Going Full TeRM: the Seminal Role of Tissue-Resident Macrophages in Organ Remodeling during Pregnancy and Lactation

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Abstract:

During pregnancy and lactation, the uterus and mammary glands undergo remarkable structural changes to perform their critical reproductive functions, before reverting to their original dormant state upon childbirth and weaning, respectively. Underlying this incredible plasticity are complex remodeling processes which rely on coordinated decisions at both the cellular and tissue-subunit levels. With their exceptional versatility, tissue-resident macrophages play a variety of supporting roles in these organs during each stage of development, ranging from maintaining immune homeostasis to facilitating tissue remodeling, although much remains to be discovered about the identity and regulation of individual macrophage subsets. Here we review the increasingly appreciated contributions of these immune cells to the reproductive process and speculate on future lines of inquiry. Deepening our understanding of their interactions with the parenchymal or stromal populations in their respective niches may reveal new strategies to ameliorate complications in pregnancy and breastfeeding, thereby improving maternal health and well-being.

Part 1: Introduction

The crucial role of the immune system in mediating healthy maternal-fetal and -neonatal interactions has received a surge of interest recently, as epitomized by the 2022 *Journal of Immunology* special issue on Maternal-Fetal Immunology, which focused on the contribution of various immune cell populations to uterine and placental function during pregnancy. To complement these articles, we have elected to shine a spotlight on tissue-resident macrophages (TRMs) and their roles in the remodeling events of maternal organs that occur before, during, and after pregnancy. In particular, we will focus our review on two key organs: the uterus and the mammary gland, which are indispensable for fetal development before and after birth, respectively.

TRMs play diverse roles across various biological settings, from conventional immune defense to mediating tissue remodeling and regeneration (1–5). These cells occupy distinct niches in their host tissue while performing their specialized functions and are typically derived from dedicated embryonic progenitors *in utero* (6–8), although monocyte-derived TRMs may arise following cell death or when new niches become available in the organ (9, 10). Since the uterus and mammary glands undergo extensive remodeling to cater to the needs of pregnancy, but must revert to a basal state postpartum (Fig. 1A, 2A) , it is not surprising that TRMs have been shown to participate in settings ranging from tissue development and estrous cycling to pregnancy and involution, while their depletion or dysregulation can lead to adverse outcomes such as impaired fetal and neonatal development and cancer (11, 12). While the function of the uterus is essentially complete upon parturition, the mammary gland's role in supporting the offspring via lactation is only just beginning; indeed, the duration of the breastfeeding stage may exceed that of the pregnancy itself. Despite exhibiting rapid involution after parturition or weaning, both organs remain poised to respond to future pregnancies.

All of these complex processes occur with remarkable efficiency and consistency, a fact which necessitates a well-choreographed interplay between parenchymal, stromal, and oftentimes immune populations. Furthermore, as a result of the unparalleled levels of remodeling experienced by these organs in the course of their reproductive function, both the quantity and nature of their TRM niches fluctuate dramatically over time as well (9). Thus, the goals of this review are to interpret existing findings in the unique context of pregnancy and lactation, as well as to highlight potentially exciting avenues of inquiry. Improving our understanding of the specific mechanisms underlying the recruitment and specialization of TRMs under healthy conditions may facilitate the development of prophylactic or therapeutic strategies targeting these cells in disease states.

Part 2: TRMs in the uterus

Steady State

The uterus serves as a mucosal barrier even in the absence of pregnancy, and TRMs constitute up to ~10% of all uterine cells at steady state (Fig. 1B) (12–14). Their numbers fluctuate in tandem with the murine estrous cycle and may be regulated by sex hormones such as estrogen and progesterone (15); these hormones also stimulate the production of myeloid cytokines and chemokines in uterine epithelial cells such as Colony Stimulating Factor 1 (CSF1), Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), and Monocyte Chemoattractant Protein-1 (MCP-1) (16–19). The uterine endometrium undergoes significant structural changes during estrous cycling, chiefly the formation of a transient decidual layer by the differentiation of stromal endometrial cells (20, 21). In the event of implantation, placentation is dependent on extravillous trophoblast (EVT) invasion and spiral artery remodeling, both of which are in turn reliant on matrix metalloprotease (MMP) activity by TRMs (11, 22). Conversely, if implantation does not occur, uterine TRMs adopt a wound-healing, alternatively activated phenotype to assist in the degradation of the decidua via MMP activity (11, 14) and phagocytosis of apoptotic epithelial cells (12). Throughout this regenerative process, pre-existing TRMs are supplemented by newly recruited monocytes, some of which differentiate into fresh TRMs (23). While TRMs as a whole have been shown in macrophage-deficient mouse models to be indispensable for successful implantation, it is important to note that these models also result in the depletion of ovarian TRMs, which leads to impaired progesterone secretion by the corpora lutea (24, 25). As a result, it is unclear to what extent decreased uterine receptivity is due specifically to the loss of decidual TRMs, and caution is advised when interpreting data from studies using total macrophage depletion models.

