Journal of Reproductive Immunology 96 (2012) 25–33

Contents lists available at SciVerse ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jreprimm

An increase in milk IgA correlates with both pIgR expression and IgA plasma cell accumulation in the lactating mammary gland of PRM/Alf mice

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ARTICLE INFO

Article history:
Received 5 March 2012
Received in revised form 25 July 2012
Accepted 13 August 2012

Keywords:
Mouse
Mammary gland
cIgA cells
Milk
Adhesion molecules
CCL28

ABSTRACT

In mice, during late pregnancy and lactation, maternal precursors of IgA-containing cells (cIgA-cells) are primed in the gut and home to the mammary gland where they secrete IgA. In turn, the ensuing increase in milk IgA mediates immune protection of the newborn gastrointestinal tract. PRM/Alf is an inbred mouse strain which exhibits a substantial post-natal intestinal lengthening which develops throughout the neonatal suckling period, suggesting that the availability of cIg-A cells and the level of protective IgA in milk might also be increased. We confirmed that PRM/Alf milk contains higher amounts of IgA than C57BL/6J throughout lactation, concomitantly with an increase of pIgR on epithelial cells and a higher density of cIgA-cells in the PRM/Alf mammary gland. Furthermore, a search for variations in cellular and humoral factors implicated in regulating cIgA-cell migration towards the mammary gland, including the vascular addressins MAdCAM-1 (mucosal addressin cell adhesion molecule-1) and VCAM-1 (vascular cell adhesion molecule-1) as well as the mucosal epithelial chemokine CCL28, did not reveal any quantitative differences in expression between PRM/Alf and C57BL/6J mice strains. Thus our results indicate that these factors are not limiting in the recruitment of cIgA-cells released from the elongated gut of PRM/Alf mice. In the context of intestinal lengthening, these findings strengthen the notion of an entero-mammary gland link, where the neonatal gut is protected by the maternal gut through the immune function of the mammary gland.

1. Introduction

In mammals, depending on the species, maternal immunity is transmitted to immunologically naive offspring before and/or after birth. During pregnancy, a transfer of IgG through the placenta confers systemic passive protection. After birth, milk is the main vector of maternal immunity provided by the mammary gland. Indeed, milk contains a variety of molecules including glycopeptides, lipoproteins, cytokines and antimicrobial peptides, as well

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http://dx.doi.org/10.1016/j.jri.2012.08.001

as large amounts of immune cells and immunoglobulins. The main immunoglobulin in maternal milk of several mammals, including humans, is secretory IgA (sIgA). sIgA is secreted as dimeric IgA (dIgA) *via* a receptor-mediated transcytotic pathway (Johansen and Kaetzel, 2011). The specificity of milk sIgA for intestinal and respiratory pathogens and/or dietary antigens (Macpherson et al., 2008) and the role of breast feeding for the prevention of neonatal infections is thought to be the result of antigenic stimulation at the digestive and upper respiratory tracts – the so-called, entero- and/or broncho-mammary links (Kleinman and Walker, 1979; Fishaut et al., 1981; Brandtzaeg, 2003; Macpherson et al., 2008; Salmon et al., 2009; Salmon, 2012).

The presence of sIgA in milk is due to either transport from the blood of serum dIgA into the milk or by sIgA secretion from local cIgA-cells. sIgA are exported into the milk *via* the polymeric Ig receptor (pIgR) found on the membrane of epithelial cells. After cleavage of the receptor, secretory component (SC) is released into the milk, as free protein or bound to dIgA. Various transport mechanisms for milk sIgA have been described in mice. Some sIgA is transported from the blood at the beginning of lactation (Halsey et al., 1982), but the bulk of mouse milk IgA is produced by local IgA-containing cells (cIgA-cells) primed in the gut, which migrate to mammary gland during late pregnancy and lactation (Roux et al., 1977; Tanneau et al., 1999; Van Der Feltz et al., 2001). Roux et al. (1977) were the first to demonstrate, by showing accumulation in the mammary gland of transferred mesenteric lymph-node I^{125} labeled cells, that cIgA-cells are derived from IgA lymphoblasts primed in the gut. This anatomical origin was later confirmed by the characterization of trafficking factors (see below). It thus appears that passive immune protection of the pup gastrointestinal tract depends upon an active migration process of cIgA-cells to the mother's lactating mammary gland.

