The definitive version of this article is published and available online as:
Sakamoto, Y.; Moran, P.; Bulmer, J.N.; Searle, R.F.; and Robson, S.C. Macrophages and not granulocytes are involved in cervical ripening. Journal of Reproductive Immunology 2005, 66, 161-173.
doi:10.1016/j.jri.2005.04.005

Macrophages and not granulocytes are involved in cervical ripening

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Short title: Macrophages and cervical ripening

ABSTRACT

OBJECTIVE: To clarify the role of leucocytes in human cervical ripening and dilatation.

METHODS: Cervical biopsies were obtained from 6 non-pregnant women, 8 women undergoing early termination of pregnancy, 18 pregnant women undergoing elective Caesarean section at term (both with and without a ripe cervix as determined by Bishop score) and 11 women after term vaginal delivery. Leucocytes were localised by immunohistochemistry labelling and quantified in subepithelial and deep stromal areas.

RESULTS: CD45+ leucocytes were more numerous in the subepithelial area of the cervix than in the deep stroma in all groups ($P<0.01$). CD14+ macrophages and CD15+ granulocytes were increased in both the subepithelial and deep stromal areas only in the vaginal delivery group ($P<0.01$). The number of macrophages in the ripening cervix (Bishop score above 4) was higher than in the unripe cervix (Bishop score 4 or less) ($P<0.05$) with no differences in other leucocyte populations. CD3+ CD8+ T cells in the subepithelial area were reduced in late pregnancy and after vaginal delivery ($P<0.01$) but showed no relationship with Bishop score.

CONCLUSION: Macrophages and granulocytes are crucially involved in the process of cervical dilatation, but macrophage infiltration into the ripening cervix before labour suggests their important role in the ripening process. Reduced numbers of CD3+ CD8+ T-lymphocytes in late pregnancy and after vaginal delivery suggests that local immunity is downregulated in the late pregnancy period. Regional differences in leucocyte subpopulations in the cervix indicate that leucocyte infiltration is likely to be regulated by local factors.

Keywords: Cervix, Ripening, Granulocyte, Macrophage, T-Lymphocyte

INTRODUCTION

For most of gestation the human uterine cervix remains rigid and closed but prior to labour it undergoes softening and effacement, a process termed ripening. Ripening occurs prior to dilatation proper, is a prerequisite for vaginal delivery and is associated with collagen remodelling and altered proteoglycan and water content (Junqueira et al 1980, Osmers et al 1993, 1995). Absence of normal ripening at term is associated with prolonged labour and post-term pregnancy, whereas premature ripening occurs as part of the preterm delivery syndrome or a second trimester abortion (Olah & Gee 1992).

There is increasing evidence that the process of cervical dilatation resembles an inflammatory response (Liggins 1981, Yellon et al 2003). Leucocytes migrate into cervical stroma and mucus during labour reaching a density 2-3 fold higher than that found in late pregnancy (Luo et al. 2000, Young et al. 2002, Osman et al. 2003); the stromal infiltrate is composed principally of neutrophils and macrophages (Osman et al. 2003). Identical findings have been reported in the lower uterine segment during labour (Thomson et al 1999, Winkler et al 1999). In contrast other workers reported an increase in macrophage and neutrophil numbers in cervical stroma during late pregnancy but no further changes during labour (Bokstrom et al 1977). This discrepancy may relate to differences in cervical ripening at the time of elective caesarean section but no clinical information on the state of the cervix was provided in any of these studies. Further it is unclear whether leucocyte distribution is different in the cervical epithelium.

To determine if leucocyte influx into the cervix is a feature of cervical ripening or follows cervical dilatation in labour we compared leucocyte numbers in the cervical

stroma and epithelium in women with and without a ripe cervix, as determined by Bishop's pelvic score.