Pregnancy

During pregnancy, the initiation of placentation demands even more drastic tissue remodeling compared to basal estrous cycling, during which TRMs perform expanded roles including degrading pre-existing stromal structures to make room for further EVT invasion and secretion of angiogenic factors to drive spiral artery remodeling, as well as maintaining a tolerogenic immune environment (Fig. 1B) (14, 26). As the uterus expands to accommodate the growing fetus(es), TRM numbers swell due to extravasation and differentiation of Ly6C^{hi} circulating monocytes, as well as in situ macrophage proliferation (27). Both processes are CSF1-dependent and occur primarily in the myometrium as opposed to the decidua, which suggests that some level of

compartmentalization may be enforced at this stage. Intriguingly, F4/80+ TRMs are the primary source of CCL2 in this tissue layer, implying that a feed forward loop exists to drive monocyte recruitment during pregnancy (27). The polarization state of these TRMs is critical for establishing a healthy pregnancy, as mice deficient for the type 2 cytokine IL-33 exhibit extensive defects in remodeling and implantation at the early stage, followed by poor pregnancy outcomes later on (28). IL-33 is expressed locally by several myometrial and decidual stromal populations, including endothelial cells, which suggests that TRMs commit to specific roles based on the signaling cues they receive from the surrounding niche (28). Trophoblasts are also a key interacting partner for TRMs; for instance, in response to bacterial infection, murine uterine epithelial cells signal to trophoblasts via CSF1 to induce recruitment of macrophages and neutrophils via CXCL1 and CXCL2 (29).

Parturition:

The onset of labor is associated with the elevated expression of inflammatory cytokines and chemokines in the myometrium, coupled with an increase of both neutrophils and F4/80+ macrophages (Fig. 1B) (30). These events may be linked to the secretion of surfactant proteins from the fetal lung and/or maternal decidua, although there have been conflicting reports on the precise nature of this mechanism (31–33). Separately, systemic diphtheria toxin-mediated depletion of CD11b+ cells (primarily targeting macrophages) in late gestation (~e16.5) resulted in an increased frequency of preterm birth and poorer offspring survival, with a concomitant increase in immune activation and inflammatory cytokine levels in the uterine decidua (34). Notably, macrophage depletion at this late timepoint did not alter plasma progesterone levels, nor was the phenotype rescued by exogenous progesterone administration, suggesting that the adverse pregnancy outcomes were primarily due to non-hormonal perturbations such as increased inflammation at the maternal-fetal interface (34). Furthermore, single-cell analysis of mice undergoing preterm labor (induced via intra-amniotic *E. coli* infection) revealed a significant influx of uterine and decidual macrophages and monocytes compared to uninfected controls, coupled with extensive transcriptional changes in inflammatory pathway genes in these cells (35). Together, these findings argue that distinct subsets of uterine TRMs may contribute to maintaining a tolerogenic environment during pregnancy, though the specific contribution of uterine and ovarian TRMs remains unclear. Regardless, this immunological balance is especially important in species such as humans and mice that exhibit hemochorial placentation which exposes maternal immune cells to fetal antigens (36). Consequently, the deliberate

disruption of this immunological balance can trigger the onset of normal labor, whereas inappropriate inflammation may lead to preterm birth.

