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Short communication

Immunophysiology of the mammary gland and transmission of immunity to the young

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The nature and extent of the transmission of immunity by the mammary gland from the mother to her offspring is related to the rate of transmission during foetal life: in mammals with immunoglobulin (Ig) permeable placenta, the systemic immunity of the mother is transferred during foetal life; in contrast, in artiodactyls with Ig impermeable placenta, systemic immunity is transmitted just after birth by the Ig enriched colostrum, and the neonate gut is permeable to Ig during the first 36 h after birth [1]. Then the transmission of mucosal immunity takes over systemic immunity. The nature and purpose of both types of immunity are different: the systemic immunity is convoyed by the Ig isotype with destroying properties in the presence of the complement, IgM and more appropriately by IgG (of lower molecular weight and thus more prone to diffuse in tissue spaces and trap the pathogen) [2]; in contrast, the mucosal protection is assured by dimeric IgA, which protects the mucosae by a non inflammatory mechanism such as the inhibition of pathogen attachment to the mucosae: in simple-stomached animals, both colostrum/milk are rich in secretory dimeric IgA (S-$(IgA)_2$): moreover, colostrum is richer in IgA than milk in species with systemic immunity protection during fetal life. Since secretory IgA in mammary secretions are specific of pathogens in contact with the mucosae, the mother thus ensures an immunity of the newborn, until weaning, at a time when the mucosal immune system of the young is able to mount a good protective immune response [3].

In the sow as in the mouse, Ig plasma cells are present in the mammary gland at delivery and during lactation [4, 5]. In the mouse, it has been shown conclusively that these plasma cells originate as cell-precursors in the gut Peyer's patches then they migrate in the blood and home into the mammary gland (the so-called entero-mammary link) at a time when this gland begins lactation [6]. Once in the mammary gland these plasma cells release in situ the dimeric IgA which will be excreted in the milk after transcytosis of the mammary epithelial cell via the IgA receptor. In species such as the mouse, the concentration of IgA in the milk increases during lactation and is accounted

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for by an increase in IgA plasma cell number [7] rather than by an increase in IgA excretion by the IgA receptor; in contrast, in ruminants the excretion of gut-borne Ig is mediated predominantly by this receptor.

With the hope to enhance the protective properties of the mother's milk for its progeny, we investigated the mechanisms of the homing of lymphocyte precursors into the mammary gland [8].

In swine, we first showed that T and B cells (as judged by the presence of IgA, IgM or IgG) differentially and inversely accumulate in pregnancy and lactation, respectively, i.e. B cells increased when T cells decreased (Fig. 1, [4, 9]). Thereafter, we looked at the mouse mammary gland and found a similar inverse relationship between T and B cells [10]. Since the different migration pathways of lymphocytes in the body may be determined by the expression of particular structures on the surface of endothelial cells of the blood vessels (vascular addressin) and complementary structures on the membranes