MATERIALS AND METHODS

Subjects

Forty-three healthy women were recruited from the Obstetric and Gynaecology wards at the Royal Victoria Infirmary, Newcastle upon Tyne. The study was approved by the Joint Ethics Committee Newcastle and North Tyneside Health Authority and written informed consent was obtained from each subject. The non-pregnant group consisted of 6 regularly menstruating women (median age 42.5 [range 40-43] years, median parity 2 [range 1-4]), undergoing hysterectomy for non-malignant disease. None of the women had cervical disease. The early pregnant group consisted of 8 women (median age 25 [range 21-34] years, median parity 1 [range 0-3]) undergoing surgical termination of pregnancy under general anaesthesia at a median gestational age of 12^{+1} [range 8^{+0}- 13^{+0}] weeks. None of the women received prostaglandin prior to surgery. The late pregnant group consisted of 18 healthy non-labouring pregnant women (median age 31 [range 23-42] years, median parity 1 [range 0-3]) undergoing elective caesarean section at a median gestational age of 39^{+0} (range 37^{+2}- 41^{+0}) weeks. The indications for caesarean section were previous caesarean section or breech presentation. The vaginal delivery group consisted of 11 women (median age 27 [range 22-33] years, median parity 1 [range 0-2]) who had just completed a vaginal delivery following spontaneous labour at term (median gestational age 40^{+2} [range 39^{+0} – 41^{+1}] weeks).

Sample collection

One or two punch biopsies (each 20-25 mg) were taken from the anterior and/or posterior cervical lips using a 6 mm biopsy needle (Stiefel Laboratories, Wooburn Green, Bucks, UK) (Ledingham et al 1999). The biopsies from non-pregnant women were taken in the operating theatre immediately after hysterectomy. Cervical biopsies from early pregnant, late pregnant or vaginal delivery groups were taken transvaginally immediately after surgical termination, Caesarean section or vaginal delivery. Biopsies were immediately snap-frozen in liquid nitrogen-cooled isopentane and stored at -70°C until assayed.

In the late pregnant group undergoing Caesarean section, the cervix was assessed by digital vaginal examination by one of the authors (PM) and scored using Bishop's pelvic score (Bishop 1964, Walker & Resnik 1993). This consisted of a score of 0 to 3 each for cervical dilatation, length, and station of the fetal head, and 0 to 2 for consistency and position. A Bishop score >4 was taken to indicate a ripening cervix while a score >8 indicated a ripe cervix. For all subjects the same obstetrician (PM) evaluated the Bishop's pelvic score to standardise clinical evaluations.

All subjects had unremarkable post operation or post partum course.

Immunohistochemistry

Immunohistochemistry was performed using an avidin-biotin-peroxidase method (Vectastain Elite; Vector Laboratories, Peterborough, UK). Primary antibodies are detailed in Table 1. Serial 7μm frozen sections of intact cervix were mounted on APES (Sigma Chemical Co., Poole, UK)-coated slides and fixed in acetone for 10 minutes at room temperature. Each biopsy was stained with haematoxylin and eosin for histological

analysis. Sections were rehydrated in 0.05 M Tris buffered 0.15 M saline, pH 7.6 (TBS) for 10 minutes and the supplied normal horse serum was applied for 10 minutes at room temperature to prevent non-specific antibody binding. The sections were then incubated with appropriately diluted primary monoclonal antibody (mAb) in TBS. Optimal dilutions and incubation times for each antibody were determined using sections of tonsil. Sections were washed then three times in TBS, incubated with biotinylated horse anti-mouse immunoglobulins for 30 minutes, washed again in TBS, and overlain with avidin-biotin-peroxidase complex for 30 minutes. Bound primary mAbs were detected by incubation with 0.05% 3,3'-diaminobenzidine-tetrahydrochloride (DAB) (Sigma Chemical Co.) containing 0.03% hydrogen peroxide for 5 minutes. Sections were counterstained in Mayer's haematoxylin (BDH, Poole, UK) and mounted in synthetic resin. Negative controls were performed for all samples and sections were incubated with normal serum instead of primary mAb. Cryostat sections of tonsil were included as positive controls in each staining run.

