J Mammary Gland Biol Neoplasia (2014) 19:229–239
DOI 10.1007/s10911-014-9324-x

Hormonal Regulation of the Immune Microenvironment in the Mammary Gland

Eleanor F. Need · Vahid Atashgaran · Wendy V. Ingman · Pallave Dasari

Received: 8 May 2014 / Accepted: 16 June 2014 / Published online: 4 July 2014

Abstract It is well established that the development and homeostasis of the mammary gland are highly dependent upon the actions of ovarian hormones progesterone and estrogen, as well as the availability of prolactin for the pregnant and lactating gland. More recently it has become apparent that immune system cells and cytokines play essential roles in both mammary gland development as well as breast cancer. Here, we review hormonal effects on mammary gland biology during puberty, menstrual cycling, pregnancy, lactation and involution, and dissect how hormonal control of the immune system may contribute to mammary development at each stage via cytokine secretion and recruitment of macrophages, eosinophils, mast cells and lymphocytes. Collectively, these alterations may create an immunotolerant or inflammatory immune environment at specific developmental stages or phases of the menstrual cycle. Of particular interest for further research is investigation of the combinatorial actions of progesterone and estrogen during the luteal phase of the menstrual cycle and key developmental points where the immune system may play an active role both in mammary development as well as in the creation of an immunotolerant environment, thereby affecting breast cancer risk.

Keywords Progesterone · Estrogen · Macrophage · T cell · Cytokine

Abbreviations

AR	Androgen receptor
COX2	Cyclooxygenase-2 enzyme
CSF	Colony-stimulating factor
ERBB1/EGFR	Epidermal growth factor receptor
ERα	Estrogen receptor alpha
IFNG	Interferon gamma
IL	Interleukin
IκB	Inhibitor of NF-κB
LPS	Lipopolysaccharide
NF-κB	Nuclear factor kappa B
PR	Progesterone receptor
RANK	Receptor activator of NF-κB
RANKL	Receptor activator of NF-κB ligand
TDLU	Terminal ductal lobular units
TEB	Terminal end buds
Th	Helper T lymphocytes
TLR	Toll-like receptor
TNFA	Tumor necrosis factor alpha
Treg	Regulatory T lymphocytes

E. F. Need · V. Atashgaran · W. V. Ingman · P. Dasari (✉)
Discipline of Surgery, School of Medicine, The Queen Elizabeth Hospital, University of Adelaide, DX465702, 28 Woodville Road, Woodville, SA 5011, Australia
e-mail: pallave.dasari@adelaide.edu.au

E. F. Need · V. Atashgaran · W. V. Ingman · P. Dasari
Robinson Research Institute, University of Adelaide, Adelaide, Australia

W. V. Ingman
School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, Australia

Introduction

It is increasingly recognized that the actions of hormones on mammary gland development and breast cancer are mediated by a number of paracrine interactions locally within the breast. Significantly, immune system cells are abundant in the stroma surrounding the mammary gland epithelium and provide essential support for hormone-driven mammary gland morphogenesis [1]. Estrogen, progesterone and prolactin each direct immune cell functions both alone and in combination, and the

actions of these immune cells drive pubertal and adult mammary gland development [2, 3]. However, the specific functions of immune cells necessary for normal development may also be important in contributing to hormone-mediated increased breast cancer risk.

The immune system is a complex network of immune organs, cells, and soluble factors such as cytokines that act locally or systemically through a variety of mechanisms, such as an immediate inflammatory response through cytokines and phagocytes, a specific tailored immune response through adaptive immune cells, or a regulated immunotolerant response. The differing, and sometimes opposing, roles of the immune system are mediated by a complex interplay of intracellular and extracellular signaling pathways. Cells of the immune system participate in a diverse number of processes in the body which can be categorized into (1) protection of the host from invading pathogens, foreign antigens and incipient tumor cells, and (2) participation in development, maintenance of homeostasis, tissue repair and regeneration for processes such as wound healing.

In the highly cited review on the hallmarks of cancer by Hanahan et al. [4], ten key biological processes are identified as being required for cancer to establish itself and metastasize in the host. Two of these hallmarks involve the immune system with (1) the tumor avoiding immune detection and destruction, and (2) inflammation promoting tumor growth. Of particular interest is how incipient tumors avoid immune detection and destruction, where evidence suggests that the ovarian hormones estrogen and progesterone can promote immune suppression [5, 6]. In addition, estrogen, progesterone and prolactin can modulate inflammation both systemically and locally within the mammary gland. In the mammary gland the effects of estrogen, progesterone and prolactin on immune cells can be either direct, or indirect through hormonally-regulated mammary gland components of mammary epithelial cells and stroma. The direct effects of the hormones on immune cells are mediated when the immune cells express receptors for estrogen, progesterone and prolactin, which are engaged by their respective ligands. The indirect effects of estrogen, progesterone and prolactin on immune cells are mediated by paracrine signals from mammary epithelial cells and stroma. How estrogen and progesterone regulate the immune microenvironment in the mammary gland is less well studied than their roles in mammary epithelia (discussed below), but there is a growing body of evidence that demonstrates that the hormonally-regulated immune environment plays an essential role in mammary gland development.

The actions of estrogen and progesterone are inextricably involved in the development and homeostasis of the normal breast and in most breast cancers [reviewed in 1, 7]. Epidemiological evidence points to a number of breast cancer risk factors associated with menstrual cycling and hormone exposure. For every year earlier of menarche there is a 5 % increased lifetime risk of breast cancer, and a 3.5 % increased lifetime risk for every year later of menopause [8]. In addition to these reproductive factors, in one large scale trial including several periods of clinical follow up, an increased risk of developing and dying from breast cancer was found in individuals who have previously taken combined hormone replacement therapy, which consists of combined exogenous estrogen and the synthetic progestin medroxyprogesterone acetate when compared to patients who were administered placebo [9, 10]. In this same trial of 16,608 women, those women who had undergone hysterectomy were administered estrogen alone, and exhibited decreased breast cancer risk in comparison to those taking placebo [11]. Extrapolation of all of these risks to biological function infers that increased risk of breast cancer is related to the timing of puberty and the increased number of menstrual cycles, and thus exposure to repeated fluctuations in ovarian hormones. This risk was acknowledged by Pike et al. in 1993, who coined "the estrogen augmented by progestogen hypothesis", which asserts that this increased breast cancer risk may be attributed to repeated cycles of progesterone-induced activity in combination with estrogen-induced activity [12].

However, although hormones act directly on the mammary gland epithelium to promote development, paracrine signals from the local immune population are essential in facilitating epithelial cell proliferation, differentiation and tissue remodeling [2, 3, 13, 14]. Together, the role of hormones in modulating breast cancer risk, combined with the role of the immune environment during dynamic structural remodeling and altered tissue composition of the normal breast may provide key mechanistic insights into factors that cause breast cancer. Hence, we outline here how estrogen, progesterone and prolactin impact on the immune microenvironment during different developmental stages of the mammary gland, and provide a summative model of how hormonally-regulated immune cells in the mammary gland impact on mammary gland development and the risk of breast cancer.

