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Immunomorphological changes in the rhesus monkey endometrium and decidua during the menstrual cycle and early pregnancy

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Abstract

Problem—Throughout the reproductive cycle and into early pregnancy, the normal endometrium undergoes changes in a range of leukocytes, epithelia, stromal fibroblasts, and vascular structures caused by intersecting effects of hormone balance and embryo implantation. The direct investigation in humans of reproductive tract responses during normal and physiologically altered cycles is not practical or feasible.

Method and Study—The aim of this study was to define immunological and morphological changes through immunohistological and morphometric evaluation of the endometrium throughout the menstrual cycle and the decidua during early gestation in the rhesus monkey, a tractable experimental animal model.

Results—A zone-dependent method for the immunohistological description of the rhesus uterine mucosa was established, and showed that leukocyte infiltration, stromal cell decidualization, glandular and vascular responses were zone- and cell type-dependent, and changed throughout the cycle and early pregnancy. Morphological heterogeneity of uterine NK cells in the cycling endometrium and gestational decidua were consistent with the recent characterization of phenotypic subsets.

Conclusions—These data establish a morphological platform upon which to further study the regulation of endometrial responses to the hormonal milieu of pregnancy, the control of local leukocyte populations, and the responses to threatened pregnancy, infection, and inflammation.

Keywords

Endometrium; NK cell; decidua; rhesus monkey

INTRODUCTION

The rhesus macaque shares many common structural and physiological features with the human female reproductive tract, including similarity of uterine gross anatomy, and generally analogous morphological changes within the endometrium during the proliferative

and secretory phases of the natural 28-day menstrual cycle.^{1–5} Despite some differences between macaque and human placentas (e.g., number of discs, extent of interstitial trophoblast invasion) their basic morphological and functional characteristics (villous, hemochorial and discoid) are very similar.^{6–10} These similarities make the rhesus monkey a valuable animal model for studies of local tissue/cellular regulation and responses in the normal cycling endometrium and in the gestational decidua.

Based on epithelial, stromal and vascular elements, Bartelmez proposed a horizontal zonation of the rhesus endometrium.¹ Secretory differentiation and menses occur in Bartelmez's zones I–III, and these zones are combined frequently and referred to as the functionalis zone. The upper region of the functionalis (zone I and part of zone II) slough away as menstruation ensues, whereas the lower level, including zones III and IV (and occasionally zone II) are retained following menses.^{4,5,11} Lying approximately between these two (upper and lower) regions is the zone of placental separation,¹² where the basal plate (the segment of the decidua that adheres to the delivered placenta) separates from the placental bed (the basal portion of the decidua attached to the myometrium, which remains in the uterus after delivery).¹³

The endometrium and the decidua include different tissue types (epithelium, stromal fibroblasts, vascular endothelium and smooth muscle, and a range of leukocytes) that respond to physiological stimuli (hormones, cytokines, embryo implantation and placental development, etc). Previous studies have shown zone-dependent cellular responses in the rhesus endometrium to the same hormone stimuli, but these studies have focused on epithelial and stromal cells,^{11,14–18} and the endothelium of the spiral arteries.^{19–21}

In the human uterus as well as in several animal species, leukocyte infiltration of endometrial and decidual stroma has been widely demonstrated. Leukocytes constitute around 10% of the total cellular content of the stroma in human proliferative and early secretory endometrium, and their number significantly increase in the late secretory phase, and contribute up to 32% in first trimester decidua.²² The proportions of different leukocyte cell types change with the menstrual cycle phase and pregnancy development. The largest subset of leukocytes, from 50% in the human proliferative endometrium and up to 80% in the early pregnant decidua, are natural killer (NK) cells. Macrophages are also present in the human endometrium throughout the cycle, with a small increase in the premenstrual period, or an increase to 38% of CD45+ cells in early pregnancy.^{22,23} The remainder of leukocytes are dendritic cells (DC), T cell subsets and neutrophils.

