Chemotactic Activity of Gestational Tissues Through Late Pregnancy, Term Labor, and RU486-Induced Preterm Labor in Guinea Pigs

Nardhy Gomez-Lopez[1], Wing-Chiu Tong[2,3], Marcia Arenas-Hernandez[1], Satomi Tanaka[2], Omar Hajar[2], David M. Olson[2], Michael J. Taggart[3], Bryan F. Mitchell[2]

[1]Department of Obstetrics and Gynecology, Perinatology Research Branch, Wayne State University, Detroit, MI, USA;
[2]Department of Obstetrics and Gynecology, University of Alberta, Edmonton, AB, Canada;
[3]Institute of Cellular Medicine, Newcastle University, Newcastle-upon-Tyne, UK

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Correspondence
Bryan F. Mitchell, HMRC 2-20, University of Alberta, Edmonton, AB, Canada T6G 2S2.
E-mail: brymitch@ualberta.ca

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Problem
Is increased leukocyte chemotactic activity (CA) from gestational tissues necessary for term or preterm labor in guinea pigs?

Method of Study
Tissue extracts were prepared from pregnant guinea pig decidua–myometrium, cervix, fetal membranes (amniochorion), and placenta during early third trimester ($n = 8$), term not in labor (TNL, $n = 5$), and term spontaneous labor (TL, $n = 6$), RU486-induced preterm labor (PTL, $n = 6$), or controls (cPTL, $n = 5$). Leukocyte CA was assessed using a modified Boyden chamber assay. Extract chemokine and maternal progesterone concentrations were quantified by enzyme immunoassay.

Results
Only the extracts from amniochorion demonstrated increased CA through late gestation and labor. In contrast, CA was decreased in extracts from amniochorion and cervix from animals after RU486-induced PTL. Maternal progesterone concentrations remained high in all groups.

Conclusion
Leukocyte CA of intrauterine tissues is increased in term spontaneous labor. However, RU486-induced preterm labor occurs in the absence of increased CA.

Introduction

Preterm birth (<37 weeks gestation) occurs in 12.2% of all pregnancies in the United States[1] and is associated with the majority of neonatal mortality and long-term serious morbidity.[2–4] The mechanisms that lead to preterm labor (PTL) are poorly understood. Currently available treatments to prevent or treat spontaneous PTL have not been shown to improve outcomes for the preterm newborn.

It is well established that labor, both at term and preterm, is accompanied by enhanced pro-inflammatory activity within intrauterine tissues. Specific inflammatory mediators (i.e., cytokines) can be produced by several intrauterine tissues.[5–9] Included among these cytokines are chemotactic factors that are able to attract leukocytes from the peripheral blood into the maternal–fetal interface.[8,10,11] Infiltrated leukocytes create a 'pro-inflammatory microenvironment' in this interface and, in a positive

feedback manner, stimulate production of more and other inflammatory mediators that have been postulated to play a role in the initiation of parturition.[12–14] We recently demonstrated that chemotactic activity (CA) is increased in rat intrauterine tissues just before normal labor,[15] in support of the concept that chemotactic production by gestational tissues is a factor for labor initiation or progression.

Preterm labor is of particular interest because of the tremendous medical and social costs that can result from preterm birth. Preterm birth can be a consequence of medical treatment for maternal or fetal complications that dictate immediate delivery of the fetus despite the preterm gestational age. Another group may be caused by intrauterine infection, for which urgent delivery is usually recommended at any gestational age. However, in approximately half of the cases of preterm birth (so-called spontaneous), the cause is unknown.[16] This latter group is the target of efforts to understand the pathophysiology such that methods can be developed to prevent or arrest preterm labor. Most research regarding preterm birth has utilized rat or mouse models treated with potent inflammatory stimuli such as bacteria, lipopolysaccharide (LPS), interleukin-1, and TNF-α.[17–21] In these rodent species, normal parturition is due to luteolysis induced by prostaglandin F, an end product of pro-inflammatory pathways, which results in a dramatic decline in maternal progesterone concentrations that activate uterine contractile mechanisms and result in parturition.[22] Thus, the effects of the potent inflammatory stimuli that lead to production of prostaglandins are not surprising and such models might better represent infection-induced preterm labor rather than the spontaneous category. However, regulation of parturition in humans is clearly not related to luteolysis or to a decline in maternal serum progesterone. For this and other reasons, we have argued that the guinea pig, which is endocrinologically more similar to the human, might be a preferable animal model for studies of preterm parturition.[22]

Although there is a clear association between increasing pro-inflammatory mediators in the uterus and the occurrence of parturition, the cause–effect relationship between these two events has not been clearly proven. We have suggested that the purpose of the enhanced pro-inflammatory activity at this time might be directed toward another target such as the healing of the placental site after delivery and the process of uterine involution that occurs in the days following parturition.

The objectives of the present study were (i) to determine the CA of intrauterine tissues of pregnant guinea pigs during the last trimester of pregnancy and around the time of normal term labor, (ii) to determine the CA of intrauterine tissues using a previously developed model of guinea pig preterm labor using the progesterone antagonist, RU486, which is not a direct inflammatory stimulus, (iii) to associate CA in the tissue extracts with concentrations of selected chemokines, and (iv) to relate these events to maternal serum progesterone concentrations. We hypothesized that the CA and chemokine profiles in spontaneous term and RU486-induced preterm labor would be similar, thus supporting a role for these events in the progress of parturition. Our results suggest that although there is increased CA in some intrauterine tissues at the time of normal spontaneous parturition, preterm birth induced by the progesterone antagonist occurs in the absence of such an increase.