Immune System Cells and Cytokines in the Mammary Gland

Macrophages

Macrophages are derived from circulating blood monocytes that migrate into tissue and differentiate into tissue specific resident macrophages. They are highly plastic cells exquisitely sensitive to cytokine signals in the surrounding microenvironment and tailor their function accordingly [15]. Their functions are considerably varied compared to other immune cells and range from classically-activated cells that express MHC class II and participate in infection control, alternatively-

activated cells for tissue remodeling that express mannose receptor, or cells involved in immune modulation. However, this historical categorization of macrophage phenotypes has long been recognized as being inadequate to convey the complexity of macrophage functional plasticity. A recent study using transcriptome analysis revealed that macrophages can be activated via numerous signals resulting in nine clusters of macrophage activation and function [16]. Such a broad scope of macrophage phenotype is yet to be determined in the macrophages resident in the mammary gland. Therefore, in this review mammary gland macrophages will be discussed from the broader categories of immunity and tissue development, while being mindful of the complexity of macrophage plasticity. The functions of macrophages, like other cells, are implemented by expression of receptors and secretion of specific molecules to direct cell response and tailor functions of adjacent cells. Macrophages are critically important immune cells in pubertal and adult mammary gland development and the function of macrophages at specific stages of development will be discussed in Hormones and the Immune Environment during Mammary Gland Development section.

T lymphocytes

T lymphocytes are immune cells that mediate the cellular, humoral and immunoregulatory branches of the immune system principally through local cytokine signals and specific cell-to-cell interactions through the T cell receptor that governs antigen-mediated immunity. A major class of T lymphocytes is the helper T cells (Th), which are categorized further into several subsets on the basis of cell surface markers and functional attributes. Th cells can be polarized to either Th1 or Th2 subsets that secrete different patterns of cytokines; Th1 cells secrete mostly pro-inflammatory cytokines such as interferon-gamma (IFNG) and tumor necrosis factor (TNF)-alpha (TNFA), and Th2 cells induce immunomodulating cytokines such as interleukin (IL)4 and IL10, which also are involved in tissue remodeling [17]. Regulatory T cells (Treg) regulate the balance between immunity and tolerance, to avoid excessive or insufficient immune function [18]. A population of intra-epithelial T cells has been identified in the normal human breast [19, 20], however studies into their function are limited. There is an absence of literature available regarding the influence of estrogen and progesterone on T lymphocytes in the mammary gland, therefore discussed below is the response of peripheral blood T lymphocytes to ovarian hormones.

Eosinophils and Mast Cells

Hormone-regulated immune cells that are necessary for mammary gland development and homeostasis also include eosinophils and mast cells. Eosinophils are large, phagocytic immune cells, which target helminthes and participate in allergic reactions [21]. Mast cells are tissue resident cells that participate in early inflammatory responses and in allergies [22].

Cytokines

In the mammary gland, cytokines are secreted by mammary epithelial cells, fibroblasts, adipocytes or immune cells. The profile of cytokines can be pro-inflammatory, anti-inflammatory, immunoregulatory or tissue growth promoting depending upon the predominant secretory profile. This cytokine microenvironment can directly affect the development and function of the mammary gland by ensuring the appropriate recruitment of immune cells such as macrophages and eosinophils at appropriate developmental phases through the secretion of factors such as colony-stimulating factor 1 (CSF1), interleukins (IL) 4 and10, and tumor necrosis factor alpha (TNFA) [2, 3, 14].

Hormones and the Immune Environment during Mammary Gland Development

Mammary Gland Development during Puberty

In mice immediately preceding puberty, the terminal end buds (TEB) form, which are the distal ends of the mammary ducts that swell as the cuboidal epithelial cells within the bud proliferate in response to rising pre-pubertal estrogen concentrations [23]. In comparison, in the human pre-pubertal mammary gland; the distal ends of the ductal structures lack these distinct spherical structures and instead form into clusters that are collectively called terminal duct lobular units (TDLU) [24]. However, both TEBs and TLDUs undergo estrogen driven bifurcation, ductal elongation and lateral ductal branching to produce the network of epithelial ducts observed in the adult mammary gland in mice, rats and humans. This process is dependent upon the presence of the transcriptional regulator of estrogen action, estrogen receptor alpha (ERα) [25]. Likewise, key ERα coregulators are also essential for the development of the mammary gland, such as SRC-3 and RIP140 [26, 27]. While activation of ERβ or androgen receptor (AR) is not essential at any stage of mammary development, these receptors have been described to contribute to mammary development by inhibition or enhancement of ductal growth during the pubertal stage [28–30].

The effects of ERα in the developing mammary gland are largely paracrine; only a subset of the mammary epithelium is required to express ERα for normal mammary gland development [25]. One of these ERα-regulated developmental paracrine signals is amphiregulin, which activates the

epidermal growth receptor (ERBB1/EGFR) in the mammary stroma [31]. Indeed, mice with deficient ERBB1 stromal signaling have impaired alveolar development similar to that observed in ERα null mice, and amphiregulin-null mice fail to develop mature ductal trees due to defects in ductal outgrowth, which may in part be due to inhibited recruitment of macrophages and eosinophils to sites of tissue remodeling [2, 3, 25, 31–33].

Estrogen-regulated production of CSF1 in the murine mammary gland is essential for pubertal development, as CSF1-regulated macrophages associate with the expanding mammary epithelial ducts and buds [34, 35], and alter the structural alignment of collagen fibers around the expanding terminal end buds [36]. In a humanized mouse model, macrophages exposed to estrogen promote the growth of the pubertal mammary stroma through paracrine effects on mammary fibroblasts [37]. Macrophages are critical at the pubertal stage as their absence, in line with the effects of the absence of ERα as discussed above, delays mammary gland development in mice [35].

In the mammary gland of pubertal female mice, the rising estrogen concentration increases EGFR signaling via upregulation of amphiregulin, thus leading to increased recruitment of eosinophils, with modulation of eotaxin concentration identified as the primary paracrine mediator of eosinophil recruitment [3]. The association of eosinophils with mammary epithelia increases ductal branching and formation of TEBs in mice [35]. Interestingly, overabundance of eosinophils in the pubertal gland in mice actually retards mammary epithelial morphogenesis, implying that their optimal abundance controls the overall number of ductal structures in mammary gland development [38]. Mast cells are also necessary for development of the mammary gland during puberty. As with eosinophils, mast cells are important for mammary epithelial cell proliferation in mice, as their absence retards growth [39]. Thus, macrophages, eosinophils and mast cells are required for expansion of the mammary gland during puberty.

Menstrual Cycling Impact on the Mammary Gland Immune Environment

Steroid Receptors and Hormones

The menstrual cycle involves the cyclical production of estrogen and progesterone, governed by endocrine signals from the hypothalamus, pituitary, ovaries and adrenal glands. During the follicular phase, maturing ovarian follicles secrete increasing amounts of estrogen until a peak is reached which leads to ovulation. In contrast, the luteal phase is characterized by rising levels of progesterone produced by the remnant of the follicle, the corpus luteum. Importantly, this increase in progesterone concentration is accompanied by a concomitant second smaller peak of estrogen during the luteal phase (Fig. 1a). The follicular phase in humans and estrus phase in mice reflect similar profiles of circulating estrogen concentration, while in the metestrus and diestrus phases, circulating progesterone concentration increases and estrogen concentration is lower. It has been appreciated for four decades that the mammary gland undergoes dynamic morphological changes during the menstrual cycle in both humans and mice, and that these stages can be categorized histologically by examining the morphological structures within the gland [40, 41] (Fig. 1b). Detailed analyses of the changes in mammary epithelial cell proliferation and apoptosis over the human menstrual cycle have revealed that proliferation of the epithelial cells is highest during the luteal phase, and is positively associated with serum progesterone concentration, while apoptosis was relatively consistent between the follicular and luteal phases in some but not all studies [42–45]. However, from a human whole mammary gland perspective, the mammary epithelial ductal structures proliferate and undergo increased branching during the luteal phase and then regress during the follicular phase [46]. Thus, declining circulating progesterone towards the end of the luteal phase does not appear to be linked with apoptosis of human mammary epithelia [43, 45], unlike the observed positive correlation of progesterone withdrawal with mammary epithelial cell apoptosis in mice [47]. This conflict may be due to sampling difficulties and the histological techniques employed in human specimens. TUNEL staining and histological measures of apoptosis used in human studies detect late stage apoptosis, and these dying cells may be rapidly phagocytosed by adjacent macrophages before exhibiting these apoptotic markers [48].