Postpartum Involution:

The early postpartum period in mice is associated with the upregulated expression of monocyte chemoattractants such as CCL2, CCL3, and CXCL1 in the myometrium and a concomitant influx of monocytes (30), although these new entrants may only be required after parturition, since CCR2-KO mice exhibit normal gestational lengths and litter sizes (37). Similarly, artificial induction of preterm labor with either lipopolysaccharide (LPS) or the progesterone receptor antagonist RU486 elicits a significant increase in macrophage numbers within 2-6 hours postpartum, but not during labor itself (30).

As parturition results in significant physical damage to the uterine lining, postpartum TRMs may play roles analogous to those of alternatively activated macrophages in other tissue settings such as wound healing, angiogenesis, and extracellular matrix remodeling (2). Indeed, postpartum TRMs can be found in close proximity to both epithelial and endothelial structures in the endometrium, which suggests that they may participate in their regeneration at this stage (38).

What Comes Next?

Analyses of human samples (primarily from first trimester elective terminations) have revealed the existence of several decidual TRM subsets that are distinguishable based on their expression of markers such as CCR2 and CD11c (39–41), all of which display a range of phagocytic and antigen presentation capabilities. Furthermore, in vitro studies have shown that human trophoblasts can secrete cytokines and chemokines in response to LPS to recruit and polarize monocytes and macrophages towards a pro-inflammatory state, thus underscoring that TRMs may be heavily influenced by local signals from other cells at the maternal-fetal interface (42–45). As with mice, human decidual TRM subsets must collectively strike a balance between providing sufficient protection against infection and maintaining an appropriate tolerogenic environment to achieve term pregnancy (46).

Notably, both recurrent miscarriage and spontaneous preterm birth in women are associated with increased frequency of M1-like / pro-inflammatory TRMs, as well as decreased PD-1/PD-L1 and increased TNF-α signaling in these cells, respectively (34, 47, 48). Furthermore, decidual TRMs can suppress inappropriate IFN-

γ responses in T cells via PD-L1, suggesting a role for myeloid regulation of adaptive immune responses at this stage (49). Together, these findings argue that inappropriate TRM activation during pregnancy may be mistaken for the normal inflammatory cascade preceding the onset of labor, resulting in the premature initiation of this process.

Separately, though both murine estrous cycling and postpartum involution are triggered by progesterone withdrawal and exhibit similar remodeling processes, i.e. endometrial resorption followed by tissue repair, it is unclear whether the TRMs recruited in both stages are functionally identical. This may be investigated by performing phenotypic and transcriptional comparisons of decidual TRMs during metestrus versus the early postpartum period. In addition, though mice do not undergo menstruation, they do exhibit similar fluctuations in progesterone levels to humans, with a notable peak and decline in circulating hormone preceding ovulation (50, 51). As such, the use of mouse models to simulate human menstruation via progesterone withdrawal (23, 52) will be invaluable for eventually translating new discoveries to patients in the clinical setting. Conversely, progesterone supplementation (via injection or implanted pellet) could be used to compensate for the reduced hormone secretion exhibited by TRM-deficient mice during various stages of pregnancy (24, 25), in order to distinguish any phenotypes arising from indirect ovarian defects, as opposed to from the depletion of decidual TRMs. Similarly, the development of genetic strategies to specifically target decidual, but not ovarian, TRMs (or vice versa) will be important for delineating the precise roles played by these two myeloid populations during pregnancy.

In summary, though many signaling pathways have been identified in TRMs which affect pregnancy outcomes in mice, further investigations are needed to verify these findings in the clinic. In particular, the identification of homologous TRM subsets between species will be invaluable for defining the regulation and function of these cells in humans, especially in the later stages of pregnancy from which patient samples are either unavailable or scarce.

Part 3: TRMs in the mammary gland

Steady State:

During the steady state, the mammary glands of non-pregnant or -lactating female mice are spatially synonymous with subcutaneous white adipose tissue (scWAT), with a parenchymal compartment consisting primarily of white adipocytes supported by stromal populations such as vascular and neuronal networks (Fig. 2B). The mammary glands also contain a skeletal framework of mammary epithelial ducts which is present from pre-puberty and grows in tandem with the surrounding tissue well into adulthood (53). During estrous cycling, these ducts undergo periodic blooms of alveolarization, which are a microcosm of the rampant expansion seen during pregnancy. TRMs are also present from the early postnatal period and have been shown to contribute to ductal morphogenesis (54–57). Upon the onset of puberty, their numbers fluctuate according to the estrous cycle and peak during metestrus and diestrus, corresponding to the height of alveolarization (58), suggesting that they may contribute to epithelial proliferation and alveolar sprouting. More recent studies used a combination of 3D imaging, flow cytometry, and single-cell transcriptomics to define several TRM subpopulations, including a subset of CX3CR1+ ductal-associated macrophages that continuously surveys both luminal and basal surfaces of the epithelium, and various stromal-associated TRMs that are located distal to the alveoli and ducts (59–61). Given that ductal TRMs play a key role in phagocytosing apoptotic epithelial cells and other debris during weaning-induced involution (59, 62), they may also perform a similar housekeeping function at this stage, albeit at a much smaller scale. In addition, TRMs are induced by Dll1-Notch signaling from basal epithelial cells to secrete Wnt ligands which maintain the mammary stem cell niche (63); a subsequent study reported a similar homeostatic role for TRM-derived TNF-α (64). Notably, constitutive genetic disruption of Notch signaling in CD11c+ myeloid cells resulted in impaired outgrowth of the ductal tree at 5-6 weeks of age (63), showing that TRM-stem cell interactions are necessary at this early developmental stage.

As a result of both tissue growth and subsequent estrous cycling, the mammary gland exists in a state of constant flux even in the steady state, which necessitates checks and balances to prevent these processes from going awry. One TRM population that may contribute to such oversight is the Lymphatic Vessel Endothelial Hyaluronan Receptor 1 (LYVE1+) subset, which localizes to hyaluronan-rich regions of the mammary gland such as the capsule and interlobular septa throughout development, pregnancy, and lactation (65, 66). As in other tissue settings (67), these TRMs contribute to tissue homeostasis by regulating turnover of hyaluronan, collagen, and other extracellular matrix components, but do not appear to be strictly necessary for ductal

development during the steady state (65). Finally, studies focusing on WAT's role as a major metabolic organ have also identified stromal TRM subsets from male or non-pregnant female mice such as a sympathetic neuron-associated population that modulates local neurotransmitter signaling (68), as well as one that secretes proangiogenic factors to promote vascularization during development (69), though it is currently unclear if these play pregnancy-related roles in the dormant mammary gland. In summary, specialized TRM populations are present in the mammary gland even in the absence of pregnancy, and contribute to parenchymal and stromal remodeling during development and estrous cycling.

Pregnancy:

Pregnancy is accompanied by an initial surge and subsequent fluctuations in key pregnancy hormones such as progesterone, estrogen, and prolactin which signal directly to mammary epithelial cells to drive mammary gland remodeling (70, 71). The composition of the TRM compartment fluctuates with each phase and may receive either direct hormone signaling or secondary cues from epithelial cells and other mammary gland populations (58, 72, 73). At the tissue level, the most salient change is the replacement of the adipocyte compartment by grape-like alveolar clusters, which involves several processes occurring in tandem: de-differentiation of adipocytes into Platelet-derived growth factor receptor A (PDGFRα+) precursors (74), sprouting of dense alveolar bunches to take their place, and the presumed expansion of vascular and neuronal support networks (Fig. 2B). During alveolarization, ductal TRMs proliferate in parallel with the mammary epithelial network in order to maintain dense coverage of both luminal and basal surfaces of ducts and alveoli (59). They interface with epithelial stem cells to promote their differentiation and proliferation, while maintaining the stem cell pool for future pregnancies (63, 64). Intriguingly, CSF1-deficient op/op mice exhibit dysfunctional mammary gland remodeling during pregnancy, namely impaired ductal branching coupled with excessive alveolarization, suggesting that TRMs may exert fine control over this process (75).

Concurrently, capsular and septal LYVE1+ TRMs persist through pregnancy and contribute to extracellular matrix remodeling, in part by mediating stromal hyaluronan homeostasis via both synthesis and degradation (65, 66). The extracellular matrix in turn provides mechanosensory cues to guide differentiating and proliferating epithelial cells (76). Besides influencing fate decisions at the cellular level, the distinctive positioning of LYVE1+ TRMs at the boundaries of both adipocyte lobules in the steady state and alveolar clusters during pregnancy suggests that these cells may help maintain subunit organization during hypertrophy of the overall tissue (77).