Materials and methods

Animals and Tissues

Animal protocols were approved by the University of Alberta Animal Care and Use Committee for Health Sciences, and the experiments were conducted in accordance with the Guidelines and Policies of the Canadian Council on Animal Care. Non-pregnant and pregnant guinea pigs (Dunkin Hartley strain; Charles River, Saint-Constant, QC, Canada) were used in this study. Non-pregnant animals were housed in the University of Alberta Health Sciences Laboratory Animal Services and time-mated on-site.

The average gestational length for our overall guinea pig program is 67.5 days (d) with a standard deviation of ±1.9. The range for spontaneous term delivery was 64–70 d. The median number of piglets was 3 with range 1–5. At predetermined time points, guinea pigs were euthanized with a lethal dose of Isofluorane (Bimeda-MTC, Cambridge, ON, Canada) by inhalation in a large beaker. Based on previous studies,[23] preterm birth was induced using RU486 (3 mg/kg body weight in corn oil) administered by daily subcutaneous injection on gestational d55 and d56. Delivery occurred at 47 ± 7 hr from the first injection of RU486.

To provide comparability to human gestation, experimental groups were allocated according to the following definitions. Third trimester was considered

>45 days gestation and was subdivided into early third trimester (3T; d46–64, $n = 8$) and term (>d64). The term group was divided into those with no signs of labor (TNL; >d64, $n = 5$) and those in active labor (TL; >d64, having delivered at least one piglet, $n = 6$). These groups were compared to those receiving RU486 to induce preterm labor as described above (PTL; $n = 6$), and a group of controls that received injections of corn oil on d55 and d56 and tissues were obtained 2–3 d later (cPTL; $n = 5$). Uterine horns and cervix were removed from each animal. Each uterine horn was cut longitudinally along the antimesenteric border. The gestational sacs and placentas were removed by gentle traction to separate them from the underlying uterine wall. The decidua–myometrium tissue samples consisted of full thickness sections of the uterine wall excised from areas between placental attachment sites. We deliberately left the decidua–myometrium intact as it is likely that there are important interactions between these two tissues that affect parturition, and it is quite difficult to precisely separate the two tissues. We chose the interplacental sites to avoid potential confounding effects of high local concentrations of placental hormones or contaminating trophoblast tissues. The fetal membranes (amnion and chorion laeve) surrounding the piglets were removed but kept together as a single unit (amniochorion). All tissue samples were immediately washed in 1× PBS and placed in liquid nitrogen until use.

Protein Extracts of Tissues

Frozen tissues were gradually defrosted, cut into fragments of ~1 cm^2, and homogenized in 1 mL of DMEM (High Glucose 1× and 1% antibiotics; Invitrogen, Grand Island, NY, USA) using a Polytron PRO200 homogenizer (PRO Scientific, Oxford CT). These extracts were centrifuged at 4°C, 12,000× g for 30 min, repeating this last step until getting a clear supernatant. The protein concentration of tissue extracts was measured using Protein Assay Reagent (Precision Red™, Cytoskelton, Denver, CO, USA) at 600 nm in the NanoDrop ND-1000 UV-Vis spectrophotometer (Thermo Fisher Scientific, Ottawa, ON, Canada). Protein extracts then were stored at −20°C until use.

Leukocyte Isolation

Leukocytes were obtained from term pregnant guinea pigs (>d64; $n = 4$). Blood was obtained by heart puncture (10 mL) using a heparinized vacuum tube (BD Vacutainer, Franklin Lakes, NJ, USA). Polymorphonuclear and mononuclear leukocytes were isolated using a Ficoll gradient (Polymorphprep; Axis-Shield, Norton, MA, USA) following the manufacturer's instructions. Total leukocytes were then washed in 1× PBS, and the pellet was resuspended in supplemented RPMI 1640 medium (1% antibiotics—5000 units of penicillin and 5000 μg of streptomycin/mL—and 10% fetal bovine serum; Invitrogen). These leukocytes were used in chemotaxis assays immediately after isolation.

Chemotaxis Assay

Chemotaxis assays were performed using a modified validated Boyden chamber method (AP48; Neuro Probe, Gaithersburg MD)[24] as we have described in detail previously[25] (Fig. 1). CA was assessed for each tissue extract using previously isolated heterologous leukocytes. Fifty microliters of leukocyte suspension containing 100,000 leukocytes were placed on top of the polycarbonate membrane (5 μm pore size; PFB5, Neuro Probe) with 25 μL of each tissue extract as chemo-attractant in the lower compartment (Fig. 1a). Chambers were incubated for 90 min at 37°C in humidified air containing 5% CO_2. Attracted leukocytes were removed from the lower compartment and centrifuged at 500× g for 5 min at room temperature. The pellet was fixed with 500 μL of OptiLyse® B Lysing Solution (Beckman Coulter, Miami, FL, USA) and counted by flow cytometry. Leukocyte subsets were identified using characterized fluorescent monoclonal antibodies as previously described.[25] The flow cytometer (FACSCANTO II) was set to analyze the samples for 100 s. The coefficients of variation of this method (inter- and intra-assay) are <5%. Leukocyte subpopulations (granulocytes, monocytes, and lymphocytes) were defined according to their size and complexity, as shown in Fig. 1a.