Macrophages

Murine studies show macrophage abundance in the mammary gland fluctuates over the estrous cycle, peaking at metestrus and diestrus phases, when progesterone concentration is highest, and is lowest at proestrus and estrus, when progesterone concentration is reduced [2]. Macrophages have dual roles that are critical for progesterone-mediated epithelial cell growth and regression in the mammary gland; an absence of macrophages results in both reduced epithelial cell proliferation and impaired regression during estrogen and progesterone-mediated cyclical morphogenesis [2].

Progesterone alters the abundance of macrophages in the mammary gland expressing MHC class II, the scavenger receptor CD204 and DNA damage recognition receptor NKG2D during the estrous cycle, and in ovariectomised mice administered exogenous estrogen and progesterone [14]. The percentage of MHC class II-expressing macrophages is reduced by estrogen and progesterone administration, and elevated following administration of the progesterone receptor antagonist RU486. MHC class II is critical for macrophages to present antigens to T lymphocytes and activate them to elicit an immune response. Therefore, the progesterone-mediated

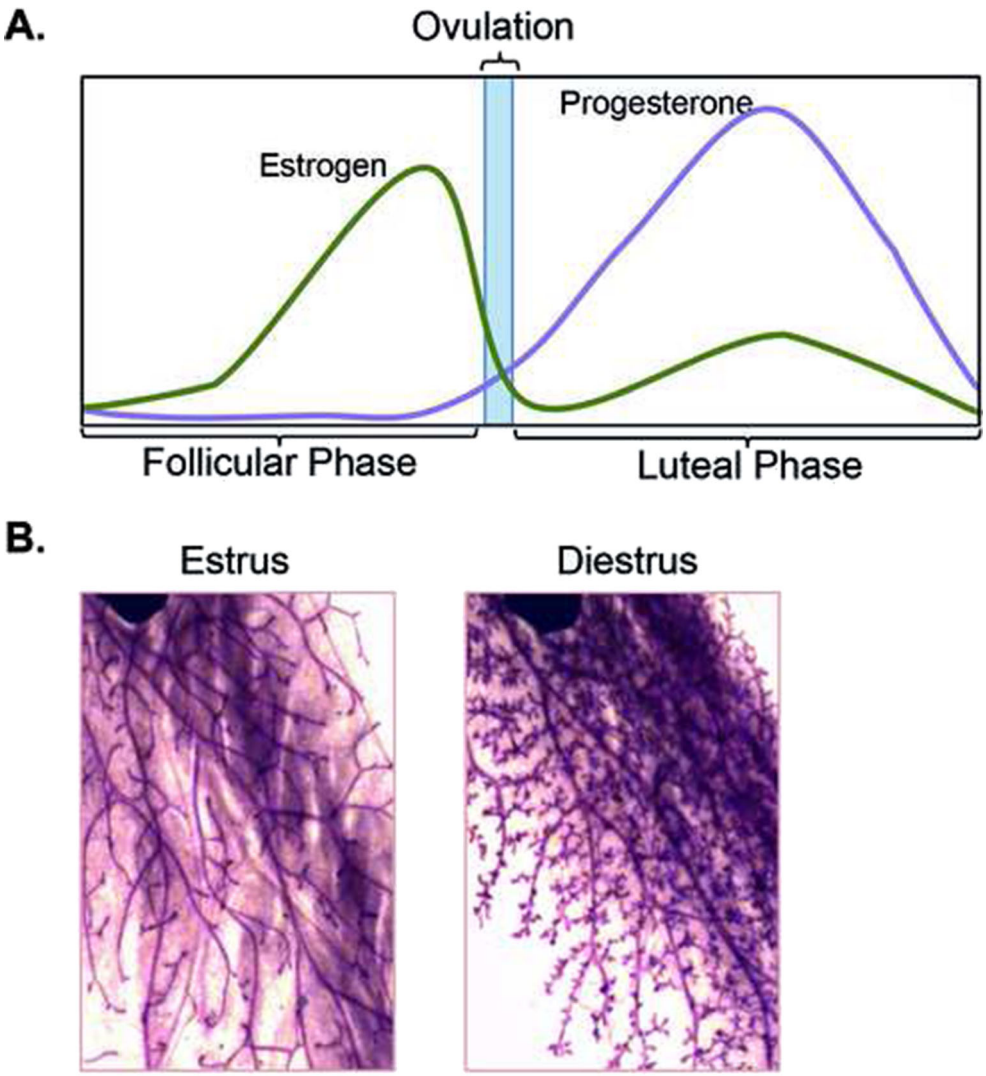

Fig. 1 The effect of ovarian hormone cycling on mammary gland remodeling. **a** The fluctuations of estrogen (*green*) and progesterone (*purple*) during the human menstrual cycle and (**b**) fluctuations in estrogen and progesterone have a profound impact on mouse mammary gland morphology during estrus (high estrogen and low progesterone) and diestrus (high progesterone and low estrogen). Modified from Hodson et al. [14] with permission

reduction of MHC class II expression by macrophages is likely to limit their ability to initiate adaptive immune responses against pathogens. On the other hand, the percentage of NKG2D-expressing macrophages is elevated in response to estrogen and progesterone and declines upon administration of RU486 [14]. Surface expression of NKG2D on macrophages enables detection of DNA damaged cells, and stimulates secretion of inflammatory mediators to recruit other immune cells to the site [49].

Human macrophages are also stimulated by estrogen via ERα resulting in suppression of the nuclear factor-kappa B (NF-κB) signaling pathway [50], which is critical for phagocytosis and drives IL4-mediated alternative activation of murine macrophages, thus dampening the inflammatory response [51]. Indeed, rapid phagocytosis of apoptotic cells dampens inflammation through inhibition of the production of inflammatory cytokines IL1B, IL8, TNFA and CSF2 [52, 53], and increasing production of IL10 [52]. IL10, an anti-inflammatory cytokine, is necessary for mammary ductal branching, as observed in *Il10* null mutant mice, suggesting that IL10 may skew macrophages towards a tissue remodeling role [54]. Thus, anti-inflammatory conditions via IL10 modulation and phagocytosis of apoptotic mammary epithelial cells during the regression of mammary epithelial cells in response to progesterone withdrawal in the context of rising estrogen may create an immunotolerant mammary environment for tumor initiation. This is supported by studies demonstrating increased murine mammary gland susceptibility to I-nitroso-I-methylurea(NMU)-induced carcinogenesis during the proestrus and estrus phases of the cycle, which exhibit declining serum progesterone concentrations and elevated estrogen, in comparison to the diestrus phase where the rat exhibits elevated serum progesterone and low estrogen [55, 56].

T lymphocytes

The immune environment of the mammary gland is dampened during different phases of the menstrual cycle in response to

hormonal stimuli. During the luteal phase of the menstrual cycle, Th cells in the blood are biased towards the Th2 type, which secrete cytokines such as IL4 and IL10 [57]. Estrogen directly increases the abundance of Treg cells in blood and enhances their immunosuppressive functions [5, 58]. In the follicular phase the numbers of Treg cells increase and correlate positively with serum concentration of estrogen [59]. Progesterone, in combination with TGFB1, a growth factor that inhibits mammary epithelial cell proliferation, also induces high numbers of Treg cells in the blood, which are highly stable and more effective in their immunosuppressive function [6]. While further investigations into T cell function and abundance in the cycling mammary gland are required, the regulation of immunosuppressive Treg cells in the blood by estrogen and progesterone suggest that the abundance of Treg cells in the mammary gland may fluctuate over the menstrual cycle.

