup>+</sup>. The eosinophil subset was further shown to be distinct from neutrophils based on both low Neu 7/4<sup>+</sup> and Gr1<sup>+</sup>.

Recruitment of monocytes is dependent on steroid hormone environment
Loss of progesterone hormone action precedes onset of parturition in humans and numerous animal species (36, 37). Our previous studies have described a lack of migration of Neu 7/4<sup>+</sup> cells into the cervical stroma of a cervical ripening defective model, the Srd5a1<sup>−/−</sup> mouse, in which cervical tissue progesterone levels remain high in the Srd5a1<sup>−/−</sup> mouse at day 18.75. All myeloid populations, with the exception of macrophages, were decreased in number in the knockout mice. B, WT animals at gestation day 15 were treated with progesterone receptor antagonist ZK98299 (ZK) for 13 h before tissue harvest. This led to a premature increase in monocytes and eosinophils. Data represent the mean ± SEM of four to seven cervices, as indicated. *, p ≤ 0.05 compared with day 15.
significantly lower in the knockout mouse cervix at day 18.75 when analyzed by flow cytometry, although macrophage numbers were unchanged (Fig. 3A). To further demonstrate the effect of progesterone on leukocyte recruitment, the progesterone receptor antagonist, ZK98299 (Onapristone; Shering), was administered to pregnant wild-type (WT) mice on gestation day 15 and myeloid cell populations were identified by flow cytometry after 13 h of treatment. Blocking progesterone action with agonist treatment causes premature cervical ripening in human and other species, and the agonist ZK98299 has previously been shown to cause a premature infiltration of leukocytes into the cervical stroma (24, 37). In the current study, ZK98299 treatment resulted in a significant increase in both monocytes and eosinophils (Fig. 3B). Taken together, these studies suggest that progesterone withdrawal, which normally begins before cervical ripening, is required for appropriate migration of specific myeloid cell populations into the cervix.

mRNA expression of genes associated with macrophage differentiation and activation is up-regulated after parturition

A decline in progesterone action is required for the migration of monocytes and eosinophils into the cervical matrix during cervical ripening. Identification of the timing and regulation of macrophage differentiation before or after birth is necessary to understand the role of these inflammatory cells in cervical ripening/dilation and/or in the PP tissue repair of the cervix. CSF1 expressed in various cell types such as fibroblasts and endothelial cells, and its receptor, Csf1r, expressed by all mononuclear phagocytes and some epithelial cells, is an important factor in normal macrophage function in the uterus (38–41). CSF1 protein and Csf1r mRNA were measured by ELISA or quantitative real-time PCR was performed to determine the change in CSF1 protein and Csf1r mRNA expression, respectively. CSF1 protein expression was highest on gestation day 15 and decreased later in gestation and PP. CSF1 receptor (Csf1r) expression increased PP with significant up-regulation by 10–12 h PP. Data represent the mean ± SEM of five to eight cervices, as indicated. Significance (*p < 0.05) is indicated by an asterisk when compared with day 15; a, compared with days 15, 18.75, and in labor (IL), and b, compared with 2–4 h PP.

FIGURE 5. Quantitative real-time PCR analysis of genes expressed by alternatively activated (M2) macrophages. Expression of IL-13ra1, Arg1, and IL-1ra was significantly up-regulated PP. YM1 expression significantly increased in labor (IL) and remained elevated up to 10 h PP. TGF-β (TGFb) had variable expression late on day 18, and a significant increase was observed between day 15 and term. NIL. TGF-β expression remained high through PP. Data represent the mean ± SEM of five to seven cervices, as indicated. Significance (*p < 0.05) is indicated by: a, compared with day 15; b, compared with day 18.75; c, compared with term, NIL (d19 NIL); and d, in labor (IL).
FIGURE 6. Temporal changes in protein expression of YM1. Western blot containing 40 μg of total cervical protein from gestation day 15, 18.75, in labor (IL), 2–4 h, and 1 day PP was probed with anti-YM1 Ab (upper panel). YM1 protein migrates at 40 kDa. To ensure equal loading, the blot was stripped and probed with anti-calnexin Ab (bottom panel). Calnexin protein migrates at 90 kDa.