Quantification of positive stained cells

Positive cells, identified by the presence of brown staining, were counted at x400 magnification using a 10x10 mm graticule covering an area of 0.0625 mm^2. Because the leucocyte populations differed in the subepithelial and deep stromal areas, these areas were assessed separately. The subepithelial area was defined as the area immediately beneath the basal layer of the squamous epithelium to an actual depth of no more than 0.25mm; counting in the deep stromal area was performed at a minimum depth of 1mm from the basal layer of the overlying squamous epithelium. Positive cells were counted in a minimum of five x400 fields and the mean count was used for statistical analysis.

Data and statistical analysis

Statistical analysis was performed with Statview (Berkeley, CA). Data are reported as mean number of cells per x400 field ± standard error of the mean (SEM). Differences in leucocyte numbers between the subepithelial and deep stromal areas were compared with Wilcoxon signed rank test. Differences in leucocyte numbers between groups were compared by Kruskal-Wallis test. When a statistically significant effect was indicated, differences between individual groups were compared using Bonferroni/Dunn's post-hoc multiple comparison. Differences in the number of leucocytes in unripe and ripening cervices were compared with Mann-Whitney's U test. The conventional level of 0.05 was taken as the limit of significance.

RESULTS

All negative controls were unstained and positive controls showed the expected immunoreactivity (data not shown). Mean numbers of the major cervical leucocyte populations in the 4 subject groups are shown in Table 2. The number of CD45+ leucocytes in the subepithelial area of the cervix was higher than in the deep stroma in all subject groups ($P<0.01$). The mean number of CD45+ leucocytes in the deep stroma was increased after vaginal delivery compared to the early pregnant group ($P<0.05$) but did not differ from the non-pregnant or late pregnant groups.

Macrophages and granulocytes

The numbers of CD14+ macrophages and CD15+ granulocytes are illustrated in Figure 1 and shown in Figure 2. The number of CD14+ macrophages in the subepithelial area in the vaginal delivery group was higher than in the non-pregnant, early pregnant and late

pregnant groups ($P<0.01$). The same pattern was seen in the deep stromal area (Figure 1, Table 2, $P<0.01$). Similarly the number of CD15+ granulocytes in the subepithelial and deep stromal areas was also higher in the vaginal delivery group compared to non-pregnant, early pregnant and late pregnant groups (Figure 1, subepithelial; $P<0.01$; deep stroma; $P<0.01$).

T-lymphocytes

T-lymphocytes were the predominant subpopulation of leucocytes in the subepithelial area. The numbers of CD3+, CD4+ and CD8+ T-lymphocytes in the subepithelial area were higher than in the deep stroma in all subject groups (Table 2; $P<0.01$, $P<0.05$ and $P<0.01$, respectively). Subepithelial CD3+ and CD8+ T-lymphocyte numbers were lower in late pregnancy and after vaginal delivery compared to the non-pregnant group (CD3+; $P<0.01$; CD8+; $P<0.01$).

B-lymphocytes, plasma cells and natural killer cells

B-lymphocytes, plasma cells and natural killer cells were rarely observed in the cervix. Although numbers were low, compared to the deep stroma, there were more CD79a+ B-lymphocytes in the subepithelial area of early and late pregnant groups (Table 2, $P<0.05$). However, there were no differences in either CD20+ or CD79a+ counts between any of the subject groups. Numbers of CD16+ or CD56+ cells in the subepithelial area of the late pregnant group were higher than in the deep-stromal area (Table 2, $P<0.01$). Comparing subject groups, the number of CD16+ cells in the subepithelial area of the late pregnant and vaginal delivery groups was greater than in the non-pregnant and early pregnancy groups (Table 2, $P<0.01$). The number of CD16+ cells in the deep stroma of the vaginal delivery group was also greater than in the non-pregnant, early and late

pregnancy groups (Table 2, $P<0.01$). There were no differences in CD56+ cell numbers between subject groups.

Leucocyte populations in women with and without a ripe cervix

Leucocyte numbers in both subepithelial and deep stromal areas in relation to Bishop score are shown in Table 3. In the late pregnancy group, five women with a ripening cervix (Bishop score of >4) had increased numbers of CD14+ macrophages in both subepithelial and deep stromal areas compared to women with an unripe cervix (Bishop score 4 or less, n=13) (Figure 1, Table 3, subepithelial $P<0.05$; deep stromal $P<0.01$). No differences were found in other leucocyte populations.