Cytokine Microenvironment

Estrogen and progesterone can have differing effects on the cytokine profile in mammary glands at the pubertal stage versus the adult stage. Mammary glands in pubertal female mice treated with only estrogen or progesterone induce expression of several pro-inflammatory genes for chemokines and cytokines including genes encoding IL17B and TNFA [3]. In contrast, in adult mice, recent findings demonstrate that the cytokine microenvironment of the murine mammary gland fluctuates over the estrous cycle, with the estrus phase skewed to a pro-inflammatory environment through increased protein secretion of CSF1, IFNG and TNFA [60]. However, the pro-inflammatory environment is dampened by the presence of progesterone, and the abundance of TNFA and IL6 in the mammary gland declines during the luteal phase [61, 62]. These results in dual hormone treated mammary glands of female mice contrast with the findings of Aupperlee et al., who report that steroid hormones invoke a response in inflammatory chemokines in the mammary gland upon sole estrogen or progesterone treatment [3].

Mammary Gland Development during Pregnancy and Lactation

During pregnancy, epithelial cells undergo dramatic proliferation followed by differentiation and enhanced survival. This dynamic remodeling is accompanied by stromal remodeling that encompasses enhanced angiogenesis, infiltration of macrophages and granulocytes, fibroblast reorganization and loss of adipocyte lipid droplets. The role of progesterone signaling in the non-neoplastic breast is most evident during pregnancy, where the PR-B isoform is required, but only in a subset of the lobuloalveolar epithelia, for the extensive proliferation of epithelial cells within the lobuloalveolar structures, thus supporting a paracrine role for PR-B in this process [63]. Another key hormone that is required for pregnancy-induced mammary development is prolactin, a peptide hormone produced by the pituitary in response to neuroendocrine signals from the hypothalamus [64]. At parturition, the fall in circulating progesterone, in the context of elevated prolactin, triggers the switch to secretory lactation, where tight junctions between epithelia close, and colostrum accumulate in the alveolar lumens for ejection from the breast.

Interestingly, the NF-κB signaling pathway, commonly known to mediate detection of invading pathogens, inflammation and cellular apoptosis, is identified as a critical mediator of lobuloalveolar development during pregnancy. Receptor activator of NF-κB ligand (RANKL), a member of the TNF cytokine family is a candidate paracrine signaling molecule in pregnancy-induced mammary gland morphogenesis. Signals from prolactin and progesterone both converge to activate the RANKL signaling and NF-κB pathways [65]. Mice with RANKL or RANK deficient lobuloalveolar buds fail to produce differentiated, milk producing alveoli but still form alveolar buds upon pregnancy, indicating that the preliminary progesterone-mediated proliferative effects in the lobuloalveolar epithelia upon pregnancy may be independent of RANK signaling, while the differentiation of the alveolar epithelial cells into mature milk secreting cells is RANK signaling dependent [66, 67]. The differentiation of alveolar epithelial cells is dependent upon NF-κB activation, as demonstrated by IKKα null mice, which is required for NK- κB activity. These IKKα null mice display mammary gland defects similar to RANKL deficient mice and do not express Cyclin D1, which has also been shown to be essential for lobuloalveolar differentiation during pregnancy, and is regulated by both progesterone and STAT5 [68, 69]. Thus, the network of PR, prolactin, NF-kB and STAT5 signaling are interlinked by mutual regulation of RANKL and its downstream effectors, resulting in differentiation or maturation of the lobuloalveolar epithelia into mature milk secreting glands.

While a role in pregnancy-induced mammary remodeling is yet to be described, cyclooxygenase 2 (COX2), an enzyme involved in the synthesis of pro-inflammatory prostanoids, has been shown to be hormonally regulated in murine mammary tissues in response to estrogen and progesterone, and is increased in expression in normal human mammary tissue during pregnancy [70]. However, there is also evidence progesterone suppresses systemic inflammatory responses; mononuclear immune cells from progesterone-treated blood, which is dominated by precursor macrophages, have decreased concentrations of NF-κB, TNFA and IL6 [71].

Macrophages

The epithelium within the ducts and alveoli of the lactating mammary gland express high concentrations of CSF1

receptor and CSF1 compared to non-pregnant, non-lactating mammary epithelium in mice [72]. During pregnancy, in the mammary gland macrophages regulate epithelial cell development, as absence of CSF1 leads to reduced ductal branching, excessive lobuloalveologenesis and precocious differentiation and milk production in mice [13]. Macrophages also play an essential role in mediating the switch from pregnancy to lactation, and mice deficient in these cells do not secrete milk. Although it is unknown how macrophages promote the switch to lactation, macrophages may regulate tight junction permeability. NF-κB activation is a key event in increasing membrane permeability during periods of inflammation in a number of tissues [73], and activation of NF-κB by the toll-like receptor 4 signaling pathway in a mouse model increases permeability of the milk-blood barrier [74]. Importantly, macrophage-mediated TLR4 activation regulates inflammation in the mammary gland [75] [76], and the decline in progesterone at parturition may affect the function of resident macrophages to promote tight junction closure.

T lymphocytes

Pregnancy places the mother in an immunotolerant, anti-inflammatory state for the survival of the allogeneic fetus [77]. Estrogen, at concentrations comparable to pregnancy, increases the proliferation and immunosuppressive ability of Treg cells in the blood [5] and also inhibits the inflammatory reaction via NF-κB in rat muscle cells [78]. As discussed above, NF-κB is upregulated during pregnancy by progesterone-driven upregulation of RANK, which also regulates Treg cells [79]. As in menstrual cycling, elevated progesterone in the mammary gland during pregnancy may increase immune tolerance while upregulating the factors which drive Treg cell function.

Limited evidence suggests a role for prolactin governing immune cell profiles during lactation in the mammary gland. A lactation deficient rat model with decreased serum prolactin does not have a skewed profile of B or T cell phenotypes, however they did observe reduced migration of T lymphocytes from the blood to the mammary gland, with reduced expression of chemokine receptors by those T lymphocytes [80]. Prolactin-induced differentiated luminal epithelial cells in mice upregulate expression of IL4 receptor and alter the immune microenvironment by altering their cytokine profile towards a Th2 bias [81]. Additionally, progesterone skews murine T lymphocytes towards the Th2 profile and inhibits the Th1 subtype while elevating secretion of IL10 by T lymphocytes [82]. Thus, both progesterone and prolactin in the pregnant/lactating mammary gland directly influence T lymphocytes through the mammary epithelial cells producing Th2-like cytokines such as IL4, IL5 and IL13.

The Involution Immune Environment

As involution commences, the extensive alveolar sacs and ductal branches undergo the dramatic process of remodeling that returns the mammary epithelia to the pre-pregnancy, basic ductal network. Initial changes can be observed within hours of the cessation of suckling, and involve a decline in the production and secretion of prolactin and oxytocin. Early cellular events within the epithelial cells in response to weaning in mice include activation of NF-κB, increased expression of proapoptotic proteins Bax and Bcl-x, a decrease in phosphorylated and cellular STAT5 and increases in STAT3 phosphorylation [83–85]. These changes are accompanied by an eight fold increase in recruitment of developmental, tissue remodeling phenotype macrophages to the mammary gland that allows remodeling of the mammary epithelial network to the earlier nulliparous state [86–88]. The immune microenvironment during the involution phase and the impact of this on breast cancer risk is reviewed elsewhere in this issue.