ELISA and quantitative real-time PCR, respectively, to determine expression in cervical tissue during pregnancy, parturition, and PP repair (Fig. 4). CSF1 expression was highest at gestation day 15 and then declined during cervical ripening (day 18.75) and PP. The receptor was expressed at all time points, although maximal expression was observed 2–4 h PP. Significant up-regulation of Csf1r by 2 h PP suggests an increase in the differentiation commitment of the monocytes to macrophages pathway occurs once labor has begun and/or during PP recovery period. Transcripts encoding Csf2 were low to undetectable in the cervix in contrast to Csf1 (data not shown).

Gene expression patterns support a role of alternatively activated M2-polarized macrophages during PP cervical tissue repair

As described in Fig. 2, macrophage numbers are unchanged during cervical ripening, parturition, and PP. Macrophage phenotypes are heterogeneous and are influenced by changes in the tissue microenvironment (31, 42). Polarization of macrophages into either M1 or M2 subpopulations within a microenvironment can influence their function. In previous studies from our laboratory, we observed an up-regulation of genes associated with classically activated (M1) macrophages after birth during PP repair of the cervix, such as Il-1α, Tnfa, and Mcp1 (24). In the current study, we sought to determine whether the increased tissue monocyte population in the cervix during ripening is followed by an up-regulation of genes associated with alternatively activated M2 macrophages, which, along with M1 macrophage subpopulations, may also contribute to the tissue repair phase of remodeling. M2 macrophages express arginase 1 (Arg1), chitinase 3-like 3 (Chi3l3, Ym1), IL-1 receptor antagonist (II-1ra), IL-13 receptor alpha 1 (II-13ral), and TGF-β (Tgfb). Expression of these genes was evaluated in the mouse cervix by quantitative real-time PCR. As described in Fig. 5, expression of II-13ral, Arg1, and II-1ra was significantly up-regulated by PP. Ym1 expression was increased in labor and remained elevated PP, whereas Tgfb had variable expression late on gestation day 18 and a significant increase was observed between day 15 and term, not in labor (NIL). YM1 protein expression was similar to mRNA expression increasing PP (Fig. 6). In contrast to induction of M2 macrophage gene expression markers in the cervix, the expression of these genes was low to undetectable in the fetal membranes on gestation days 15, 18.75, and 19 NIL (data not shown). These data, along with our previous studies reporting increased expression of genes expressed by classically activated M1 macrophages, suggest that M2 macrophages play little role in remodeling of fetal membranes during parturition.

Characterization of myeloid cells in peripheral blood during pregnancy and PP

To determine whether changes in myeloid cell populations observed in cervical tissue during pregnancy and parturition were similarly reflected in peripheral blood, staining for myeloid markers was conducted using whole blood obtained from mice before, during, and after cervical ripening. Neutrophil numbers were significantly increased PP similar to the pattern in the cervix (Fig. 7).

In contrast, monocyte numbers in peripheral blood did not change during pregnancy or PP, suggesting quantification of these cell types in blood is not reflective of local changes in the cervix during ripening, dilation, and PP repair.

Discussion

Temporal changes in immune cell phenotype during pregnancy, parturition, and PP in the mouse cervix reveal dynamic and multifaceted functions of inflammatory cells during cervical remodeling. The data from this study suggest that the contribution of macrophages to matrix remodeling predominates in the PP tissue repair phase of remodeling rather than the cervical ripening phase before birth, whereas eosinophils may contribute to cervical dilation or PP repair. Matrix remodeling of the cervix in preparation for birth requires extensive disorganization of the collagen-rich matrix, whereas PP remodeling requires removal of inappropriately assembled matrix molecules that contribute to matrix disarray. Appropriate and efficient PP remodeling is necessary to protect the entire reproductive tract from pathogens and to allow subsequent pregnancy. This work advances our understanding of the presence and contribution of myeloid cells to specific phases of cervical remodeling.

Cervical tissue monocytes are increased in number during cervical ripening, and this migration is dependent on the withdrawal of progesterone. Both morphology and surface marker expression (Neu 7/4+ and F4/80+) of these cells are similar to blood monocytes and distinct from macrophages (Neu 7/4− and F4/80−) (43). In contrast to the cervix, monocytes in blood were not increased during cervical ripening, indicative that changes in this tissue are not reflected in the peripheral blood. Because perfusion of mice at gestation day 18.75 did not alter the retrieval of monocytes, it is unlikely that changes in monocytes arise from alterations in the cervical vasculature, and therefore, the increases are a real determination of the changes within the tissue (data not shown).