DISCUSSION

Despite their important role in the local immune response, knowledge of the distribution of leucocyte populations in the human cervix is limited. This study has found marked regional differences in leucocyte distribution between the subepithelial and deep stromal areas of the cervix regardless of the reproductive status, suggesting that leucocyte infiltration into the cervix is, at least in part, regulated by local factors. It is likely that there are more leucocytes in the subepithelial area compared with the deep stromal area for local immunity in the cervix. Moreover this study detected increased number of macrophages and granulocytes after vaginal delivery, indicating that leucocytes are crucially involved in the process of cervical dilatation, but the presence of increased numbers of macrophages in the ripening cervix before the onset of labour indicates their important role in the ripening process.

Although activated neutrophils and macrophages contain inflammatory mediators such as plasminogen activators, eicosanoids, collagenase, interleukin-1 and tumor necrosis

factor-α (Nathan 1987, Osmers et al 1992, Casatella 1995), their precise role in the processes of cervical ripening and dilatation is unclear. Other potential triggers include cyclic mechanical stretch, relaxin, nitric oxide, insulin like growth factor 1, matrix metalloproteinase-8 and dithiol redox enzymes as well as cytokines/chemokines including IL-1α, IL-6, IL-8, MCP-1 and PAF (Kelly et al 2001, Sugano et al 2002, Yoshida et al 2002, Ekman-Ordeberg et al 2003, Lysell et al 2003, Sennstrom et al 2003, Sakamoto et al 2004, Vaisanen-Tommiska et al 2004). Stjernholm et al (1996, 1997) have also shown that labour is associated with a down-regulation of oestrogen and progesterone receptor levels in the cervix and a switch to a more ERβ influenced state, ERβ localising with CD45 leucocyte antigen and CD68 macrophage specific antigen expression in the cervix. The present study has demonstrated that CD45+ leucocytes, namely granulocytes and macrophages, dramatically increased after vaginal delivery but did not increase significantly in the late pregnant prelabour group compared with early pregnancy. Moreover these findings mirror local pro-inflammatory cytokine production (Sakamoto et al 2004) as IL-8 levels also increase dramatically in both cervical stroma and epithelium after labour and vaginal delivery. These infiltrating granulocytes and macrophages express IL-8 receptors inferring that IL-8 may play a crucial role in the recruitment of leucocytes in an autocrine or paracrine manner during labour (Sakamoto et al 2004).

To determine whether leucocytes are involved in the events of cervical ripening, leucocytes were compared in late pregnant prelabour women with and without a ripe cervix. The number of CD14+ macrophages in the ripening cervix (Bishop score above 4) was significantly higher than in the unripe cervix (Bishop score 4 or less), whereas granulocytes were sparse even in the ripening cervix. These findings indicate not only

that macrophages and granulocytes are crucially involved in the process of cervical dilatation, but also that macrophages are likely to play an important role in the ripening process since their infiltration into the cervix proceeds labour. Ideally we should have examined women with very favourable cervices but in practice it proved difficult to recruit these women (Bishop score >8), and therefore we could not clarify the precise role of macrophages in the final ripening process. Importantly we cannot exclude the possibility that granulocytes may also increase late in the ripening process. An alternative approach would be to sample women in preterm labour who often have favourable cervices but this has the disadvantage that the mechanisms involved in cervical ripening and dilatation may differ from those in spontaneous term labour.

B-lymphocytes and plasma cells were sparse in the cervix regardless of sample group. A recent immunohistochemical study of human non-pregnant cervix reported that B-lymphocytes are few in number and are found only in lymphoid aggregates, and that plasma cells are present in lamina propria (Johansson et al 1999). The present findings concur with these findings and indicate that B-lymphocytes and plasma cells are not involved in the process of cervical ripening or dilatation.