In macaques, as in women, only a few scattered leukocytes may be found in the proliferative and early secretory stroma, but the differentiation of the fibroblastic stroma into predecidual/decidual cells with postovulatory hormonal secretion and embryo implantation coincides with abundant leukocyte infiltration of the endometrium.^{24,25} The majority of rhesus decidual leukocytes, as with human early pregnancy, are NK cells and macrophages, with modest numbers of T cells.^{26,27} NK cells and macrophages are present in the proliferative and secretory non-pregnant endometrium and dramatically increased in early pregnant decidua, while DC-SIGN cells were observed only in the pregnant endometrium and their numbers increased during the first weeks of gestation.²⁸ It has been also shown that macrophages and DC-SIGN+ cells are distributed in close vicinity to the implantation site.^{27,28} However, the zonal distribution of leukocytic subsets in rhesus endometrial/decidual tissue remains undefined.

Thus, the aim of this study was to define the zone-dependent immunomorphological changes in rhesus monkey endometrium throughout the menstrual cycle, and the decidua during early gestation. The results obtained demonstrated differential distribution of leukocytes in the

rhesus endometrium and decidua, as well as morphologically distinct endometrial and decidual NK cell subpopulations, suggesting differential trafficking and/or progenitor expansion and differentiation during the reproductive cycle.

MATERIAL AND METHODS

Animals and tissue preparation

Adult female rhesus monkeys (*Macaca mulatta*) were from the colony maintained at the National Primate Research Center, University of Wisconsin, Madison. All experimental procedures were performed in accordance with the NIH guidelines for Care and Use of Laboratory Animals and under the approval of the University of Wisconsin Graduate School Animal Care and Use Committee. Eleven non-pregnant uteri were obtained from cycling animals: 3 animals on days 7–8 of the cycle (middle follicular phase (dF7–8), 1 = the first day of menses), one each on luteal phase day 2 (dL2) and dL7 (early-mid luteal; dL0 = the day after the ovulatory luteinizing hormone peak as determined by radioimmunoassay), and 6 on dL10–14 (late luteal). Rhesus monkey implantation sites were obtained from normal pregnant animals on gestational day (gd) 18–20 (4 animals) and gd24–25 (4 animals) (day 0 = the day after the ovulatory luteinizing hormone peak, gd ~ 165 = term). All tissues were treated identically in a standardized manner. Intact nonpregnant uterine tissue and uteroplacental units were collected from euthanized animals following perfusion with 2% paraformaldehyde/PBS, as previously described.²⁹

Immunohistochemistry

Paraffin sections (5 μ m) were stained with primary antibodies and visualized using a Vectastain ABC peroxidase complex kit and Vector NovaRed substrate kit for peroxidase (Vector Laboratories, Burlingame, CA) generally as described previously.^{28,30} The antibodies used and their working concentrations are given in Supplemental Table I. Mouse IgG1, IgG2, or normal rabbit serum (Sigma-Aldrich Inc, St. Louis, MO) served as negative controls.

Double Perforin/CD3 immunostaining was performed using a sequential immunoenzymatic labeling technique as previously described.²² Anti-perforin mAb was first visualized using a Vectastain ABC peroxidase complex Kit and Vector NovaRed substrate kit for peroxidase. After washing, the sections were incubated with polyclonal rabbit anti-CD3, which was visualized using Vectastain ABC-AP Kit and Vector Blue Alkaline Phosphatase Substrate Kit III (Vector Laboratories, Burlingame, CA)

Quantitative analysis

To reveal endometrial zone-dependent changes in the distribution of leukocyte subsets, we divided the rhesus endometrium and decidua into two zones. The upper zone (UZ) includes Bartelmez's zone I and part of zone II, which sloughs during menses and resembles the basal plate in the decidua. The lower zone (LZ) comprises zones III and IV and occasionally zone II, which survives menses and corresponds to the placental bed in pregnancy (Fig. 1). Histological analysis revealed that clusters of coiled spiral artery cross-sections with a surrounding dense lymphoid infiltrate were present in that part of the endometrium, which remained in the uterus after menses (Fig. 1D). The apical points of those clusters may serve as a distinguishing histological marker for the border between the UZ and LZ in the rhesus monkey endometrium.