The number of attracted leukocytes was considered as the number of events acquired by flow cytometry (Fig. 1a). Leukocytes attracted by the medium control were subtracted in all cases. The number of attracted leukocytes by each tissue extract was normalized to its protein concentration (Fig. 1b). To compare relative CA among all the samples, all values were normalized to the average of attracted leukocytes by tissues from four animals at d50–54 (the earliest gestational age points, considered as a reference sample; Fig. 1c,d). At this gestational age,

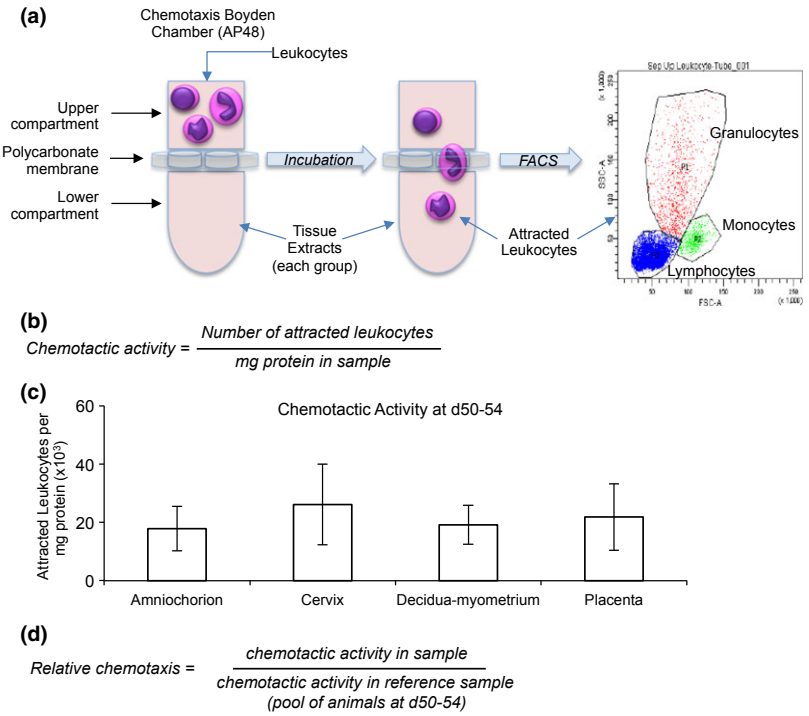

Fig. 1 Chemotaxis assay. (a) Schematic representation of the chemotaxis assay. Heterologous leukocytes from term pregnancy were placed on the top of the polycarbonate membrane, and tissue extracts were placed on the bottom. Leukocytes that were attracted by the tissue extract migrate through the membrane where they are counted and analyzed by flow cytometry. (b) Formula used to calculate the chemotactic activity. (c) Chemotactic activity of reference tissues at d50–54. (d) Formula used to calculate the relative chemotactic activity in each sample.

we did not find any difference in chemotactic activities between the tissues (Fig. 1c). Therefore, the relative CA estimates are comparable across similar tissues and between different groups of tissues.

Histology to Assess Leukocyte Infiltration

Immediately following collection, samples of the decidua–myometrium from each group of animals were washed gently in 1× PBS, embedded in Tissue-Tek® media (Sakura Finetek, Torrance, CA, USA), and frozen to −80°C. At the time of processing for histology, embedded frozen tissues were cut into 7-μm-thick sections and placed onto SuperFrostR Plus microscope slides (Fisher Scientific, Pittsburgh, PA, USA), dried overnight at room temperature, and stored at −80°C. Tissue sections were fixed with precooled acetone for 10 min. Following fixation, tissue sections were maintained at room temperature for 30 min, and these were rinsed twice in 1× PBS. The Leica Bond Max autostainer (Leica Microsystems, Wetzlar, Germany) and Bondtm Polymer Refine Detection Kit (Leica Microsystems) were used for immunostaining and chromogenic detection, respectively. Mouse antiguinea pig anti-CD45 primary antibody (Novus Biologicals, Littleton, CO, USA) was used in the dilution of 1:100 for 10-min incubation. Tissue sections were dehydrated in three changes of alcohol [95%, 100% (2x)], then cleared in three changes of xylene. Cover slips were applied using the Tissue-Tek SCA automated coverslipper (Sakura Finetek). Leukocyte infiltration (CD45+ cells) was assessed the next day.

ELISAs for Chemokines

We chose to assess concentrations of four chemokines in each extract as representatives of potential classes of chemotactic factors: CXCL8 (IL-8) and CXCL1 (GRO1 or GROα) are important chemotactic factors primarily for neutrophils and also other granulocytes; CXCL10 (interferon-γ-induced protein, IP-10) and CCL2 (monocyte chemotactic protein-1, MCP-1) have chemo-attractant activity for monocytes/macrophages as well as T lymphocytes. ELISAs for guinea pig CXCL8 (#GI0063, NEO BioLab, Cambridge, MA, USA), CXCL1 (#GG0369, NEO BioLab), CXCL10 (#GC0047, NEO BioLab), and CCL2 (#GM0253, NEO BioLab) were performed in tissue extracts from decidua–myometrium, cervix, and amniochorion following the manufacturer's instructions. Inter- and intra-assay coefficients of variation were 5–10%.

Chemokine concentrations were normalized to protein concentrations.