Estrogen-Mediated, Progesterone Fuelled Signaling and the Immune Environment in Breast Cancer Risk

Increasing evidence highlights progesterone-regulated immune system factors in breast cancer risk, with RANKL and NF-κB as key signaling mediators. Progestin increases RANKL expression in the murine mammary gland, which is required for DMBA-induced carcinogenesis [89, 90]. Progesterone also induces RANKL expression in human mammary glands [91]. Combination hormone replacement therapy (estrogen plus medroxyprogesterone acetate) in ovariectomised cynomolgus macaques induces a gene expression profile remarkably similar to that exhibited during the differentiation of the TDLU in late pregnancy or during the luteal phase of the menstrual cycle, where estrogen plus medroxyprogesterone acetate caused upregulation of PRL/STAT5, ERBB1 and RANKL pathways in conjunction with increased proliferation within the glands, an effect not observed in macaques treated with estrogen alone [92]. Likewise, an analysis of gene expression in cynomolgus macaques over several life stages revealed remarkable transcriptional similarities between the mammary glands of luteal phase and pregnant or lactating monkeys, including elevated expression of the RANKL-associated differentiation factor ELF5 [93]. Together, these results suggest that the actions of progestogens (synthetic progestins or naturally occurring progesterone) in the presence of estrogen activate NF-κB-mediated differentiation in estrogen-primed, progestogen-fuelled paracrine proliferation of mammary epithelial cells (Fig. 2).

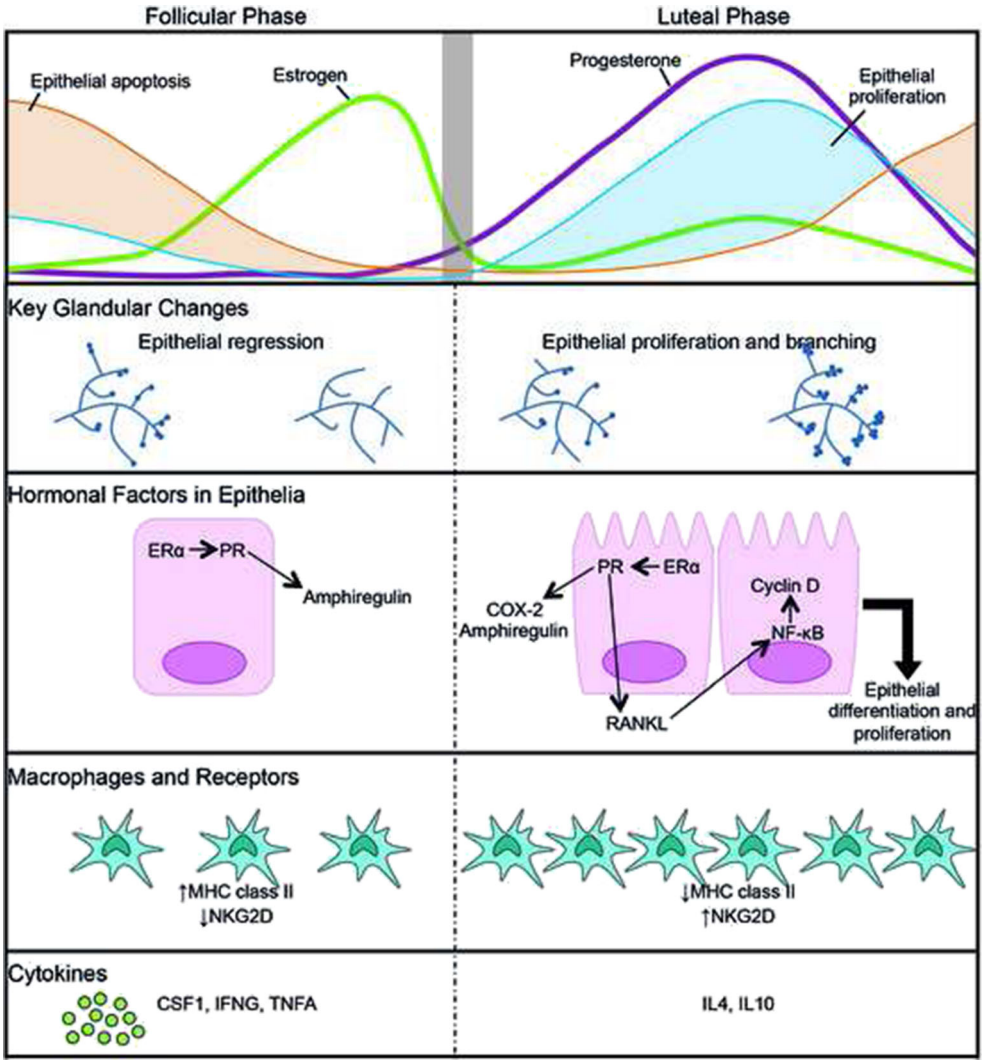

Fig. 2 Summative model of immune microenvironment in cycling hormonal mammary gland. Shown in orange and blue are the net apoptosis and net proliferation in the gland, respectively, during the menstrual phases [97]. Key glandular changes occurring in the gland are represented, as well as key alterations in mammary epithelial cells that relate to immune cell function. The relative abundance of macrophages in the gland is represented over the cycle, with fluctuations in cytokines between the follicular phases and luteal phases also depicted. Increasing evidence suggests a high level of integration between each level of the figure, but the precise mechanisms of integration remain to be defined

RANKL signaling and NF-κB activation have profound effects on directing immune system responses that can promote carcinogenesis by subverting tumor immunity and driving persistent inflammation [94]. RANKL is expressed by Treg cells [95] and increases their local abundance [96]. Expression of RANKL by Treg cells is critical for *Erbb2*+ tumors to metastasize [95]. The RANKL-mediated tumor metastasis operates through Treg cells, though exogenous RANKL is equally effective. The elevated expression of RANKL in metastatic tumors is dependent on local populations of Treg cells in the adjacent stroma. Collectively these results suggest an underappreciated level of interplay between mammary epithelial cells and Treg cells governing cancer-associated inflammation.

The fluctuations of estrogen and progesterone during the menstrual cycle have direct and indirect effects on the local mammary gland immune system. The immune microenvironment during the luteal phase and pregnancy are characterized by high concentrations of serum estrogen and progesterone. The principal effects of estrogen and progesterone on the local immune system of the mammary gland are the recruitment of immune cells via CSF1, TGFB and IL10 which skew the cells towards a developmental role in mammary morphogenesis [6, 13, 52], thus reducing the active immune surveillance of the mammary epithelia. An additional effect of progesterone-driven mammary epithelia remodeling is the immune dampening by phagocytosis of apoptotic cells, which releases the immunomodulatory cytokines, IL4 and IL10. Estrogen and progesterone also have direct effects on the function of immune cells. Macrophages are directed into a tissue remodeling phenotype which downregulates expression of critical immune markers such as MHC class II and decreases the secretion of inflammatory cytokines. Collectively, these findings indicate that the combined effect of estrogen and progesterone in the mammary gland result in a net dampening of local immune surveillance, toward an environment exhibiting immune tolerance, which may lead to enhanced tumorigenesis due to decreased cancer cell detection and eradication.

Conclusion

The mammary gland undergoes hormonally mediated continual cycles of tissue remodeling, alongside cyclical modulation of the immune system between states of inflammation and immunotolerance. The cumulative effects of this process, which involves luteal phase-driven mammary epithelial cell proliferation and differentiation, may enhance tumor growth after the initial stage of genetic aberration in mammary epithelial cells that is necessary for tumorigenesis. If this stage occurs in a proliferating immunotolerant mammary gland that is ill-equipped to detect and eliminate aberrant cells, then breast cancer risk is likely to be exacerbated. In conclusion, immune system cells and cytokines have essential roles in hormonally-driven mammary gland development. Future studies investigating the precise relationship between estrogen, progesterone, mammary epithelial cell function and the immune environment are required in order to fully appreciate their combined influence on breast cancer risk.

Funding Support EFN is supported by The Hospital Research Foundation Early Career Research Fellowship. WVI is a THRF Associate Professor and National Breast Cancer Foundation Early Career Fellow. PD is supported by an Australian Breast Cancer Research Postdoctoral Fellowship. We thank Mr Peter Adams for his expert illustrative assistance with the figures.