Leukocyte infiltration was examined separately in the UZ and LZ of the non-pregnant endometrium and gestational decidua. Cell and vascular counts were obtained from digital images captured from 15 different fields of each zone at 400x final magnification,

maximizing stromal tissue and limiting glandular and epithelial content. Images for the estimation of gland diameter and density were taken at 100x. A Leica MMIRB microscope, equipped with a MagnaFire digital camera and software was used. Images were taken of slides free of fixation, sectioning or staining artifacts, and analyzed for specific immunolabeled cells using NIH ImageJ software.³¹ The number of uNK cells (perforin+ and CD56+), T cells (CD3+), macrophages (CD68+), DC-SIGN+ cells, polymorphonuclear leukocytes (PMNL, neutrophils) (neutrophil elastase+), and stromal cells (hematoxylin/eosin staining) were counted within each measured field (100×100 μm , 0.01 mm^2) using a grid overlay on each image. Mean cell density per square millimeter of stromal tissue was calculated.^{32,33}

The maximal diameter of glands was determined by measuring the distance between the basement membranes on opposite sides of a glandular profile. To evaluate vascular and glandular density all vessels (immunostaining with anti-Von Willebrand Factor mAb) or glands (immunostaining with anti-Cytokeratin mAb) were counted per unit area in a similar region within each specimen. Vascular and glandular densities were calculated as the ratio of vascular or glandular surface per mm^2 (mm^2/mm^2) of decidual stroma.^{34,35}

Endometrial thickness³⁶ was assessed by measuring in digital images captured at 50x magnification the distance from the luminal surface in the non-pregnant uterus, and from the trophoblast-endometrial junctional zone³⁷ in the pregnant uterus, to the apical points of the coiled spiral artery cross-sections clusters to define the UZ, and from the latter to the endometrial-myometrial border to define the LZ. The sum of the thicknesses of the UZ and LZ (total endometrial thickness) and the UZ or LZ to total thickness ratio was calculated. All measurements in pregnancy were taken from the central part of the implantation site.

Statistical analysis of the data was performed using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA). Mean $\pm$ SD were calculated for each group and the statistical significance of the differences between pairs of groups was assessed using t-test.

RESULTS

Leukocytic populations in the endometrium and decidua

CD56+ and perforin+ NK cells, CD3+ T cells, CD68+ macrophages, and elastase+ PMNL are present in both UZ and LZ of the rhesus monkey endometrium, and their number varied throughout the menstrual cycle and early pregnancy (Fig. 2). A distinguishing sign of rhesus pregnancy vs. the non-pregnant endometrium was the appearance of DC-SIGN+ cells among the decidual leukocytes (Fig. 2). Except for a slight (but significant) decrease of CD56+ cells in the LZ in the first part of the secretory stage compared to the proliferative ($p<0.05$), leukocytic populations did not change significantly during the follicular and early-mid luteal phases of the cycle in both zones. In the UZ and LZ, macrophages and T cells, respectively, are the major subsets through proliferative-mid secretory stages. By the late luteal stage (dL10–14), the number of NK cells was significantly increased in the UZ, and along with CD68+ macrophages, constitute the majority in both the UZ and LZ (Fig. 2). The number of T cells in the UZ is lower than in the LZ, where there is also an increase in early gestation. In the LZ, we noted that CD3+ T cells in the late luteal phase (Supplemental Fig. 1) typically form dense perivascular infiltrates, as we have previously shown at gd18–24.^{27,29} Only modest numbers of PMNL were present in either endometrial level throughout the cycle. However, DC-SIGN+ cell and PMNL (Supplemental Fig. 1) infiltration increased dramatically at gd24–25 in the UZ, although in the LZ their density remained very low. This suggests that in the UZ PMNL trafficking and macrophage differentiation is selectively promoted.