Although monocytes are increased during cervical ripening and CSF1 is expressed during pregnancy, the mRNA expression of the monocyte to macrophage differentiation factor receptor Csf1r, as well as gene markers specific for both polarized activation states of macrophage, was not up-regulated until labor began and/or PP. This would suggest an increase in monocyte to macrophage differentiation during labor and PP. The recruitment of monocytes to cervix, however, was not followed by an increase in macrophage numbers presumably due to the increased turnover of activated macrophages during this process.

Macrophages show significant heterogeneity in function as the microenvironment influences their properties and activation state.

Downloaded from http://www.jimmunol.org by guest on September 13, 2017
(42, 44). Macrophage activation can be described as a continuum between two different polarization phenotypes, M1 and M2. Numerous biological systems exist in which both macrophage subsets coexist and a shift in the balance of the two subsets occurs upon changes in physiological or pathophysiological conditions (45–47). The M1s are classically activated by proinflammatory mediators such as LPS and IFN-γ, and produce proinflammatory cytokines such as IL-6, IL-12, and TNF-α (42, 44). In contrast, M2 macrophages termed alternatively activated are activated by IL-4 and IL-13 and express anti-inflammatory cytokines such as IL-10 and TGF-β along with IL-1ra and YM1. M2 macrophages also produce Arg1, which reduces the amount of the substrate arginine required for NO production and increases the production of ornithine necessary for collagen synthesis. M2 macrophages, therefore, are involved in down-regulation of the inflammatory response and help promote tissue repair.

Although macrophages are present in the cervix before cervical ripening (gestation day 15), it is of interest that neither M1 nor M2 markers are expressed at this time. Similar to the recently described endometrial NK cell that is present, but inactive until pregnancy, these macrophages may be a unique subset of cells that are inactive or inert until parturition or else infection ensues (48). Our previous studies report an up-regulation of proinflammatory cytokines, IL-1α and TNF-α, as well as chemoattractants (MCP-1) in the cervix as early as 2 h after birth (24). This would suggest that M1 macrophages are present at this time. More recently, we have determined little to no mRNA expression of Ifng, Il-6, Il-12, and iNos in the cervix before or after birth (data not shown), suggesting that M1 proinflammatory macrophages may play a minor role in remodeling during either cervical ripening or PP. Up-regulation of Ym1, Arg1, and Il-13ra1 mRNA and protein expression in labor and PP from this investigation suggests that M2 macrophages are also present in the PP cervix. The expression of CsF1 in the cervix may also predispose recruited monocytes or resident macrophages toward the M2 phenotype, as has been described in other systems (44, 49). Flow cytometry using surface markers specific for M1 and M2 macrophages will be required to quantify the relative numbers of M1 vs M2 macrophages in PP remodeling.

M2 macrophages may be activated by IL-13 or IL-4. IL-13 expression in the cervix was low to undetectable (data not shown) in whole tissue protein extracts. Furthermore, neither IL-4 nor the immunosuppressive cytokine IL-10 were detectable in the cervix during ripening or PP, emphasizing the fact that macrophage phenotypes are distinct within the cervix microenvironment when compared with macrophages in other tissues. A mixed population of macrophages displaying phenotypes across both polarization states may have evolved to ensure optimal PP cervical remodeling. We hypothesize that M1s would be necessary to protect against microorganism invasion during delivery as well as facilitate removal of ECM components, whereas macrophages with a polarized activation state similar to M2 would function to suppress excessive proinflammatory responses and to promote tissue repair.

Evidence for the contribution of both M1- and M2-polarized macrophages to maintenance of pregnancy and parturition is mounting. Macrophages present in first trimester human decidua express markers of M2-polarized macrophages consistent with their role in immune tolerance at the maternal-fetal interface (45). In contrast, remodeling of the fetal membranes during parturition may require primarily an M1 activation phenotype. Increased expression of proinflammatory genes occurs in term fetal membranes (Il-1α, Ccl5, Tnf, and Cxcl2) (24). More recently, we have evaluated the expression of Ym1, Arg1, and Il-1ra in gestation day 18.75 fetal membranes and found little to no expression of these M2 marker genes (data not shown). At the end of pregnancy, the fetal membranes undergo remodeling, resulting in rapid loss of tensile strength and membrane rupture (50). Because fetal membranes need not be preserved once parturition is complete, we speculate that the immunosuppressive and repair actions of M2 macrophages are unnecessary as compared with the cervix, whose integrity must be rapidly regained. Tissue-specific macrophage phenotypes are required for maintenance of pregnancy and successful parturition, and clearly emphasize the varied functions of immune cells in this critical process.