References

1. Coussens LM, Pollard JW. Leukocytes in Mammary Development and Cancer. Cold Spring Harbor Perspectives in Biology. 2011;3(3). doi:10.1101/cshperspect.a003285.
2. Chua AC, Hodson LJ, Moldenhauer LM, Robertson SA, Ingman WV. Dual roles for macrophages in ovarian cycle-associated development and remodelling of the mammary gland epithelium. Development. 2010;137(24):4229–38. doi:10.1242/dev.059261.
3. Aupperlee MD, Zhao Y, Tan YS, Leipprandt JR, Bennett J, Haslam SZ et al. Epidermal growth factor receptor (EGFR)-signaling is a key mediator of hormone-induced leukocyte infiltration in the pubertal female mammary gland. Endocrinology. 2014:en20131933. doi:10.1210/en.2013-1933.
4. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74. doi:10.1016/j.cell.2011.02.013.
5. Prieto GA, Rosenstein Y. Oestradiol potentiates the suppressive function of human CD4 CD25 regulatory T cells by promoting their proliferation. Immunology. 2006;118(1):58–65. doi:10.1111/j.1365-2567.2006.02339.x.
6. Lee JH, Lydon JP, Kim CH. Progesterone suppresses the mTOR pathway and promotes generation of induced regulatory T cells with increased stability. Eur J Immunol. 2012. doi:10.1002/eji.201142317.
7. Brisken C. Progesterone signalling in breast cancer: a neglected hormone coming into the limelight. Nat Rev Cancer. 2013;13(6):385–96. doi:10.1038/nrc3518.
8. Cancer CGoHFiB. Menarche, menopause, and breast cancer risk: individual participant meta-analysis, including 118 964 women with breast cancer from 117 epidemiological studies. Lancet Oncol. 2012;13(11):1141–51. doi:10.1016/S1470-2045(12)70425-4.
9. Chlebowski RT, Anderson GL, Gass M, et al. EStrogen plus progestin and breast cancer incidence and mortality in postmenopausal women. JAMA. 2010;304(15):1684–92. doi:10.1001/jama.2010.1500.
10. Heiss G, Wallace R, Anderson GL, Aragaki A, Beresford SA, Brzyski R, et al. Health risks and benefits 3 years after stopping randomized treatment with estrogen and progestin. JAMA. 2008;299(9):1036–45. doi:10.1001/jama.299.9.1036.
11. LaCroix AZ, Chlebowski RT, Manson JE, et al. Health outcomes after stopping conjugated equine estrogens among postmenopausal women with prior hysterectomy: A randomized controlled trial. JAMA. 2011;305(13):1305–14. doi:10.1001/jama.2011.382.
12. Pike MC, Spicer DV, Dahmoush L, Press MF. Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. Epidemiol Rev. 1993;15(1):17–35.
13. Pollard JW, Hennighausen L. Colony stimulating factor 1 is required for mammary gland development during pregnancy. Proc Natl Acad Sci U S A. 1994;91(20):9312–6.
14. Hodson LJ, Chua AC, Evdokiou A, Robertson SA, Ingman WV. Macrophage phenotype in the mammary gland fluctuates over the course of the estrous cycle and is regulated by ovarian steroid hormones. Biol Reprod. 2013;89(3):65. doi:10.1095/biolreprod.113.109561.
15. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol. 2008;8(12):958–69. doi:10.1038/nri2448.
16. Xue J, Schmidt Susanne V, Sander J, Draffehn A, Krebs W, Quester I, et al. Transcriptome-Based Network Analysis Reveals a Spectrum Model of Human Macrophage Activation. Immunity. 2014;40(2):274–88. doi:10.1016/j.immuni.2014.01.006.
17. Mosmann TR, Sad S. The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today. 1996;17(3):138–46.
18. Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell. 2008;133(5):775–87. doi:10.1016/j.cell.2008.05.009.
19. Lwin KY, Sloane JP, Zuccarini O, Beverley PC. An immunohistological study of leukocyte localization in benign and malignant breast tissue. Int J Cancer. 1985;36(4):433–8.
20. Zuk JA, Walker RA. Immunohistochemical analysis of HLA antigens and mononuclear infiltrates of benign and malignant breast. J Pathol. 1987;152(4):275–85.
21. Rothenberg ME, Hogan SP. The eosinophil. Annu Rev Immunol. 2006;24:147–74. doi:10.1146/annurev.immunol.24.021605.090720.
22. Metcalfe DD, Baram D, Mekori YA. Mast cells. Physiol Rev. 1997;77(4):1033–79.
23. Russo J, Tay LK, Russo IH. Differentiation of the mammary gland and susceptibility to carcinogenesis. Breast Cancer Res Treat. 1982;2(1):5–73.
24. Cardiff R, Wellings S. The Comparative Pathology of Human and Mouse Mammary Glands. J Mammary Gland Biol Neoplasia. 1999;4(1):105–22. doi:10.1023/a:1018712905244.
25. Mallepell S, Krust A, Chambon P, Brisken C. Paracrine signaling through the epithelial estrogen receptor α is required for proliferation and morphogenesis in the mammary gland. Proc Natl Acad Sci U S A. 2006;103(7):2196–201.
26. Nautiyal J, Steel JH, Mane MR, Oduwole O, Poliandri A, Alexi X, et al. The transcriptional co-factor RIP140 regulates mammary gland development by promoting the generation of key mitogenic signals. Development. 2013;140(5):1079–89.
27. Xu J, Liao L, Ning G, Yoshida-Komiya H, Deng C, O'Malley BW. The steroid receptor coactivator SRC-3 (p/CIP/RAC3/AIB1/ACTR/TRAM-1) is required for normal growth, puberty, female reproductive function, and mammary gland development. Proc Natl Acad Sci. 2000;97(12):6379–84.
28. Krege JH, Hodgin JB, Couse JF, Enmark E, Warner M, Mahler JF, et al. Generation and reproductive phenotypes of mice lacking estrogen receptor β. Proc Natl Acad Sci. 1998;95(26):15677–82.