Perforin-positive cells in the endometrium and decidua

Based on cell and nucleus size, nuclear/cytoplasm ratio, and number and distribution of cytoplasmic granules (Fig. 3), we identified three populations of perforin-positive uNK cells that differentially populated the uterine mucosa throughout the cycle and early pregnancy. Small granular lymphocytes (SGL) were identified as small cells (diameter (d) = 5.2–7.6 μm) with small nuclei (d =4.0–6.4 μm) and few perforin granules in a scant cytoplasm (Fig. 3A, D). These CD3[−] cells had a similar appearance to decidual CD3⁺/perforin⁺ T lymphocytes (Supplemental Fig. 2A). The subtype of medium granular lymphocytes (MGL), similar to SGL, had a similar small nucleus (d =4.7–6.9 μm), but expanded cytoplasm (d =8.0–13.0 μm) densely packed with perforin, which formed a compact aggregate, often localized to one pole of the cell (Fig. 3D, E). By comparison, the majority of perforin⁺ lymphocytes in iliac lymph nodes (LN) (Fig. 3G, H) and spleen (Fig. 3I) were SGL, with only a few MGL. A third subset of uterine perforin⁺ cells had a morphological appearance of classic human large granular lymphocytes (LGL) (Fig. 3C, F) with large nuclei (d =7.2–13.0 μm), and a higher number of perforin granules than other subtypes dispersed throughout a wide cytoplasm (d =11.5–22.1 μm). This resembled the broad perforin granular distribution seen in perforin⁺/CD3[−] peripheral blood lymphocytes occasionally observed in decidual vessels (Supplemental Fig. 2B). Supplemental Table II summarizes the morphological features of uterine NK cells.

SGL and MGL were found in both levels of the endometrium throughout the cycle and early pregnancy (Fig. 4). Their density was steady during the cycle, and elevated in early gestation. In contrast, LGL were not found in either region in the follicular phase or in the LZ in early-mid luteal phase. Only a few cells of this subset were present later in the cycling endometrium. The earliest that LGL appeared in the endometrium was dL7 (mid luteal stage). LGL comprise the majority of uNK in the early gestational decidua, where their density increased dramatically, while in the non-pregnant endometrium, MGL are the significantly most numerous perforin⁺ lymphocytes (Fig. 4).

Endometrial structures

Stromal cell density did not change throughout the endometrium during proliferative and early-mid secretory stages (not shown). With the onset of predecidualization in the subepithelial stroma in late luteal phase, spindle-like stromal cells (Fig. 5A, C) become enlarged, oval to polygonal, and widely separated, forming a broad mantle around UZ vessels beneath the luminal epithelium (Fig. 5B, D). Quantitatively, stromal cell density significantly decreased in the UZ, while in the LZ, where stromal fibroblasts show little change, density remained high (Fig. 6). In early pregnancy, decidual stromal cell density greatly decreased in both regions of the implantation site, compared with the luteal endometrium (Fig. 6). In the UZ, density decreased due to decidual transformation of stromal cells, which are large, rounded, and continue to be surrounded by extracellular matrix (ECM) (Fig. 5E). Decidualization does not develop extensively at the periphery of the site of placental attachment. In the LZ, the decrease in cell density was less striking than in the UZ, and was due to edema of the ECM, rather than cell transformation (Fig. 5F).

We also quantified the vascular and glandular density with the cycle and early pregnancy (Figs. 6,7). The vascular density of the LZ did not significantly change throughout the cycle or early gestation, however in the UZ it significantly increased during gestation, compared with the secretory endometrium (Fig. 6). Glandular density as well as dilatation increased in the luteal phase, in comparison with the follicular phase in both the LZ and UZ, although the increase was significant only in the LZ. In early gestation, the area occupied by glands gradually decreased in the UZ without discernable changes in the LZ, where glands were significantly enlarged (Fig. 7). Note that the relative glandular density in the UZ

corresponded to stromal decidualization and thus the expanded area of the stroma alters the apparent glandular density of the overall UZ tissue.