Enzyme Immunoassay for Progesterone

Progesterone concentrations were measured in maternal serum samples using the DRG enzyme immunoassay kit (EIA-1561, DRG International, Mountainside, NJ, USA). Immediately following collection, blood samples were centrifuged to obtain serum and stored at −20°C. At the time of assay, each sample was diluted in assay buffer and each dilution run in triplicate with a standard curve on each assay plate. The required dilution was determined in preliminary assays and varied according to the gestational age at which the sample was collected. The dilution ranged from 1:1 for non-pregnant and post-partum samples to 1:160 for samples at mid-gestation and 1:40 in late gestation. The assays were performed according to the manufacturer's instructions. Assay sensitivity was 0.05 ng/mL, and inter- and intra-assay coefficients of variation were 7.6 and 5.3%, respectively.

Statistical Analyses

Analyses were performed using SPSS, version 19.0 (IBM, Armonk, NY, USA). For comparisons among more than two groups, data were analyzed using one-factor ANOVA. If the Shapiro–Wilk normality test failed to confirm normal distribution, data were analyzed using the Kruskal–Wallis test. Unpaired *t*-tests or Mann–Whitney U-tests were used to evaluate statistical differences between two specific groups. Statistical significance was accepted when P was ≤ 0.05. For clarity of presentation, we have presented all data as means ± standard error of the means.

Results

The relative CA profiles of the tissues from all of the study groups are illustrated in Fig. 2. In the amniochorion tissues, there was a statistically significant progressive increase in CA for the total leukocyte population as well as the granulocyte and lymphocyte subpopulations through late normal gestation (from the 3T through the TL groups; Fig. 2a). There were positive trends toward increased chemotaxis for total leukocytes and granulocytes in the extracts from cervix and decidua–myometrium, but these failed to achieve statistical significance (Fig. 2b,c). There were no significant changes in extracts from placental tissue (data not shown). There was no change in chemotactic attraction for monocytes in any of the tissues.

For the RU486-induced preterm labor group, delivery occurred at 47 ± 7 hr from the first injection of RU486. The newborns from the RU486-treated preterm group weighed significantly less than the term piglets but were similar to the untreated controls and to the 3T piglets from approximately the same gestational age (Table I). Contrary to our expectations, there were no significant increases in CA from any of the tissue extracts from the animals receiving the antiprogestin when compared to the gestational age-matched, placebo-treated control group or to the early third trimester animals. Indeed, the amniochorion and cervical extracts from the RU486-induced preterm labor group contained significantly lower CA for total leukocytes and granulocytes in comparison with the spontaneous TL animals (Fig. 2a,b). In the extracts from the cervix, RU486 treatment resulted in decreased CA compared to the gestational age-matched animals treated with vehicle only. There were no statistically significant changes in CA in the PTL animals for lymphocytes or monocytes or for any of the leukocyte populations with extracts from decidua–myometrium (Fig. 2c) or placenta (data not shown).

We chose to measure the concentrations of CXCL8, CXCL1, CXCL10, and CCL2 as these chemokines have demonstrated leukocyte CA and have been associated with leukocyte recruitment into intrauterine tissues in humans and rodents.[10,12,15,26] These data are presented in Fig. 3. As we found no changes in chemotactic activities in placental extracts, we did not measure chemokine concentrations in these extracts. There were significant increases in the amniochorion extracts (Fig. 3a) for CXCL1 and CXCL10 at the time of spontaneous term parturition and in CXCL8, CXCL1, and CCL2 following RU486-induced preterm birth. In the cervix (Fig. 3b), there were no significant increases in any of the cytokines prior to spontaneous term delivery or in the RU486-induced preterm delivery compared to their controls. However, concentrations of CXCL1 were higher at induced preterm labor than at spontaneous term parturition, a pattern similar to that seen in the extracts from cervix and decidua–myometrium (Fig. 3b,c). In the decidua–myometrium, CXCL8 also was increased in RU486-induced labor compared to term labor. However, in the latter