29. Yeh S, Tsai M-Y, Xu Q, Mu X-M, Lardy H, Huang K-E, et al. Generation and characterization of androgen receptor knockout (ARKO) mice: An in vivo model for the study of androgen functions in selective tissues. Proc Natl Acad Sci. 2002;99(21):13498–503. doi:10.1073/pnas.212474399.
30. Peters AA, Ingman WV, Tilley WD, Butler LM. Differential effects of exogenous androgen and an androgen receptor antagonist in the peri- and postpubertal murine mammary gland. Endocrinology. 2011;152(10):3728–37. doi:10.1210/en.2011-1133.
31. Ciarloni L, Mallepell S, Brisken C. Amphiregulin is an essential mediator of estrogen receptor α function in mammary gland development. Proc Natl Acad Sci. 2007;104(13):5455–60.
32. Wiesen JF, Young P, Werb Z, Cunha GR. Signaling through the stromal epidermal growth factor receptor is necessary for mammary ductal development. Development. 1999;126(2):335–44.
33. Gallego MI, Binart N, Robinson GW, Okagaki R, Coschigano KT, Perry J, et al. Prolactin, Growth Hormone, and Epidermal Growth Factor Activate Stat5 in Different Compartments of Mammary Tissue and Exert Different and Overlapping Developmental Effects. Dev Biol. 2001;229(1):163–75. doi:10.1006/dbio.2000.9961.
34. Van Nguyen A, Pollard JW. Colony stimulating factor-1 is required to recruit macrophages into the mammary gland to facilitate mammary ductal outgrowth. Dev Biol. 2002;247(1):11–25. doi:10.1006/dbio.2002.0669.
35. Gouon-Evans V, Rothenberg ME, Pollard JW. Postnatal mammary gland development requires macrophages and eosinophils. Development. 2000;127(11):2269–82.
36. Ingman WV, Wyckoff J, Gouon-Evans V, Condeelis J, Pollard JW. Macrophages promote collagen fibrillogenesis around terminal end buds of the developing mammary gland. Dev Dyn. 2006;235(12):3222–9. doi:10.1002/dvdy.20972.
37. Fleming JM, Miller TC, Kidacki M, Ginsburg E, Stuelten CH, Stewart DA, et al. Paracrine interactions between primary human macrophages and human fibroblasts enhance murine mammary gland humanization in vivo. Breast Cancer Res. 2012;14(3):R97. doi:10.1186/bcr3215.
38. Sferruzzi-Perri AN, Robertson SA, Dent LA. Interleukin-5 transgene expression and eosinophilia are associated with retarded mammary gland development in mice. Biol Reprod. 2003;69(1):224–33. doi:10.1095/biolreprod.102.010611.
39. Lilla JN, Werb Z. Mast cells contribute to the stromal microenvironment in mammary gland branching morphogenesis. Dev Biol. 2010;337(1):124–33. doi:10.1016/j.ydbio.2009.10.021.
40. Fanger H, Ree HJ. Cyclic changes of human mammary gland epithelium in relation to the menstrual cycle—an ultrastructural study. Cancer. 1974;34(3):574–85.
41. Vogel P, Georgiade N, Fetter B, Vogel F, McCarty Jr K. The correlation of histologic changes in the human breast with the menstrual cycle. Am J Pathol. 1981;104(1):23.
42. Fata JE, Chaudhary V, Khokha R. Cellular turnover in the mammary gland is correlated with systemic levels of progesterone and not 17β-estradiol during the estrous cycle. Biol Reprod. 2001;65(3):680–8.
43. Navarrete M, Maier CM, Falzoni R, Quadros L, Lima GR, Baracat EC, et al. Assessment of the proliferative, apoptotic and cellular renovation indices of the human mammary epithelium during the follicular and luteal phases of the menstrual cycle. Breast Cancer Res. 2005;7(3):R306–13.
44. Söderqvist G, Isaksson E, von Schoultz B, Carlström K, Tani E, Skoog L. Proliferation of breast epithelial cells in healthy women during the menstrual cycle. Am J Obstet Gynecol. 1997;176(1):123–8.
45. Potten CS, Watson R, Williams G, Tickle S, Roberts SA, Harris M, et al. The effect of age and menstrual cycle upon proliferative activity of the normal human breast. Br J Cancer. 1988;58(2):163.
46. Ramakrishnan R, Khan SA, Badve S. Morphological changes in breast tissue with menstrual cycle. Mod Pathol. 2002;15(12):1348–56. doi:10.1097/01.MP.0000039566.20817.46.
47. Fata JE, Chaudhary V, Khokha R. Cellular turnover in the mammary gland is correlated with systemic levels of progesterone and not 17beta-estradiol during the estrous cycle. Biol Reprod. 2001;65(3):680–8.
48. Walker NI, Bennett RE, Kerr JF. Cell death by apoptosis during involution of the lactating breast in mice and rats. Am J Anat. 1989;185(1):19–32.
49. Diefenbach A, Jamieson AM, Liu SD, Shastri N, Raulet DH. Ligands for the murine NKG2D receptor: expression by tumor cells and activation of NK cells and macrophages. Nat Immunol. 2000;1(2):119–26.
50. Murphy AJ, Guyre PM, Pioli PA. Estradiol suppresses NF-kappa B activation through coordinated regulation of let-7a and miR-125b in primary human macrophages. J Immunol. 2010;184(9):5029–37. doi:10.4049/jimmunol.0903463.
51. Ribas V, Drew BG, Le JA, Soleymani T, Daraei P, Sitz D, et al. Myeloid-specific estrogen receptor alpha deficiency impairs metabolic homeostasis and accelerates atherosclerotic lesion development. Proc Natl Acad Sci U S A. 2011;108(39):16457–62. doi:10.1073/pnas.1104533108.
52. Voll RE, Herrmann M, Roth EA, Stach C, Kalden JR, Girkontaite I. Immunosuppressive effects of apoptotic cells. Nature. 1997;390(6658):350–1. doi:10.1038/37022.
53. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. J Clin Invest. 1998;101(4):890–8. doi:10.1172/jci1112.
54. Masso-Welch PA, Merhige PM, Veeranki OL, Kuo SM. Loss of IL-10 decreases mouse postpubertal mammary gland development in the absence of inflammation. Immunol Investig. 2012;41(5):521–37. doi:10.3109/08820139.2012.684193.
55. Lindsey WF, Das Gupta TK, Beattie CW. Influence of the Estrous Cycle during Carcinogen Exposure on Nitrosomethylurea-induced Rat Mammary Carcinoma. Cancer Res. 1981;41(10):3857–62.
56. Smith M, Freeman M, Neill J. The Control of Progesterone Secretion During the Estrous Cycle and Early Pseudopregnancy in the Rat: Prolactin, Gonadotropin and Steroid Levels Associated with Rescue of the Corpus Luteum of Pseudopregnancy 1 2. Endocrinology. 1975;96(1):219–26.
57. Faas M, Bouman A, Moesa H, Heineman MJ, de Leij L, Schuiling G. The immune response during the luteal phase of the ovarian cycle: a Th2-type response? Fertil Steril. 2000;74(5):1008–13.
58. Polanczyk MJ, Carson BD, Subramanian S, Afentoulis M, Vandenbark AA, Ziegler SF, et al. Cutting edge: estrogen drives expansion of the CD4+ CD25+ regulatory T cell compartment. J Immunol. 2004;173(4):2227–30.
59. Arruvito L, Sanz M, Banham AH, Fainboim L. Expansion of CD4+ CD25+and FOXP3+ regulatory T cells during the follicular phase of the menstrual cycle: implications for human reproduction. J Immunol. 2007;178(4):2572–8.
60. Dasari P, Sharkey DJ, Noordin E, Glynn DJ, Hodson LJ, Chin PY, et al. Hormonal regulation of the cytokine microenvironment in the mammary gland Manuscript submitted. 2014.
61. Brannstrom M, Friden BE, Jasper M, Norman RJ. Variations in peripheral blood levels of immunoreactive tumor necrosis factor alpha (TNFalpha) throughout the menstrual cycle and secretion of TNFalpha from the human corpus luteum. Eur J Obstet Gynecol Reprod Biol. 1999;83(2):213–7.
62. Angstwurm MW, Gartner R, Ziegler-Heitbrock HW. Cyclic plasma IL-6 levels during normal menstrual cycle. Cytokine. 1997;9(5):370–4. doi:10.1006/cyto.1996.0178.
63. Mulac-Jericevic B, Lydon JP, DeMayo FJ, Conneely OM. Defective mammary gland morphogenesis in mice lacking the progesterone receptor B isoform. Proc Natl Acad Sci. 2003;100(17):9744–9.