Finally, the zonal and total thickness of the endometrium did not change significantly from the mid-follicular through the luteal phase, although there was a slight increase of absolute and proportional size of the UZ in the late secretory endometrium (Fig. 8). With the onset of gestation, both the UZ and LZ of the decidua significantly increased in size, although their relative proportions remained similar to the follicular and early-mid luteal endometrium (Fig. 8).

DISCUSSION

The endometrial remodeling that occurs during the transition from the menstrual cycle to gestation is accompanied by significant changes in the population of uterine mucosal leukocytes. We have described a zone-sensitive method for the estimation of the immune cell dynamics in the rhesus uterine mucosa, which allowed the identification of region-specific changes in leukocyte subsets in endometrial and decidual tissue throughout the cycle and early pregnancy. Leukocyte subsets (NK cells, T lymphocytes, macrophages and PMNL) remained consistent from the proliferative up to the middle secretory phase throughout the whole endometrium, but dramatically changed beginning with the late luteal phase, as reported in other studies in the human and nonhuman primate uterus.^{22,23,27,28,38,39} Early local cellular immune stability may be explained by little fluctuation in the endometrial stroma before the onset of differentiation in the predecidual phase,³ whereas the period from the mid- to late luteal phase is the critical time when the endometrial decision whether to decidualize or prepare for menses is made.⁴⁰

The populations of leukocytes in the two endometrial zones were differentially regulated. In the endometrial LZ, T cells and macrophages remain the predominant leukocyte subpopulations, with slightly lower numbers of CD56+ lymphocytes. While the number of CD3+ cells remained higher in the LZ than in the UZ, the density of other leukocyte subsets in the LZ was substantially lower than in the UZ. The major leukocyte subsets in early pregnancy increased corresponding 3–5-fold in the UZ and approximately 2-fold in the LZ, if compared with the mid-luteal stage. This confirms an earlier suggestion regarding less variation in immune cell populations in the basal endometrium than in the functional zone.³⁹

The follicular and early-to-mid luteal stages were characterized by the prevalence of macrophages in the endometrial UZ. This suggests that macrophages play an important role in endometrial repair and regeneration during the proliferative phase.⁴¹ We previously demonstrated that DC-SIGN+ macrophages are specific to pregnancy in the rhesus decidua.²⁸ In the current study, both macrophage and DC-SIGN+ cell infiltrates increased during early gestation throughout the decidua, with approximately a 2-fold higher density in the UZ than in the LZ. Interestingly, the increasing number of CD68+ macrophages after the initiation of pregnancy closely reflects the increasing in DC-SIGN+ cells, confirming the previous conclusion that the latter are a distinct subpopulation of decidual macrophages of early pregnancy.²⁸

Polymorphonuclear leukocytes (PMNL) as a rich source of ECM-degrading proteases are thought to be involved in tissue breakdown and repair at the time of menstruation, as well as in promoting placental abruption and preterm birth.^{23,42} In human pregnancy, PMNL increase during the first trimester of gestation, and neutrophils are located near the placenta where they are associated with phagocytosis of decidual debris related to implantation and trophoblastic invasion.^{25,43} It has been shown in the rhesus monkey that massive infiltration of blood-derived cells, including neutrophils, appears by 24–48 h after onset of menses,⁴⁴

however PMNL distribution in the nonhuman primate cycling endometrium or in pregnancy has not been described. While a slight increase in PMNL numbers in the late luteal phase supports a role of these cells in perimenstrual tissue remodeling, the increase in their density during gestation was highest in the UZ on gd24–25, when cells of the epithelial plaque undergo degeneration,^{9,29} also suggesting the importance of neutrophils in cell and tissue debris elimination during the implantation process.