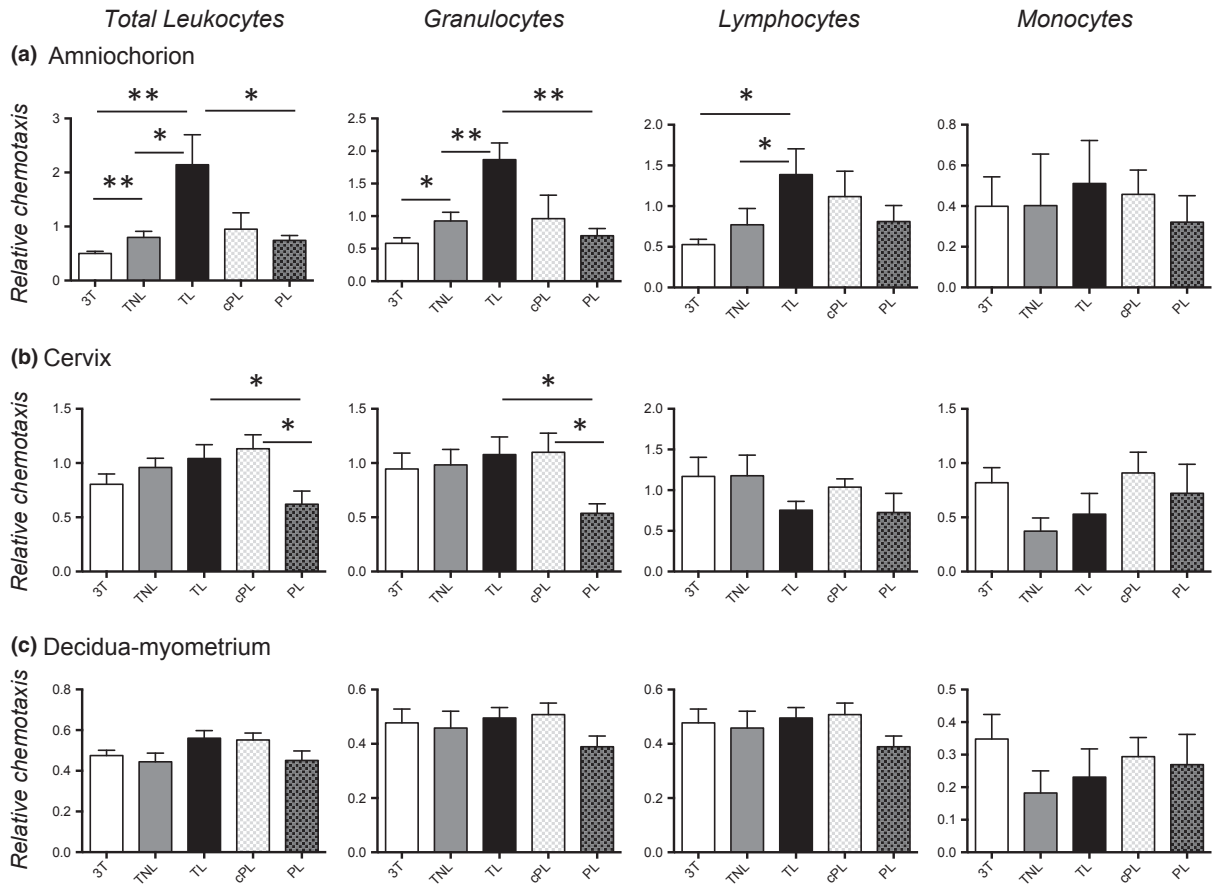

Fig. 2 Relative chemotactic activity (as calculated from formula in Fig. 1d) of tissue extracts. Extracts from amniochorion (a), cervix (b), and decidua–myometrium (c) from early third trimester (3T; $n = 8$); term not in labor (TNL; $n = 5$); term in labor (TL; $n = 6$); vehicle-control preterm labor (cPL; $n = 5$); and RU-486 induced preterm labor (PL; $n = 6$). Chemotactic activity was determined for the total leukocyte population as well as the granulocyte, lymphocyte, and monocyte subpopulations. Data are expressed as means ± S.E.M. Statistically significant differences between groups are indicated (* when $P \leq 0.05$ and ** when $P \leq 0.01$).

Table I Characteristics of the Study Groups (Means ± S.E.M.)

	3T ($n = 8$)	TNL ($n = 5$)	TL ($n = 6$)	cPTL ($n = 5$)	PTL ($n = 6$)
Gestational length (days)	60.1 ± 1.8[a]	66.6 ± 0.2[b]	67.7 ± 0.6[b]	55.8 ± 1.5[a]	57.8 ± 0.3[a]
Weight of dams (g)	1137 ± 56	1179 ± 34	1156 ± 29	1042 ± 54	1038 ± 27
Number of piglets*	3 (3, 4)	4 (3, 4)	3.5 (3, 4.3)	4 (2.5, 5.5)	4 (3.8, 4.8)
Weight of piglets (g)	81.2 ± 8.9[a]	106.8 ± 6.4[b]	99.7 ± 7[b]	67.0 ± 5.7[a]	67.1 ± 3.6[a]
Progesterone (ng/mL)[†]	190.6 ± 16.3	218.4 ± 33.5	173 ± 31.9	135 ± 27.7	221.3 ± 62.5

3T, early third trimester; TNL, term not in labor; TL, term in labor; cPTL, vehicle-control preterm labor; PTL, RU486-induced preterm labor.
*Non-continuous data presented as medians with 25th and 75th percent quartiles in parentheses.
[†]Measured at the time of tissue sampling.
[a,b]Different superscripted letters represent significant differences between groups of animals as assessed using Mann–Whitney tests.

tissue, CXCL1, CXCL10, and CCL2 tended to decline through late normal gestation with no increase at spontaneous parturition.

For additional support of our estimates of chemotactic activity, the pattern of infiltration of leukocytes into the decidua–myometrium was assessed using