The increase in cervical eosinophils during labor that was observed in this study has previously been described and is preceded by an increased expression of the eosinophil chemoattractant, eotaxin (10, 14, 24). Although classically thought to be involved in host protection against parasites, eosinophils more recently are appreciated to be multifunctional leukocytes involved in inflammatory responses as well as modulators of innate and adaptive immunity (51). Eosinophils are a prevalent cell population in the female reproductive tract, are regulated by steroid hormones, and thought to be important in uterine preparation for pregnancy (51, 52). Although their presence in the cervix at the time of labor may have a function in late stages of remodeling, their functions are not essential to this process because mice depleted of eosinophils (eotaxin null mice) have normal parturition (52). Further studies are required to appreciate the specific functions that eosinophils may play in cervical remodeling.

These findings propose a paradigm shift from the idea that inflammatory cells orchestrate changes in the extracellular matrix required for initiation of cervical ripening. During cervical ripening, monocytes extravasate to the cervical tissue in a progesterone-regulated manner, and after a delay of several hours this influx is followed by increased gene or protein expression of M2-polarized macrophage markers and to a lesser extent M1-polarized macrophage markers during labor or within a few hours PP. These data support a model in which macrophage recruitment and heterogeneity facilitate efficient recovery and repair of the cervix after birth, thus ensuring protection of the entire reproductive tract from microbial infection and the ability to initiate and maintain a subsequent pregnancy. Future studies will focus on identifying progesterone-regulated factors that allow monocyte migration to the cervix during ripening and studies to better understand the relative contributions of both alternatively activated M2 and classically activated M1 macrophages to PP cervical remodeling.

Acknowledgments

We thank Angela Mobley for invaluable assistance with the flow cytometry and cell sorting. We thank Dr. Petra Cravens and Dr. David Farrar for helpful discussions and critical reading of the manuscript. The technical assistance of Monika Ruschinsky and Joseph Davis is also gratefully acknowledged.

Disclosures

The authors have no financial conflict of interest.

References

1. Ananth, C. V., K. S. Joseph, Y. Oyelese, K. Demissie, and A. M. Vintzileos. 2005. Trends in preterm birth and perinatal mortality among singletons: United States, 1989 through 2000. Obstet. Gynecol. 105: 1084–1091.
2. Bramen, A. M., and K. C. Schoendorf. 2002. Changing patterns of low birthweight and preterm birth in the United States, 1981-98. Paeat Prev. Perinat. Epidemiol. 16: 8–15.
3. Leppert, P. C. 1995. Anatomy and physiology of cervical ripening. Clin. Obstet. Gynecol. 38: 267–279.
4. Read, C. P., R. A. Word, M. Ruschinsky, B. C. Timmons, and M. S. Mahendroo. 2007. Cervical remodeling during pregnancy and parturition: molecular characterization of the softening phase in mice. Reproduction 134: 327–340.
5. Mahendroo, M. S., A. Porter, D. W. Russell, and R. A. Word. 1999. The parturition defect in steroid 5a-reductase type 1 knockout mice is due to impaired cervical ripening. Mol. Endocrinol. 13: 981–992.
18. Ledingham, M. A., A. J. Thomson, F. Jordan, A. Young, M. Crawford, and Timmons, B. C., and M. S. Mahendroo. 2006. Timing of neutrophil activation.

24. Sakamoto, T., P. Moran, R. F. Searle, J. N. Bulmer, and S. C. Robson. 2004. Prostaglandins and the myometrium and cervix. Prostaglandins Leukotrienes Essent. Fatty Acids 70: 207–222.

22. Hertelendy, F., and T. Zakar. 2004. Prostaglandins and the myometrium and cervix. Prostaglandins Leukotrienes Essent. Fatty Acids 70: 207–222.

23. Junqueira, L., M. Zugab, G. Montes, O. M. Toledo, R. Krisztan, and K. Shiguahara. 1980. Morphologic and histochemical evidence for the occurrence of collagenolysis and for the role of neutrophilic polymorphonuclear leukocytes during cervical dilation. Am. J. Obstet. Gynecol. 138: 273–281.

21. Knudsen, U. B., N. Uldbjerg, T. Rechberger, and K. Fredens. 1997. Eosinophils and expression of proinflammatory markers do not support a role for neutrophils in human cervical ripening.