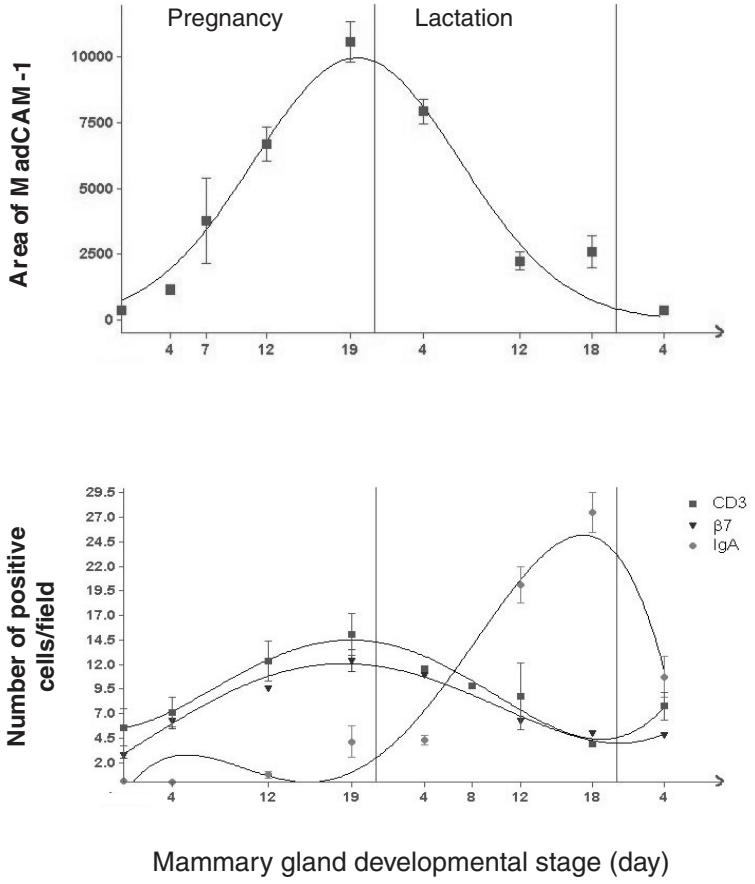

Figure 1. A comparison of the expression of MadCAM-1 (upper graph) and the accumulation of T cells (CD3, and β7) and IgA B cells in pregnancy and lactation (lower graph), respectively. Note that whilst T cells increase in pregnancy correlatively to MadCAM-1 (vascular addressin) and β7 integrin (homing receptor onto lymphocyte), they decrease in lactation. In contrast IgA B cells which nearly are absent in pregnancy, increase in lactation, whilst the expression of MadCAM-1 decreases.