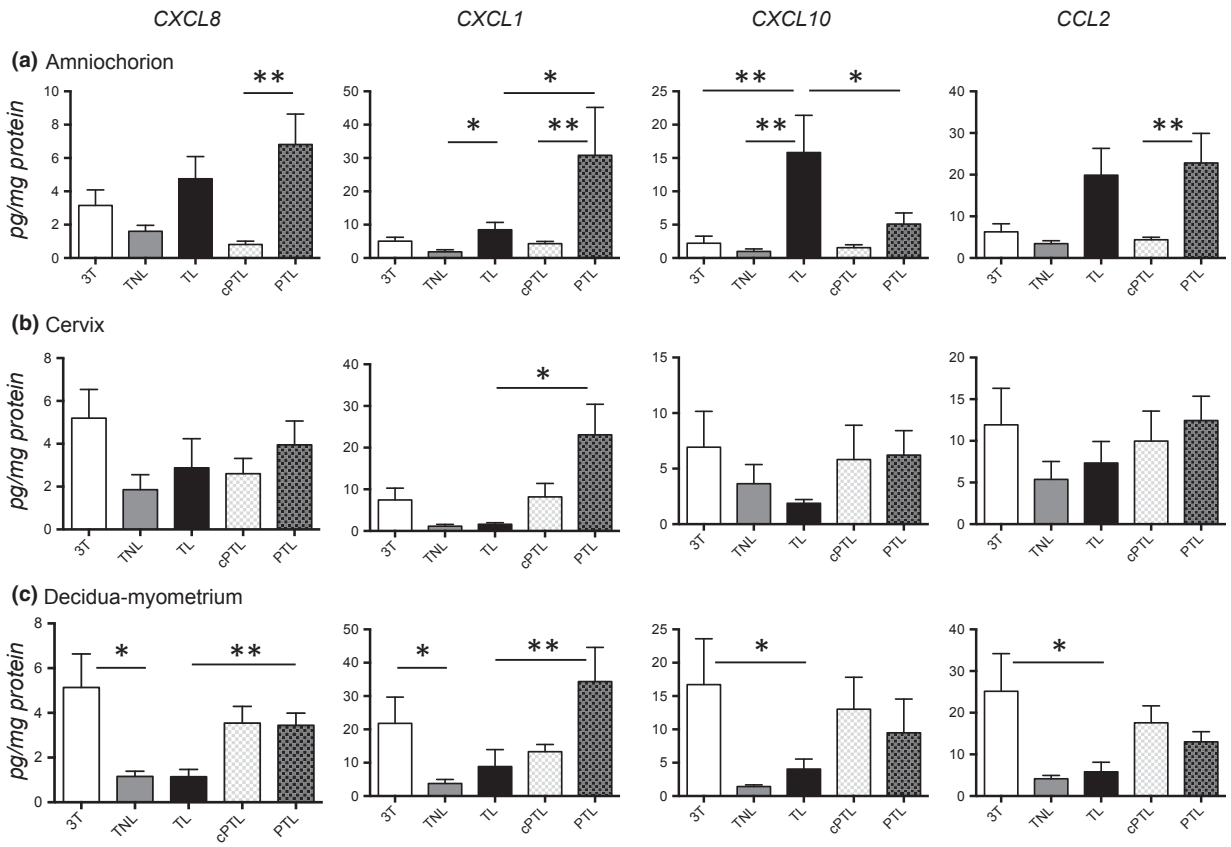

Fig. 3 Concentrations of chemokines (CXCL8, CXCL1, CXCL10, and CCL2) in tissue extracts from amniochorion (a), cervix (b), and decidua–myometrium (c) from early third trimester (3T; $n = 8$); term not in labor (TNL; $n = 5$); term in labor (TL; $n = 6$); vehicle-control preterm labor (cPTL; $n = 5$); and RU-486 induced preterm labor (PTL; $n = 6$). Data are expressed as means ± S.E.M. Statistically significant differences between groups are indicated (* when $P \leq 0.05$ and ** when $P \leq 0.01$).

immunohistochemistry. The qualitative findings (Fig. 4) were consistent with the data from the chemotactic assays. Leukocyte infiltration in the amniochorion appeared higher in TL in comparison with other groups. Leukocyte infiltration into all tissues in the PTL group was very limited.

Maternal serum P4 levels were measured when the animals were euthanized (Table I). In contrast to rats and mice, maternal P4 concentrations did not decline significantly through the last 17 days of gestation.

Discussion

Over the past decade, many studies have clearly demonstrated an association between the process of parturition and increased production and concentrations of pro-inflammatory cytokines in maternal serum and a variety of intrauterine tissues.[5–9,16,27–31] However, there is less clarity regarding the causative or enhancing role of this pro-inflammatory state to the process of parturition. Intrauterine sepsis or administration of large amounts of inflammatory stimulants (LPS, other bacterial products) will provoke parturition in many species,[18,20] but the underlying mechanism is unclear. Conversely, intraperitoneal or intravenous administration of pro-inflammatory cytokines (TNFα, IL-1β, IL-6) to late pregnant Sprague-Dawley rats to achieve concentrations similar to those noted at term or preterm labor in the absence of infection does not appear to hasten the timing of parturition or alter uterine contractility.[32–34] It remains unclear whether there are strain or species differences in the nature and extent of the inflammatory response associated with parturition and whether this plays a role in the mechanisms of the process.

Our primary goal in this study was to determine the CA of intrauterine tissues in the guinea pig for