CD16 and CD56 are mainly expressed on NK cells, but CD16 is also weakly expressed by granulocytes and macrophages. While the present findings of an increase in CD16+ cells in the vaginal delivery group may suggest a role for NK cells in cervical dilatation, the fact that there was no concomitant increase in CD56+ cells suggests that this increase is more likely to reflect the increased numbers of macrophages and granulocytes. Interestingly NK cells express receptors for interleukin-8 (IL-8) (Chuntharapai et al 1994), and IL-8 levels in the cervix are dramatically increased during labour (Sennstrom

et al 2000, Sakamoto et al 2004). Further studies are needed to clarify the role of NK cells in the process of cervical ripening and dilation.

CD3+ T-lymphocytes were the predominant lymphocyte population in the cervix, with numbers of both CD4+ and CD8+ subsets being consistently higher in the subepithelial area compared to the deep stroma. These findings are consistent with those of Johansson et al (1999) who suggested that T-lymphocytes concentrate directly beneath the epithelium in order to make an effective response against genital pathogens or epithelial neoplasia. Both progesterone and estrogen have been shown to suppress T-lymphocyte function (Ahmed et al 1985, Szekeres-Bartho et al 1985) and a decline in cervical T cell numbers in pregnancy may suggest down regulation of the local immune response (Billington 1992).

The decline in the number of subepithelial CD3+ T lymphocytes during pregnancy, principally secondary to a fall in CD8+ suppressor/cytotoxic T cells, suggests that T cells are not involved in cervical ripening or dilatation. In contrast Bokstrom et al (1997), using monoclonal antibodies with the same specificity as those in the present study, reported an increase in CD8+ cell numbers in late pregnancy, relative to early pregnancy, and then a fall during labour, while CD4+ numbers were stable during pregnancy but increased after labour. It is worth noting, however, that the total number of CD4+ and CD8+ cells exceeded that of CD3+ cells, raising the possibility that other cells that express CD4, such as macrophages, may have been included in the CD4 population. Furthermore, whereas in the present study leucocytes were separately quantified in both subepithelial and deep stromal areas, Bokstrom and coworkers specified only that areas with blood vessels were excluded.

In summary, this study has demonstrated that macrophages and granulocytes are crucially involved in the process of cervical dilatation, and that the infiltration of macrophages into the cervix before labour may play a key role in the process of cervical ripening. B-lymphocytes and plasma cells are not involved in the process of cervical ripening or dilatation, but further studies are needed to clarify the role of NK cells in the process. The decrease in the number of CD3+ CD8+ T-lymphocytes in the subepithelial area in the cervix in the late pregnancy and vaginal delivery groups suggests the down regulation of local immunity in the cervix in the late pregnancy period. The regional differences in leucocyte subpopulations between the subepithelial and deep-stromal areas indicate that leucocyte infiltration into the cervix is likely to be regulated by local factors.

Acknowledgement

We thank Mrs. Barbara Innes for assistance with immunohistochemistry and all staff in the labour and operating ward of the Royal Victoria Infirmary for their assistance with sample collection. Dr Sakamoto was supported by the Researcher Overseas Programme of Nara Medical University, Japan.

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Table 1: Primary monoclonal antibodies

Monoclonal antibody	Specificity	Dilution	Source	Clone
CD45	Leucocytes	1:100	Novocastra[1]	X16/99
CD3	T-lymphocytes	1:200	Novocastra	UCHT1
CD4	T subset (helper/inducer)	1:75	Novocastra	Edu-2
CD8	T subset (suppressor/cytotoxic)	1:50	Dako[2]	UCH-T4
CD20	B-lymphocytes	1:600	Dako	L26
CD79a	B-lymphocytes, plasma cells	1:50	Novocastra	HM47/A9
CD14	Macrophages	1:25	Novocastra	M-M-42
CD15	Granulocytes	1:10	BD Bioscience[3]	BY87
CD16	Natural killer cells, T subset, macrophages, granulocytes	1:10	Novocastra	3G8
CD56	Natural killer cells, T subset	1:100	Novocastra	ERIC-1