64. Leong DA, Frawley LS, Neill JD. Neuroendocrine control of prolactin secretion. Annu Rev Physiol. 1983;45(1):109–27.
65. Srivastava S, Matsuda M, Hou Z, Bailey JP, Kitazawa R, Herbst MP, et al. Receptor activator of NF-κB ligand induction via Jak2 and Stat5a in mammary epithelial cells. J Biol Chem. 2003;278(46): 46171–8.
66. Beleut M, Rajaram RD, Caikovski M, Ayyanan A, Germano D, Choi Y, et al. Two distinct mechanisms underlie progesterone-induced proliferation in the mammary gland. Proc Natl Acad Sci. 2010;107(7):2989–94. doi:10.1073/pnas.0915148107.
67. Fernandez-Valdivia R, Mukherjee A, Ying Y, Li J, Paquet M, DeMayo FJ, et al. The RANKL signaling axis is sufficient to elicit ductal side-branching and alveologenesis in the mammary gland of the virgin mouse. Dev Biol. 2009;328(1):127–39.
68. Cao Y, Bonizzi G, Seagroves TN, Greten FR, Johnson R, Schmidt EV, et al. IKKα provides an essential link between RANK signaling and cyclin D1 expression during mammary gland development. Cell. 2001;107(6):763–75.
69. Fantl V, Stamp G, Andrews A, Rosewell I, Dickson C. Mice lacking cyclin D1 are small and show defects in eye and mammary gland development. Genes Dev. 1995;9(19):2364–72.
70. Fornetti J, Jindal S, Middleton KA, Borges VF, Schedin P. Physiological COX-2 Expression in Breast Epithelium Associates with COX-2 Levels in Ductal Carcinomain Situand Invasive Breast Cancer in Young Women. Am J Pathol. 2014;184(4):1219–29.
71. Zhu Y, Wu M, Wu CY, Xia GQ. Role of progesterone in TLR4-MyD88-dependent signaling pathway in pre-eclampsia. J Huazhong Univ Sci Technol Med Sci. 2013;33(5):730–4. doi:10.1007/s11596-013-1188-6.
72. Sapi E, Flick MB, Rodov S, Carter D, Kacinski BM. Expression of CSF-I and CSF-I receptor by normal lactating mammary epithelial cells. J Soc Gynecol Investig. 1998;5(2):94–101. doi:10.1016/s1071-5576(97)00108-1.
73. Günzel D, Alan S. Claudins and the modulation of tight junction permeability. Physiol Rev. 2013;93(2):525–69.
74. Kobayashi K, Oyama S, Numata A, Rahman MM, Kumura H. Lipopolysaccharide Disrupts the Milk-Blood Barrier by Modulating Claudins in Mammary Alveolar Tight Junctions. PLoS One. 2013;8(4):e62187. doi:10.1371/journal.pone.0062187.
75. Gonen E, Vallon-Eberhard A, Elazar S, Harmelin A, Brenner O, Rosenshine I, et al. Toll-like receptor 4 is needed to restrict the invasion of Escherichia coli P4 into mammary gland epithelial cells in a murine model of acute mastitis. Cell Microbiol. 2007;9(12): 2826–38. doi:10.1111/j.1462-5822.2007.00999.x.
76. Glynn DJ, Hutchinson MR, Ingman WV. Toll-Like Receptor 4 Regulates Lipopolysaccharide-Induced Inflammation and Lactation Insufficiency in a Mouse Model of Mastitis. Biol Reprod. 2014. doi: 10.1095/biolreprod.114.117663.
77. Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol. 2004;5(3):266–71.
78. Xing D, Oparil S, Yu H, Gong K, Feng W, Black J, et al. Estrogen modulates NFkappaB signaling by enhancing IkappaBalpha levels and blocking p65 binding at the promoters of inflammatory genes via estrogen receptor-beta. PLoS One. 2012;7(6):e36890. doi:10.1371/journal.pone.0036890.
79. Sigl V, Penninger JM. RANKL/RANK—From bone physiology to breast cancer. Cytokine Growth Factor Rev. 2014;25(2):205–14. doi: 10.1016/j.cytogfr.2014.01.002.
80. Mackern-Oberti JP, Valdez SR, Vargas-Roig LM, Jahn GA. Impaired mammary gland T cell population during early lactation in hypoprolactinemic lactation-deficient rats. Reproduction (Cambridge, England). 2013;146(3):233–42. doi:10.1530/rep-12-0387.
81. Khaled WT, Read EK, Nicholson SE, Baxter FO, Brennan AJ, Came PJ, et al. The IL-4/IL-13/Stat6 signalling pathway promotes luminal mammary epithelial cell development. Development. 2007;134(15): 2739–50. doi:10.1242/dev.003194.
82. Miyaura H, Iwata M. Direct and indirect inhibition of Th1 development by progesterone and glucocorticoids. J Immunol. 2002;168(3): 1087–94.
83. Radisky DC, Hartmann LC. Mammary involution and breast cancer risk: transgenic models and clinical studies. J Mammary Gland Biol Neoplasia. 2009;14(2):181–91.
84. Chapman RS, Lourenco PC, Tonner E, Flint DJ, Selbert S, Takeda K, et al. Suppression of epithelial apoptosis and delayed mammary gland involution in mice with a conditional knockout of Stat3. Genes Dev. 1999;13(19):2604–16.
85. Bierie B, Gorska AE, Stover DG, Moses HL. TGF-β promotes cell death and suppresses lactation during the second stage of mammary involution. J Cell Physiol. 2009;219(1):57–68.
86. O'Brien J, Martinson H, Durand-Rougely C, Schedin P. Macrophages are crucial for epithelial cell death and adipocyte repopulation during mammary gland involution. Development. 2012;139(2):269–75.
87. O'Brien J, Martinson H, Durand-Rougely C, Schedin P. Macrophages are crucial for epithelial cell death and adipocyte repopulation during mammary gland involution. Development. 2011. doi:10.1242/dev.071696.
88. O'Brien J, Lyons T, Monks J, Lucia MS, Wilson RS, Hines L, et al. Alternatively activated macrophages and collagen remodeling characterize the postpartum involuting mammary gland across species. Am J Pathol. 2010;176(3):1241–55. doi:10.2353/ajpath.2010.090735.
89. Schramek D, Leibbrandt A, Sigl V, Kenner L, Pospisilik JA, Lee HJ, et al. Osteoclast differentiation factor RANKL controls development of progestin-driven mammary cancer. Nature. 2010;468(7320):98–102.
90. Gonzalez-Suarez E, Jacob AP, Jones J, Miller R, Roudier-Meyer MP, Erwert R, et al. RANK ligand mediates progestin-induced mammary epithelial proliferation and carcinogenesis. Nature. 2010;468(7320): 103–7.
91. Tanos T, Sflomos G, Echeverria PC, Ayyanan A, Gutierrez M, Delaloye J-F, et al. Progesterone/RANKL Is a Major Regulatory Axis in the Human Breast. Sci Transl Med. 2013;5(182):182ra55. doi:10.1126/scitranslmed.3005654.
92. Wood CE, Branstetter D, Jacob AP, Cline JM, Register TC, Rohrbach K, et al. Progestin effects on cell proliferation pathways in the postmenopausal mammary gland. Breast Cancer Res. 2013;15(4):R62.
93. Stute P, Sielker S, Wood CE, Register TC, Lees CJ, Dewi FN, et al. Life stage differences in mammary gland gene expression profile in non-human primates. Breast Cancer Res Treat. 2012;133(2):617–34.
94. Dep Prete A, Allavena P, Santoro G, Fumarulo R, Corsi MM, Mantovani A. Molecular pathways in cancer-related inflammation. Biochemia Medica. 2011;21(3):264–75.
95. Tan W, Zhang W, Strasner A, Grivennikov S, Cheng JQ, Hoffman RM, et al. Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL-RANK signalling. Nature. 2011;470(7335):548–53. doi:10.1038/nature09707.
96. Loser K, Mehling A, Loeser S, Apelt J, Kuhn A, Grabbe S, et al. Epidermal RANKL controls regulatory T-cell numbers via activation of dendritic cells. Nat Med. 2006;12(12):1372–9.
97. Anderson T, Ferguson D, Raab G. Cell turnover in the“ resting” human breast: influence of parity, contraceptive pill, age and laterality. Br J Cancer. 1982;46(3):376.