20. Raynes, J. G., J. C. Anderson, R. J. Fitzpatrick, and H. Dobson. 1988. Increased level of matrix metalloproteinases 2 and 9 in the ripening process of the cervix and after local application of prostaglandins.

19. Luque, E. H., M. M. Munoz de Toro, J. G. Ramos, H. A. Rodriguez, and O. D. Sherwood. 1998. Role of relaxin and estrogen in the control of eosinophilic invasion and collagen remodeling in rat cervical tissue at term. Biol. Reprod. 59: 795–800.

18. Vassalli, P. 1992. The pathophysiology of tumor necrosis factors.

17. Pillinger, M. H., and S. B. Abramson. 1995. The neutrophil in rheumatoid arthritis. Rheum. Dis. Clin. N. Am. 21: 691–714.

16. Rath, W., B. C. Adelmann-Grill, R. Osmers, and W. Kuhn. 1989. Enzymatic degradation, and Hormonal Regulation.

15. Luque, E. H., M. M. Munoz de Toro, J. G. Ramos, H. A. Rodriguez, and O. D. Sherwood. 1998. Role of relaxin and estrogen in the control of eosinophilic invasion and collagen remodeling in rat cervical tissue at term. Biol. Reprod. 59: 795–800.

14. Buhimschi, I. A., L. Dussably, C. S. Buhimschi, A. Ahmed, and C. P. Weiner. 2004. Prostaglandins and the myometrium and cervix. Prostaglandins Leukotrienes Essent. Fatty Acids 70: 207–222.

13. Osmers, R. G., J. Blaser, W. Kuhn, and H. Tschesche. 1995. Interleukin-8 synthesis and the onset of labor.

12. Rath, W., B. C. Adelmann-Grill, R. Osmers, and W. Kuhn. 1989. Enzymatic degradation, and Hormonal Regulation.

11. Osmers, R. G., J. Blaser, W. Kuhn, and H. Tschesche. 1995. Interleukin-8 synthesis and the onset of labor.

10. Junqueira, L., M. Zugab, G. Montes, O. M. Toledo, R. Krisztan, and K. Shiguahara. 1980. Morphologic and histochemical evidence for the occurrence of collagenolysis and for the role of neutrophilic polymorphonuclear leukocytes during cervical dilation. Am. J. Obstet. Gynecol. 138: 273–281.

9. Junqueira, L., M. Zugaib, G. Montes, O. M. Toledo, R. Krisztan, and J. E. Norman. 2001. Cell adhesion molecule expression in the cervix and myometrium during pregnancy and parturition. Obstet. Gynecol. 86: 223–229.

8. Hertelendy, F., and T. Zakar. 2004. Prostaglandins and the myometrium and cervix. Prostaglandins Leukotrienes Essent. Fatty Acids 70: 207–222.

7. Strach, K. J., M. J. Shelton, J. A. Richardson, V. C. Hascall, and M. S. Mahendroo. 2005. Regulation of neutrophil expression during cervical ripening. Glycobiology 15: 55–65.

6. Ruschinsky, M. C., D. De la, M. Mahendroo. 2008. Hyaluronan and its binding proteins during cervical ripening and parturition: dynamic changes in size, distribution and temporal sequence. Matrix Biol. 27: 487–497.

5. Sakamoto, T., P. Moran, R. F. Searle, J. N. Bulmer, and S. C. Robson. 2004. Prostaglandins and the myometrium and cervix. Prostaglandins Leukotrienes Essent. Fatty Acids 70: 207–222.

4. Nakamura, K., N. Uldbjerg, T. Rechberger, and K. Fredens. 1999. Neutrophils in human cervical ripening. Eur. J. Obstet. Gynecol. Reprod. Biol. 72: 165–168.

3. Nakamura, K., N. Uldbjerg, T. Rechberger, and K. Fredens. 1999. Neutrophils in human cervical ripening. Eur. J. Obstet. Gynecol. Reprod. Biol. 72: 165–168.

2. Ledingham, M. A., A. J. Thomson, F. Jordan, A. Young, M. Crawford, and Timmons, B. C., and M. S. Mahendroo. 2006. Timing of neutrophil activation.

1. Ledingham, M. A., A. J. Thomson, F. Jordan, A. Young, M. Crawford, and Timmons, B. C., and M. S. Mahendroo. 2006. Timing of neutrophil activation.