In addition, PDGFRα signaling can oppose ductal branching under tumor conditions, implying the existence of crosstalk between adipocyte precursors and epithelial cells (78). Furthermore, as hyaluronan is an important extracellular matrix component of macrophage-stem cell interactions in diverse tissue and tumor settings (79, 80), it may play a role in attracting LYVE1+ TRMs to the mammary stem cell niche. In a similar vein, perivascular LYVE1+ TRMs may also engage in extracellular matrix remodeling to facilitate angiogenesis as the vascular network expands to envelop each individual alveolus in a mesh-like capillary 'basket', which will be essential for rapid access to bloodborne nutrients and oxytocin later on (69, 81–83).

As for the adipocyte compartment, the signals driving de-differentiation, and by extension any specific roles for TRMs, remain undefined. Although pregnancy hormones are the primary suspects (74), mammary epithelial cells may also be involved, as they can drive adipocyte de-differentiation under tumor conditions (84). Nevertheless, numerous studies have shown that TRMs engage in extensive crosstalk with adipocytes and adipocyte precursors in male scWAT and gonadal WAT (69, 85–87), and it may be possible to extrapolate these findings to the setting of pregnancy-induced remodeling. For instance, scWAT TRMs help to recycle excess lipids in obese male mice (88, 89), and thus TRM subsets may perform a similar lipid-clearing function during adipocyte de-differentiation in the mammary gland.

Lactation:

True lactation (aka 'Lactogenesis II'), as opposed to colostrum production (aka 'Lactogenesis I'), begins shortly after parturition, and represents the culmination of the mammary gland's extensive remodeling efforts. Following milk production by alveolar epithelial cells, milk expression takes place via the let-down reflex, in which myoepithelial contractions pump the milk out during breastfeeding (90). The role of TRMs during this phase is somewhat understudied, although one recent report unveiled the existence of a predominantly monocyte-derived, CD11c+ MHC-II+ CX3CR1+ Dectin-1+ subset that is present from parturition and undergoes proliferation to become the dominant myeloid population during the middle to late stages of lactation (Fig. 2B) (61), and may overlap with the previously described ductal TRM population (59). Perturbing CSF1 signaling, either systemically using an anti-Csf1r antibody (59) or specifically via CD11c-cre-mediated excision (61), results in the depletion of these ductal- and lactation-associated TRMs, thereby underscoring the importance of this key signaling pathway. These maternal TRMs require the presence of a maternal microbiome and

extravasate into the breastmilk during nursing, which raises the tantalizing possibility that they may influence microbiota transmission from mother to offspring (61).

As nursing exposes the mammary gland to increased risk of infection by opportunistic microbes, the luminal epithelium becomes a transient mucosal barrier site (91) and is constantly patrolled by TRMs expressing various key markers such as CX3CR1, SOCS3, and HSPH1 (59, 60). Murine models of mastitis show that TRMs use Toll-like receptors (TLRs) to detect invading pathogens and release a barrage of cytokines and chemokines to thwart the infection (92, 93). However, this is a double-edged sword, as over-exuberant inflammation can lead to mastitis and premature involution, directly impairing the mammary gland's capacity for lactation and breastfeeding (93, 94). Notably, immune defense is not the sole responsibility of TRMs, and other immune subsets may be recruited as needed (93). For instance, neutrophil numbers increase dramatically during LPS-induced mastitis, though this appears to be partly CSF1-dependent, thereby implicating lactation-induced TRMs in this process as well (61). In addition, lactation-induced and pre-existing TRM subsets can phagocytose bacteria in vitro and are diminished in numbers during mastitis, which is consistent with the so-called 'macrophage disappearance reaction' seen under other inflammatory conditions (9, 61). Improving our understanding of which inflammatory and/or tolerogenic signals modulate activation of specific TRM subsets during infection will be important for promoting proportionate immune responses in the clinical setting. These studies will be greatly enabled by the development of tools to deplete specific TRMs by targeting signature markers such as Dectin-1 which is expressed by the lactation-induced subset (61).