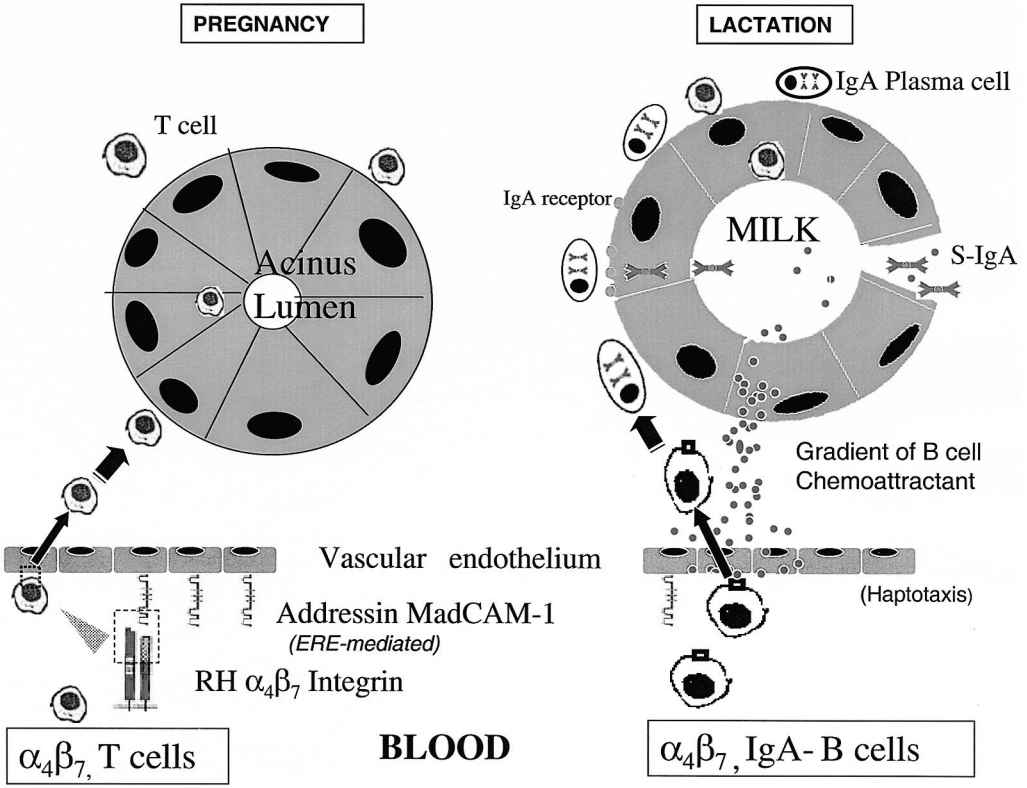

Figure 2. Cellular and humoral factors regulating the increase of homing into the mammary gland of T cells in pregnancy and IgA B cells in lactation. In pregnancy, MadCAM-1 could be the unique rate determining factor to govern the extravasations (arrow) of gut-derived $\alpha_4\beta_7$ T cells; the expression of this addressin is regulated by an ERE (suggesting an hormonal regulation): the interaction between the vascular addressin MadCAM-1 (molecule of the superIg family) and the homing receptor (HR), $\alpha_4\beta_7$ integrin dimer, is depicted in the rectangle with interrupted lines. In contrast, B cells and IgA plasma cells, whose 30% are $\alpha_4\beta_7$, need an additional factor to be extravasated in the mammary gland, such as a milk chemokine or a chemoattractant. It is hypothesised that such soluble factor (plain circle) delivered by the epithelial cells is reexpressed by haptotaxis onto the luminal side of the endothelium, and thus may interact with the corresponding ligand receptor (small rectangle) onto the lymphocyte membrane. Once, the IgA lymphoblast has crossed (thin arrow) the endothelium, it transforms (wide arrow) into plasma cells delivering the IgA antibody. Note that T and B cells exhibiting $\alpha_4\beta_7$ derive from the gut where they have been stimulated and hence the plasma cells excrete IgA in the milk of the same antibody specificity as those of the gut IgA. This is the basis of the entero-mammary immune link.

of lymphocytes (the ligand as the "homing receptor") [11], we looked at the expression kinetics of both addressins and homing receptors.

We showed that MadCAM-1 (the mucosal addressin cell adhesion molecule, specific of gut blood vessels), and $\alpha 4\beta 7$ integrin (the corresponding homing receptor) were correlative of the T cell number (Fig. 1). Thus the $\alpha 4\beta 7$ T lymphocytes (mainly CD8 T cells located in the epithelium) of gut origin accumulate in the mammary gland during pregnancy in the proportion of the MadCAM-1 expression – an expression ascertained either by RT-PCR [12] or by immunohistochemistry [10] – while the $\alpha 4\beta 7$ plasma cells accumulated later in lactation when MadCAM-1 started to decline (Fig. 1).

This last result, together with the homing in the mammary gland of β_7 [13] and β_7 and L-selectin knockout mice [14] suggested that additional factors may be necessary for the homing of IgA B cells into the mammary gland; thus this led us to seek for a factor of epithelial origin and specifically chemoattractant for IgA lymphoblasts. Such a factor should be excreted or delivered in the parenchyma, transported through the endothelium and be reexpressed at the luminal surface of endothelial cells, by a process known as haptotaxis (Fig. 2) [15]. Our results showed that such a factor is present in a sow milk ultrafiltrate (10 kDa) [16]. This factor may be tentatively different from the chemokines, already identified in milk [17]. We have not yet, however, explored the possibility of negative and positive interactions [18] between this factor and hormones.

As expected with a tertiary lymphoid organ, the peripheral lymph-node vascular addressin (PNAd) is not present in the mammary gland, whatever the developmental stage, in keeping with the absence of lymphocytes expressing L-selectin: this suggests that only memory cells migrate into the mammary gland. Interestingly, one of the ligands of L-selectin, Glycam-1 is not present on the endothelial cells of blood vessels, but in the epithelial cells and is excreted in milk; however this protein lacks the sulfate-modified carbohydrate necessary to interact with L-selectin [19]. In addition, the mammary gland lymph node looks like a somatic lymph node by the presence of PNAd on the endothelial cells and the absence of MadCAM-1.

Thus, in the mouse, differences in the recruitment of T and B cells, in gestation and lactation respectively, is accounted for by differences in the development of vascular addressins and chemoattractants (Fig. 2). Although there could be some variations between mammals due to the various kinetics of Ig lymphocyte colonisation, these results strengthen the idea that one could (1) increase the protection of the mammary gland itself while acting on the expression of MadCAM-1 via its ERE (estrogen-responsive element) [20] by hormonal modulation to recruit more CD8 lymphocytes and (2) enhance the mucosal protection of the newborn by a milk richer in IgA. For this, a better recruitment of IgA plasma cells may be assured through the increase of both MadCAM-1 and chemoattractant factor expressions.

In conclusion, these results illustrate the various means used by the mammary gland to integrate physiological and immunological functions to protect the neonate and to ensure the perpetuity of the species. Lastly, the presence of chemoattractants in the milk may lead to the research of milk biopeptides to enhance mucosal protection.

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