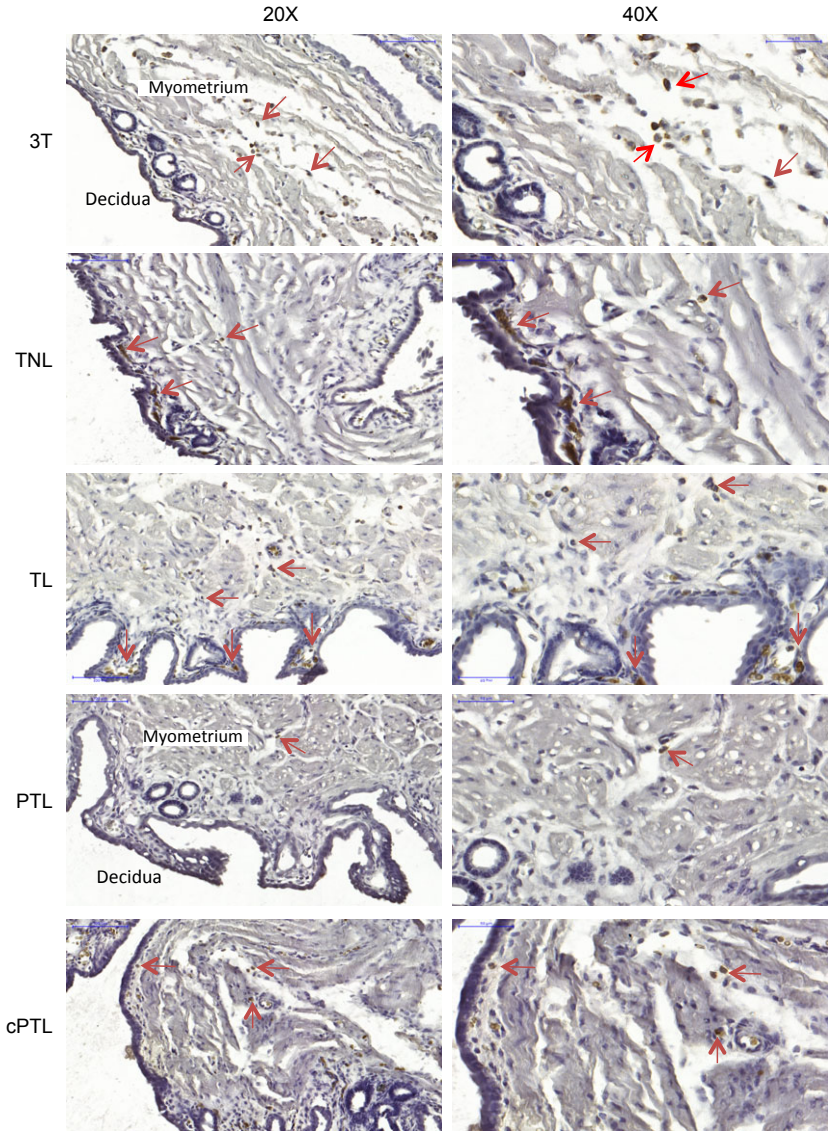

Fig. 4 Leukocyte infiltration in the decidua–myometrial tissues. Representative photomicrograph of decidua-myometrium in each group of tissues: 3T, early third trimester; TNL, term not in labor; TL, term in labor; PTL, RU-486 induced preterm labor; and cPTL, vehicle-control preterm labor. Leukocytes (CD45+) are indicated with arrows. Bar, 100 μm. Optical Microscopy: magnification ×200. Data are representative of three or more independent experiments for each group.

comparison to data from the human and from other rodent species, which are commonly used as animal models. We chose the guinea pig model because of its many similarities to humans regarding the cellular and molecular mechanisms of parturition, particularly with respect to maternal progesterone concentrations.[22]

In the guinea pig, extracts from amniochorion membrane had the highest levels of leukocyte CA and had the greatest changes through late gestation. The CA was primarily directed toward granulocytes and lymphocytes. This fits well with the chemokine data showing increased concentrations of CXCL1 and CXCL10. CXCL1 has chemo-attractant activity for neutrophils[35] and CXCL10 for lymphocytes,[36] which were the recruited leukocyte subsets observed in the CA assay. By comparison, human amniochorion also demonstrates increased CA for granulocytes and lymphocytes as well as monocytes through late gestation and this is associated with increased concentrations of CXCL8, CXCL10, CCL2, and CCL3.[10,37–39] In the Long-Evans rat, there is no increase in CA from the amniochorion, but there was a significant increase in CXCL1 and CCL2 mRNA and protein levels through day 20–22.[15]

The lack of change in CA in extracts from guinea pig decidua–myometrium through late gestation was surprising, as was the significant decline in

concentrations of all four chemokines measured. In contrast, the human uterus has increased CXCL8 and CCL2 at term labor[40,41] and biopsies of the human decidua showed increased CXCL8 at spontaneous labor compared to term non-labor.[8] Similarly, in the rat, there is a significant increase in CA from extracts from decidua–myometrium with an associated increase in CCL2 mRNA but not protein.[15]

Some of the discrepancy between our guinea pig results and human studies could relate to sample collection techniques. Because of convenience in collection of samples, human choriodecidua often is studied as a unit. These tissues have significantly increased CA at term labor compared to term non-labor,[10,11] and early studies showed increased CXCL8 in these tissues with spontaneous labor at term.[37] A potential cause of failure to find significant changes in CA or chemokine production between experimental groups could result from 'diluting' the effects of a small amount of a highly productive tissue (e.g., decidua) with a larger amount of a tissue with low and unchanging production (e.g., myometrium). Further, it is possible that our separation of the chorion from the decidua–myometrium could have disrupted amplification signals between these tissues. A more comprehensive recent study of human decidua measuring mRNA expression confirmed the increase in CXCL8 as well as CXCL1 and CXCL10, in addition to 21 other chemokines.[8] CXCL8 and CXCL10 protein levels also were significantly increased compared to term non-labor. This study also showed that CXCL10 levels in human decidua were lower in preterm than term labor, similar to our findings in the guinea pig. Immunohistochemical studies suggested that the human decidual stromal cells were the major sites of production.[8]

In the guinea pig cervical extracts, there were no significant changes in CA or chemokine concentrations through late gestation or at term labor. This is in contrast to the human where there is increased CXCL8 at the protein and mRNA levels.[42] It is important to note that in our guinea pig studies, all experimental groups are from relatively late gestation and it is possible in both the cervical and decidua–myometrium tissues that CA levels were already increased by the early third trimester, thus masking a potential significant increase prior to term parturition. In the rat, there were no changes in CA[15] between gestation d17 and d22, but there was increased attraction of leukocytes obtained from d22 compared to d17 or d20, emphasizing the importance of responsiveness of the leukocytes to the CA as an equally important factor. There have been extensive studies of the cervix using the mouse model. Some studies have noted increasing leukocyte infiltration (mostly macrophages) of the cervix over the course of pregnancy, parturition, and post-partum.[43,44] There also is invasion of the cervix by neutrophils, but this occurs only in the immediate post-partum period and is not a requisite for normal term parturition.[45,46] Prostaglandins are important mediators of cervical ripening, and it remains possible that activity of the prostaglandin pathway was increased despite the absence of increased leukocyte infiltration. Unfortunately, we did not measure elements of this pathway.