Lactogenesis in itself poses a unique metabolic challenge, whereby essential nutrients, antibodies, and other constituents of milk are translocated from the maternal bloodstream into the alveolar lumen, while excluding unwanted material such as red blood cells. This highly selective process is mediated by a host of solute-specific transporter proteins (95), though given their strategic positioning, it is possible that endothelial- or epithelial-associated TRMs may take part as well. Furthermore, given that TRMs in WAT and other tissues can adopt different activation states depending on their usage of specific metabolic pathways (96), this raises the possibility that mammary TRMs fine-tune their function based on local nutrient availability. Finally, macrophages can express the oxytocin receptor under certain inflammatory conditions (97), which suggests that a subset of TRMs may help regulate local oxytocin levels, though this possibility remains to be investigated.

Post-Weaning Involution:

Following weaning, exogenous cues such as milk stasis initiate the involution process, which consists of the coordinated apoptosis and clearance of the alveolar epithelial cells and the re-population of the tissue by re-differentiated adipocytes, returning it to a mostly pre-pregnant state (Fig. 2B) (98). TRMs play a well-characterized role in this process, with numerous studies showing that the depletion of these cells via genetic or chemical methods results in delayed involution and adipocyte repopulation, while reconstitution with fresh macrophages ameliorates these defects (59, 99). Subsequent reports have shown that the CX3CR1+ ductal TRMs play a preeminent role at this stage by phagocytosing both cellular debris and residual milk (59), in concordance with their intimate association with the alveolar epithelium. After performing this critical duty, ductal TRMs recede into the background, while other stromal TRM populations re-emerge in the tissue as the lactation machinery returns to a dormant state. Notably, incomplete clearance of lactation-associated detritus can lead to increased risk of postpartum breast cancer in women (99, 100), and tumor-associated macrophages in the *PyMT-MMTV* mouse model appear phenotypically and transcriptionally similar to CX3CR1+ ductal TRMs (59), thus implicating this population in both the onset and perpetuation of tumorigenesis. Concurrently, other TRM subsets may also provide differentiation signals to quiescent adipocyte precursors, though once again existing evidence is limited to metabolism-focused studies in male mice (69, 85).

Lastly, in addition to these changes at the cellular level, the mammary gland also shrinks back to its pre-pregnancy size and exhibits comparable lobular architecture to that of nulliparous tissue, all of which implies the existence of some kind of ‘spatial memory’. Whether this process could be encoded within specific TRM subsets whose precise positioning and extracellular matrix-modulating ability may be important for re-establishing the pre-existing subunit architecture of the mammary gland (65, 66, 77) warrants further examination.

What Comes Next?

Though tumor-associated macrophages have been characterized extensively in breast cancer, TRMs are relatively understudied in the context of human pregnancy and lactation, owing to the understandable paucity of healthy patient samples. A few recent reports circumvented this limitation by focusing on macrophages in human breastmilk, one of which also identified several subsets that had similar transcriptional profiles to those of murine lactation-associated TRMs (61, 101). In addition to shedding a light on the potential homology between mammary TRMs in humans and mice, this raises the possibility of using these breastmilk macrophages

as a proxy for ductal-associated TRMs, similar to how stool samples are used as a readout for gut microbiota. These strategies may prove invaluable for diagnosing and treating inflammation-associated breastfeeding disorders like mastitis (102). Similarly, the presence of specific TRM subsets in breastmilk during the weaning period could be developed as a biomarker of the thoroughness of the involution process, and by extension, a predictor of future cancer risk in women who have given birth (99, 100).

Part 4: Conclusion

Pregnancy is a biologically demanding process that elicits major physiological changes throughout the female body, none more so than in the reproductive organs, which undergo wholesale remodeling both during and after this period. Broadly, these changes encompass the expansion of a transient parenchymal compartment, i.e. the alveoli and the decidua in the mammary gland and uterus, respectively, as well as the concomitant reshaping of pre-existing stromal support networks such as the vasculature. Following parturition or weaning, both the uterus and mammary gland revert to their original quiescent states and sizes, which involves the controlled eviction of pregnancy-induced structures by the original tenant populations, such as adipocytes in the mammary gland. When viewed in conjunction with the low-level remodeling seen during estrous cycling, these are arguably two of the most plastic organs in mammalian biology.