[1] Novocastra Laboratories Ltd.; Newcastle upon Tyne, UK

[2] Dako; Ely, UK

[3] BD Biosciences; Lexington, USA

Table 2: Leucocyte numbers[+] in non-pregnant, early pregnant, late pregnant and vaginal delivery groups

Leucocyte population	Non-pregnant	Early pregnant	Late pregnant	Vaginal delivery
Sub-epithelial area				
CD45	54.70 ± 4.00	49.30 ± 3.10	42.10 ± 3.30	44.70 ± 4.50
CD14	**1.17 ± 0.96**	**0.46 ± 0.22**	**1.10 ± 0.25**	**9.27 ± 2.99*[3]**
CD15	**0.08 ± 0.08**	**0.28 ± 0.13**	**0.73 ± 0.17**	**2.82 ± 0.18*[3]**
CD3	**48.50 ± 2.60**	32.00 ± 4.20	**26.80 ± 3.20*[1]**	**17.80 ± 3.20*[1]**
CD4	14.10 ± 5.30	7.30 ± 2.40	10.30 ± 1.40	4.50 ± 1.30
CD8	**33.20 ± 6.30**	22.10 ± 3.60	**15.30 ± 1.60*[1]**	**12.20 ± 1.80*[1]**
CD20	0.53 ± 0.24	0.74 ± 0.30	1.14 ± 0.49	0.15 ± 0.08
CD79a	1.36 ± 0.86	0.76 ± 0.26	1.72 ± 0.67	0.17 ± 0.05
CD16	**0.06 ± 0.06**	**0.08 ± 0.04**	**0.55 ± 0.10*[2]**	**0.70 ± 0.13*[2]**
CD56	0.27 ± 0.14	0.18 ± 0.07	1.17 ± 0.24	0.62 ± 0.18
Deep stromal area				
CD45	7.60 ± 0.72	**6.61 ± 0.62**	9.88 ± 0.64	**14.10 ± 2.80*[4]**
CD14	**0.80 ± 0.25**	**0.50 ± 0.13**	**1.79 ± 0.38**	**8.15 ± 1.44*[3]**
CD15	**0.08 ± 0.05**	**0.11 ± 0.03**	**0.15 ± 0.06**	**9.78 ± 4.05*[3]**
CD3	2.52 ± 0.48	3.30 ± 0.51	3.99 ± 0.66	2.51 ± 0.54
CD4	1.18 ± 0.25	0.70 ± 0.26	1.45 ± 0.27	1.19 ± 0.73
CD8	1.75 ± 0.29	2.25 ± 0.36	3.21 ± 0.51	1.72 ± 0.34
CD20	0.38 ± 0.11	0.18 ± 0.07	0.40 ± 0.18	0.14 ± 0.09
CD79a	0.12 ± 0.06	0.08 ± 0.06	0.21 ± 0.06	0.19 ± 0.08
CD16	**0.03 ± 0.03**	**0.01 ± 0.01**	**0.21 ± 0.07**	**4.39 ± 1.70*[3]**
CD56	0.17 ± 0.11	0.21 ± 0.06	0.43 ± 0.13	2.13 ± 1.04

+ Leucocyte numbers are presented as mean ± SEM

Significant differences are highlighted

*[1] P <0.01 v. non-pregnant group

*[2] P <0.01 v. non-pregnant and early pregnant group

*[3] P <0.01 v. non-pregnant, early pregnant and late pregnant group

*[4] P <0.05 v. early pregnant group

Table 3 Leucocyte numbers[+] in low (4 or less) and high (> 4) Bishop score cases