While the number of CD56+ cells significantly increased in the late luteal phase throughout the endometrium, their density was higher in the UZ, where they formed aggregates around spiral arteries,²⁷ than in the LZ. The location of NK cells in close proximity to spiral arteries may reflect cell trafficking of their precursors from the peripheral blood⁴⁵ and is consistent with a role in promoting endometrial vascularization by producing angiogenic factors.^{46,47} Although NK cell density was 2-fold higher in the UZ than in the LZ, NK cell levels in both layers increased from the late luteal phase to gd18–20 through to gd24–25, possibly in response to abundant chemokine secretion by placental trophoblasts.⁴⁸

Striking morphological heterogeneity (significant variation in cell and nuclear size, shape and chromatin content and the number and size of cytoplasmic granules) with increasing granularity during early gestation was previously described in rhesus monkey^{24,26} and human²² decidual NK cells. We have further refined here the morphological differences among rhesus uNK cells, based on cell and nuclear size, nucleus/cytoplasm ratio, the number and distribution of cytoplasmic perforin-containing granules, and have categorized them in three readily distinguished subtypes: SGL, MGL and LGL. These may be analogous to the Type II, III, and IV NK cells of the mouse decidua,^{49–51} identified by DBA lectin staining and light and electron microscopy. SGL and MGL were also revealed in spleen and lymph node, while LGL were not found in secondary lymphoid organs in pregnant or non-pregnant animals. As reported previously in the human endometrium,⁵² the rhesus uterine NK cells in the proliferative endometrium are smaller: most of them belong to the MGL subtype, that is, mid-sized cells with compact, densely packed perforin granules, which are prevalent during the cycle through whole endometrium. A few SGL, small cells with few perforin granules, are also present. SGL and MGL cell density remains steady throughout the menstrual cycle, and then increases in early pregnancy in both endometrial levels. The earliest appearance of LGL in the cycling endometrium in the rhesus was the mid-luteal phase (dL7). This may suggest their appearance is linked to the secretory transformation of the uterine mucosa during the window of implantation, which includes the initiation of predecidual changes of stromal cells, vascular remodeling, and ongoing glandular reorganization.

The proportion of SGL and MGL in the upper decidua was lower than in the LZ during first trimester, when LGL became the major subset in both levels. These results could suggest a process of maturation of the uNK within the uterus, in which the lower decidua is a source of immature NK cells. Based on the distribution of NK cell subtypes in both cycling endometrium and gestational decidua, we could hypothesize that pre-uNK cells traffic from the blood⁵³ during the post-ovulatory stage and during early pregnancy, into the lower uterine mucosa. During their final maturation, cells migrate into the upper level, where they reach their terminal differentiation stage. It has also been suggested in a previous study in the mouse that uterine NK cells increase during pregnancy related to local proliferation of those cells, rather than to influx of precursor cells.⁵⁴ Alternatively, there could be preferential trafficking of the precursors of LGL from the blood directly to the UZ under the influence of implantation-specific signals. We have also shown substantial proliferation among decidual NK cells,⁵⁵ however more careful studies are needed to determine the dynamics of uNK cell proliferation and the importance of trafficking from the peripheral blood in the primate endometrium.