The importance of myeloid subsets as a whole in reproduction has been known for decades, thanks to seminal reports by pioneers such as the late Jeffrey Pollard (103, 75, 17, 29, 54–56). Since then, advances in techniques such as transcriptomics, flow cytometry, and microscopy have enhanced the resolution with which we can characterize the precise functions of specific TRMs (59–61), though there remains much to discover, especially in the uterus. For instance, in addition to canonical axes such as CSF1 and CCL2, which signals are responsible for the recruitment, polarization, and maintenance of particular TRM subsets and their monocyte precursors? How are these mechanisms perturbed in the settings of metabolic or inflammatory conditions, and what are the consequences for mother and offspring? Furthermore, while much work has focused on the interactions between TRMs and parenchymal compartments such as the mammary alveoli, their involvement in the remodeling of crucial stromal infrastructure such as nerves and vasculature remains relatively understudied.

Finally, though this review focuses on studies performed in mice, we would be remiss if we did not emphasize the limitations of translating findings from mouse models to the clinical setting. For instance, the estrous (murine) and menstrual (human) cycles are not directly comparable, while the uteri of both species are structurally non-homologous. In addition, human mammary glands are found solely in females, whereas their murine counterparts exist in both males and females in the form of scWAT and are largely indistinguishable in the steady state, apart from the presence of a skeletal ductal network in the latter. In the face of these intrinsic tissue differences, TRMs have been shown to occupy homologous functional niches in both species during reproduction (61) and thus represent an attractive target for pharmaceutical or cellular intervention. Expanding our knowledge of how specific TRM subsets contribute to the complex ballet of pregnancy and involution may

reveal new avenues for diagnosing and designing therapies for diverse human diseases such as breast or endometrial cancer, mastitis, and preeclampsia.

Conflict of Interest

The authors declare no conflict of interest.

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Figure legends:

Fig. 1: Overview of TRM functions in the uterus across stages of the reproductive cycle

(A) The uterus expands during pregnancy to accommodate the growing fetuses, before involuting back to its original size during the postpartum period.

(B) In the steady state, TRMs modulate the extracellular matrix during the formation and resorption of the decidual layer during estrous cycling. If implantation is successful, this role is expanded to facilitate key processes such as spiral artery remodeling and EVT invasion. During pregnancy, TRMs expand in proportion with the uterus and help maintain a tolerogenic environment; as labor is associated with increased inflammatory tone, the disruption of this balance can lead to preterm birth. Following parturition, additional monocyte precursors are recruited to the uterus are presumed to differentiate into new TRMs; these in turn contribute to postpartum involution of the decidua by driving extracellular matrix remodeling and clearance of apoptotic cells. Figure was created using BioRender.com.

Fig. 2: Overview of TRM functions in the mammary gland across stages of the reproductive cycle

(A) During pregnancy, the mammary gland grows significantly, especially in the last week leading up to birth. Unlike the uterus, it continues to maintain its size during the postpartum period as long as lactation is needed. Following ~2 days of milk stasis, the involution process begins, resulting in a gradual reversion of the tissue to its original size.

(B) During prepubertal development, TRMs play essential roles in the proper formation of mammary ducts in the mammary gland, including maintaining the mammary stem cell pool via Dll1-Notch signaling. They also contribute to alveolar sprouting during the estrous cycle, and may also be involved in the clearance of apoptotic epithelial cells during proestrus. In addition, a CX3CR1+ TRM subset conducts immune surveillance of the ductal regions. During pregnancy, these TRMs expand in tandem with extensive ductal branching and alveolarization, and continue to patrol the epithelial surfaces. Other stromal TRMs may be involved in remodeling of neuronal and vascular networks, as well as maintaining compartmentalization while the mammary gland undergoes significant hypertrophy. Following parturition, the onset of lactation is associated with the appearance of CX3CR1+ Dectin-1+ TRMs in both the mammary gland and milk. TRMs help to mount myeloid responses against invading pathogens, at the risk of developing mastitis. Upon weaning of offspring, TRMs play

important housekeeping roles in clearing apoptotic epithelial cells and milk debris, as well as in stromal remodeling during the involution of the surrounding mammary gland tissue. Figure was created using BioRender.com.