The most surprising and interesting findings came from the RU486-induced preterm labor animals. In this guinea pig model, there was no evidence of increased CA in any of the tissues studied when comparing the induced labor animals to their gestational age-matched controls or to the early third trimester group which were of similar gestational age but did not receive the injections of vehicle. Indeed, in the cervix, CA was significantly lower in treated compared to controls. Despite the fact that there were no increases in CA in the extracts from amniochorion in the treated animals, there were significant increases in CXCL1, CXCL8, and CCL2. This suggests low sensitivity to these chemokines of the leukocytes (from term pregnant rats) used in the CA assay. It is possible that these leukocytes have not yet developed the increased responsiveness that might occur immediately prior to parturition or their failure to respond could be related to a direct effect of the antiprogestin treatment.

In most rodent species, withdrawal of progesterone in late gestation is both necessary and sufficient to cause parturition.[47,48] Our results regarding maternal serum progesterone concentrations provide strong confirmation of earlier data using different methodologies[49] and show that in the guinea pig, as in the human, there is no decline in maternal serum progesterone concentrations during late gestation. However, this does not preclude a role for progesterone withdrawal in regulation of parturition in guinea pigs or humans as the changes in progesterone might occur in a paracrine fashion within intrauterine tissues and not be reflected in the maternal circulation.[22] Indeed, in human pregnancy, antiprogestin treatment early in pregnancy causes abortion and late in pregnancy results in some enhancement

of uterine contractility.[50,51] However, the antiprogestin itself usually is insufficient to complete the process of parturition and requires addition of a uterotonic agent to complete the process. This suggests that human parturition at term or preterm is not simply the result of progesterone withdrawal alone, which is consistent with earlier studies using RU486 in guinea pigs.[23] As we noted in previous studies,[52] antiprogestin treatment might interfere with feedback relationships between gonadotrophins from the hypothalamic-pituitary axis and the corpora lutea, which results in elevated maternal serum progesterone despite the blockade in progesterone effects.

RU486 is a progesterone receptor blocker. The fact that RU486 treatment did not result in increased CA from intrauterine tissues suggests that progesterone withdrawal is not the mechanism responsible for the increased CA at term parturition in this species. However, it is noteworthy that progesterone concentrations actually increased in maternal serum and this would be compatible with the concept that progesterone might suppress CA activity in a non-receptor-mediated mechanism.

A limitation to our study is that we did not quantitatively assess the infiltration of leukocytes into the intrauterine tissues. This leukocyte invasion is central to the potential role of the immune system in the initiation of parturition and is likely dependent on several factors including the production of chemokines as well as the sensitivity of leukocytes to these chemotactic factors. However, from a qualitative perspective, our immunohistochemical findings in the decidua–myometrium reflect the differences we observed for the chemotactic activity. Low numbers of leukocytes (mostly macrophages) were observed in the 3T and TNL decidua–myometrium. At term labor, there were many more leukocytes including many neutrophils. In the RU486-induced preterm labor group, only a few leukocytes were noted (again, mostly macrophages) and this was similar to the preterm controls. The surprising lack of enhancement of CA by, and leukocyte infiltration into, intrauterine tissues in the RU486-induced preterm labor model suggests that the inflammatory events that accompany preterm labor in the guinea pig are likely quite distinct from those at term, as has been noted in human studies as well.[8,40,46] Of interest, administration of glucocorticoids to pregnant women in preterm labor cause significant suppression of chemokine expression without affecting the timing of delivery.[8] These latter data support the multitude of randomized, placebo-controlled clinical trials where high doses of immunosuppressive glucocorticoids were administered to women at high risk of preterm birth without changing the interval to delivery.[53]

Conclusions

Many studies have documented that parturition, at term or preterm and in many species including human, is accompanied by a pro-inflammatory response within gestational tissues. However, no unique or consistent pattern has been described for leukocyte infiltration into gestational tissues or of chemokine production from specific tissues. The lack of unique and consistent patterns is likely a reflection of variability in experimental approaches regarding animal models, methods to assess CA, types of tissues assessed, and chemokines measured. Our data in guinea pig demonstrate that preterm parturition can occur without increased production of CA, and this is supported by other recent studies that demonstrate in mice that neutrophil infiltration into the cervix[45,46] or decidua[21] is not necessary for normal parturition. In many respects, the guinea pig appears to be a good model for the immunological events of human parturition. Further, these studies support the concept that the mechanism of LPS-induced 'inflammatory' preterm parturition, in which cervical leukocyte infiltration occurs, might differ significantly from the antiprogestin-induced preterm parturition model or normal term parturition in mice[21,45,46] and humans.[8] More studies will be necessary to determine whether the pro-inflammatory environment present at term parturition is merely a temporal association or a key regulator of parturition. Accumulating data, including the present studies, show that parturition can occur in the absence of a pro-inflammatory response and thus are compatible with the concept that the pro-inflammatory milieu at term parturition is directed toward other objectives such as remodeling of the cervix back to its non-gravid state,[45] healing of the decidual wound following placental separation, and the process of uterine involution.[22]

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