A recent study of gd35–38 rhesus decidual NK cells⁵⁵ revealed heterogeneity among these cells, based on their phenotypic and functional parameters: the predominant subset was CD56^{bright}/CD16+/NKp30[−] cells, followed by CD56^{dim}CD16-NKp30+ cells, and a small population of CD56^{neg}CD16+ NK cells. CD56^{dim} but not CD56^{bright} NK cells expressed interferon-gamma (IFNG), suggesting by analogy to the mouse⁵⁶ a key (although not yet demonstrated) role in the rhesus uterine response to blastocyst implantation. Dambaeva et al.⁵⁵ also demonstrated differences between the rhesus dNK subtypes as defined by perforin content. CD56^{dim} dNK revealed low perforin expression in comparison with other dNK cell subpopulations and pNK cells. Another report⁵⁷ in rhesus macaques indicated that the three subpopulations (CD56+CD16−, CD56+CD16+ and CD56−CD16−) of NK cells found in mucosal and secondary lymphoid tissue contain intracellular perforin. Perforin was lower in CD16− cells (CD56+/-), while the highest level was noted in CD16+ cells, at levels similar to pNK cells, consistent with a more cytotoxic phenotype.⁵⁷ Our immunohistochemical results demonstrated that LGL with large nuclei and the highest number of perforin granules dispersed throughout a wide cytoplasm dynamically increased in both upper and lower decidual levels during early pregnancy. We suggest that rhesus LGL have a CD56^{bright}CD16+NKp30[−] immunophenotype, the most abundant type of decidual NK cells detected by flow cytometric analysis.⁵⁵ The two other NK subtypes, MGL and SGL, could represent the CD56^{dim}CD16^{+/-} and CD56^{neg} phenotype. Further investigation, including in vivo experiments with anti-CD16 depletion,⁵⁸ may help understand the relationships among these three NK cell populations. Perforin has proven to be a reliable marker for immunohistochemical (IHC) analysis of dNK cells,⁵⁹ and may be used as a general marker of all mucosal NK cells. Analysis of its intracellular distribution could also provide a finer immunomorphological distinction of NK subsets in endometrial/decidual tissue.

Histological evaluation of endometrial development through the menstrual cycle and early pregnancy also revealed zone-dependent responses of stromal cells, vasculature and endometrial glands. Most changes appeared in the late luteal stage, when stromal cells in the rhesus undergo predecidualization, manifested by a decrease in stromal cell density in the UZ (but not the LZ), which became more dramatic in early pregnancy with extensive decidualization of the endometrium, as well as an increase in LZ edema. During the cycle, endometrial thickness increased only in the UZ, already committed to menstruation, without changes in stromal, glandular and vascular components in the LZ. Vascular density of the endometrium also increased in the UZ in early gestation and the quantitative changes were greater in the UZ compared to the LZ. Interestingly, in early gestation the thickness of the endometrium increased proportionally in both the UZ and LZ, compared with the late luteal phase non-conception endometrium. These data suggest the involvement of both endometrial zones in successful implantation and early placentation.

Zone- and cell type-dependent changes in local uterine cell immunity and development throughout the cycle and early gestation suggest that hormones, cytokines and other soluble factors, produced by the placenta and endometrium/decidua, are involved in recruiting leukocyte populations or their precursors into the uterus from the circulation, as well as perhaps directing intramucosal immune cell maturation and trafficking. Previous studies in the mouse and human endometrium indicated a regulatory role of dendritic cells and NK cells in the transformation of endometrial stromal cells and vascular responses associated with embryo implantation,^{40,47,56,60,61} which together with glandular epithelial changes are key features of decidualization. It is also likely that the endometrial LZ plays a role in the support of immunological stability during gestation, and its reconstitution after menstruation or labor and delivery. Thus, while the UZ is the most morphologically and functionally malleable endometrial layer, the UZ relies on the responses of the LZ to the endocrine milieu for its cyclic renewal and reestablishment of receptivity for implantation. Some studies of the endometrium have found differences in the number of uterine NK cells and

macrophages in patients suffering recurrent IVF failure and recurrent miscarriage.^{62,63} A more precise understanding of the maturation of these endometrial leukocytes may prove important for understanding their role in miscarriage,⁶⁴ and successful pregnancy outcome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Depiction of rhesus monkey endometrial and decidual zonation as defined by Bartelmez (Bartelmez, 1957) in red at the left (I – superficial, II – principal, III – transitional and IV – basal) and in black, as upper and lower zone designation in the current study. Panel A: rhesus monkey decidua, gd24. Panel B: Rhesus monkey endometrium, dL 11. Panel C: Rhesus monkey endometrium, menstrual phase. Panel D: Representative nest of a coiled spiral artery (SA) at the junction of the upper and lower zones in rhesus monkey endometrium. Hematoxylin & eosin staining.

Fig. 2.