Leucocyte population	Low Bishop Score (4 or less)	High Bishop Score (>4)
Sub-epithelial area		
CD14	**0.69 ± 0.16**	**2.14 ± 0.77***
CD15	0.79 ± 0.21	0.50 ± 0.25
CD45	49.20 ± 4.30	37.60 ± 2.80
CD3	28.80 ± 3.80	19.40 ± 1.70
CD4	10.70 ± 1.70	9.10 ± 1.40
CD8	16.50 ± 1.70	11.10 ± 3.30
CD20	1.26 ± 0.06	0.63 ± 0.26
CD79a	1.98 ± 0.81	0.66 ± 0.47
CD16	0.51 ± 0.09	0.68 ± 0.38
CD56	1.27 ± 0.29	0.78 ± 0.22
Deep stromal area		
CD14	**1.20 ± 0.18**	**3.85 ± 1.16****
CD15	0.14 ± 0.07	0.21 ± 0.11
CD45	16.50 ± 1.20	16.50 ± 2.40
CD3	3.58 ± 0.59	5.43 ± 2.26
CD4	1.56 ± 0.33	1.08 ± 0.37
CD8	2.91 ± 0.41	4.24 ± 1.87
CD20	0.47 ± 0.23	0.15 ± 0.10
CD79a	0.21 ± 0.07	0.21 ± 0.14
CD16	0.18 ± 0.08	0.31 ± 0.20
CD56	0.42 ± 0.16	0.46 ± 0.20