Quantitation of endometrial/decidual leukocytes during the menstrual cycle and early gestation. The number of cells per unit area is presented for the upper and lower zones of the designated stage of the cycle/pregnancy. Statistical comparisons: * $P < 0.05$, compared with dF7–8; + ($P < 0.05$), ++ ($P < 0.01$), +++ ($P < 0.001$) compared with dL2–7; ## ($P < 0.01$), ### ($P < 0.001$) compared with dL10–14; ^ ($P < 0.05$), ^^ ($P < 0.01$), ^^^ ($P < 0.001$) compared with gd18–20. The mean data of 15 separate fields of each zone are shown for each animal analyzed: dF7–8 ($n=3$), dL2–7 ($n=2$), dL10–14 ($n=6$), gd18–20 ($n=4$), gd24–25 ($n=4$).

Fig. 3. Representative morphology of SGL, MGL, and LGL immunostained with anti-perforin in sections of rhesus tissue, gd24. All scale bars = 5 μ m. Panels D–F are higher magnification fields of cells similar to Panels A–C. Panels J, K: Quantitative determination of nuclear diameter (N), cellular diameter (C), and nuclear/cytoplasmic (N/C) ratio in SGL, MGL and LGL. *** $P < 0.001$.

Fig. 4.

Density of perforin-positive cells in the endometrium and decidua, classified as SGL, MGL, or LGL. The upper and lower zone cells are presented separately. Statistical comparisons: + ($P < 0.05$), ++ ($P < 0.01$), +++ ($P < 0.001$) compared with dL2-7; # ($P < 0.05$), ## ($P < 0.01$), ### ($P < 0.001$) compared with dL10-14; ^ ($P < 0.05$), ^^ ($P < 0.01$) compared with gd18-20. The mean data of 15 separate fields of each zone are shown for each animal analyzed: dF7-8 ($n=3$), dL2-7 ($n=2$), dL10-14 ($n=6$), gd18-20 ($n=4$), gd24-25 ($n=4$).

Fig. 5. Stromal cell appearance and decidualization in the menstrual cycle and early pregnancy. Representative photomicrographs illustrating spindle-like stromal cells in the proliferative phase (A, C) and the expansion of subepithelial stromal cell cytoplasm in the late secretory phase (B, D) endometrium. Representative photomicrographs also illustrate stromal density of the upper (E) and the lower (F) gd24 decidua. Hematoxylin & eosin staining.

Fig. 6.

Stromal cell and vascular density in the upper and lower endometrial and decidual zones. Statistical comparisons: + ($P < 0.05$), ++ ($P < 0.01$), +++ ($P < 0.001$) compared with dL2-7; # ($P < 0.05$), ## ($P < 0.01$), ### ($P < 0.001$) compared with dL10-14. The mean data of 15 separate fields of each zone are shown for each animal analyzed: dF7-8 ($n=3$), dL2-7 ($n=2$), dL10-14 ($n=6$), gd18-20 ($n=4$), gd24-25 ($n=4$).

Fig. 7.

Glandular diameter and density in the cycle and early pregnancy in upper and lower endometrial and decidual zones. * ($P < 0.05$) compared with dF7–8; + ($P < 0.05$) compared with dL2–7; # ($P < 0.05$), ## ($P < 0.01$), ### ($P < 0.001$) compared with dL10–14. The mean data of 15 separate fields of each zone are shown for each animal analyzed: dF7–8 ($n=3$), dL2–7 ($n=2$), dL10–14 ($n=6$), gd18–20 ($n=4$), gd24–25 ($n=4$).

Fig. 8.

Endometrial thickness (total and zonal) and zonal to total ratio through the menstrual cycle and early gestation. Statistical comparisons: + ($P < 0.05$), ++ ($P < 0.01$), +++ ($P < 0.001$) compared with dL2–7; # ($P < 0.05$), ## ($P < 0.01$), ### ($P < 0.001$) compared with dL10–14. The mean data of 15 separate fields of each zone are shown for each animal analyzed: dF7–8 ($n=3$), dL2–7 ($n=2$), dL10–14 ($n=6$), gd18–20 ($n=4$), gd24–25 ($n=4$).