+ Leucocyte numbers are presented as mean ± SEM

Significant differences are highlighted

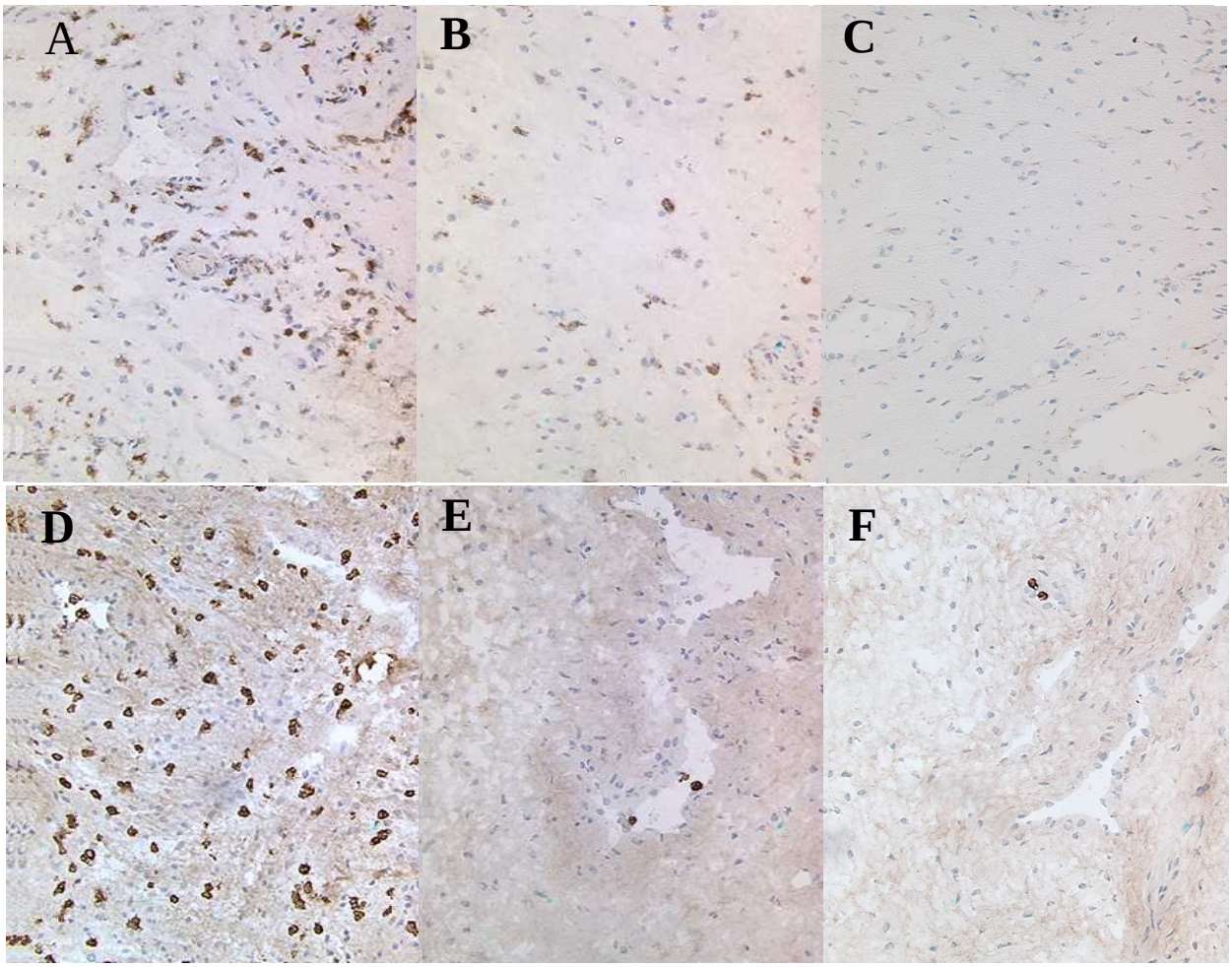
* $P < 0.05$

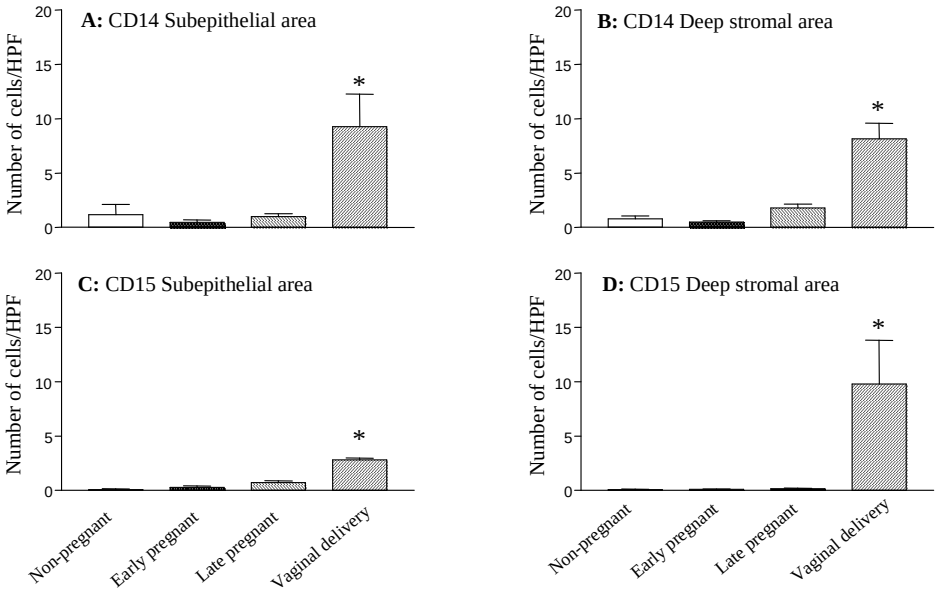
** $P < 0.01$

FIGURE LEGENDS

Figure 1: Avidin-biotin-peroxidase immunostaining for CD14+ macrophages showing their dramatic increase in cervical stroma of the vaginal delivery group (A). Increased numbers of macrophages were also observed in the late pregnant ripening cervix (Bishop score >4) (B), whilst they were sparse in the late pregnant unripe cervix (Bishop score 4 or less) (C). CD15+ granulocytes were dramatically increased in the vaginal delivery group (D) but were localized only within blood vessels in the other groups (E: late pregnant ripening cervix, F: late pregnant unripe cervix).
Original magnification x200.

Figure 2: CD14+ macrophages (A, B) and CD15+ granulocytes (C, D) in the subepithelial and deep stromal areas. Each column represents the mean and SEM. The number of macrophages or granulocytes was significantly higher in both subepithelial and deep stromal areas of the vaginal delivery group compared with the other groups (* $P<0.01$).


A
B
C
D
E
F


A: CD14 Subepithelial area
B: CD14 Deep stromal area
C: CD15 Subepithelial area
D: CD15 Deep stromal area
Number of cells/HPF
0
5
10
15
20
*
Non-pregnant
Early pregnant
Late pregnant
Vaginal delivery