The tissue-specific migration of leukocytes, including lymphocytes and lymphoblasts, is mediated by a combination of adhesion molecules as well as cytokines, chemokines and hormones (Butcher and Picker, 1996). We have previously shown that the vascular addressin MAdCAM-1 is expressed by endothelial cells in the murine mammary gland and is correlated with T cells expressing a high level of the corresponding β_7 integrin-ligand (Tanneau et al., 1999). Interestingly, after a maximum at the end of pregnancy, the expression of MAdCAM-1 decreases during lactation whereas the number of IgA plasma cells increases (Tanneau et al., 1999), suggesting that additional factors, such as chemokines, might be involved in the homing of cIgA-cells to the mammary gland. Among epithelial chemokines, CCL28/MEC (mucosae-associated epithelial chemokine) is expressed by epithelial cells in a variety of mucosal tissues, including the small and large intestine, trachea, tonsil, nasal mucosae, salivary and mammary gland. CCL28 has been suggested to function as a unifying mucosal mechanism for circulating IgA plasmablast homing and localization within CCL28-expressing mucosal sites. CCL28 binds to the cognate receptor CCR10, which was shown to be expressed by cIgA-cells (Kunkel et al., 2003; Pabst et al., 2004; Meurens et al., 2006; Berri et al., 2008). *Ccl28* expression was further shown to be up-regulated during lactation in mice (Wilson and Butcher, 2004; Bourges et al., 2008) whereas CCL28 protein was shown to be excreted into the milk (Hieshima et al., 2003). The role of CCL28 in homing was further demonstrated by Wilson and Butcher (2004) who showed that *in vivo* treatment with anti-CCL28 antibody blocks cIgA-cells accumulation in the mammary gland, which in turn inhibits both IgA secretion into the milk and the subsequent presence of antibody in the gastrointestinal tract of nursing neonates. Gene knock-out experiments have shown that CCR10 is critical for efficient localization and accumulation of cIgA-cells to the lactating mouse mammary gland (Morteau et al., 2008).

In this study, we took advantage of the PRM/Alf mouse model to study mechanisms of IgA production in the mouse mammary gland. Indeed, PRM/Alf is an inbred mouse strain showing a huge intestinal lengthening (Aubin-Houzelstein et al., 2003; Bellier et al., 2005). A strong maternal effect explains approximately 40% of the difference in gut length, in agreement with the fact that it develops over the course of the suckling period, before weaning. As the passive humoral protection of the neonatal gut is afforded by the presence of milk IgA, we sought to evaluate (in addition to other factors, Boumahrou et al., unpublished) the possibility of higher concentrations of sIgA in the milk of PRM/Alf mice in comparison to C57BL/6J mice. Presently, two non-mutually exclusive hypotheses could explain such a maternal effect. Firstly, the milk of PRM/Alf females could contain intestine-trophic factor(s) transmitted from the nurse-dam to her pups. Secondly, the intestinal microbiota, also transmitted from the nurse-dam to her pups, may contain micro-organisms capable of inducing a lengthening of the intestine, either directly or indirectly after bacterial selection by milk IgA (Kramer and Cebra, 1995).

Here, we show that the higher IgA content in PRM/Alf milk correlates with higher pIgR, SC, and cIgA-cells in the mammary gland in comparison with C57BL/6J milk. However, tissue expression of the cellular (MAdCAM-1, VCAM-1) and humoral factors (CCL28) known to be involved in cIgA-cell migration into the mammary gland did not show any differences in the PRM/Alf mice. These factors therefore must not be limiting in the recruitment of cIgA-cells. Rather, it appears likely that the lengthened intestine of the PRM/Alf mother can export more cIgA-cells to the mammary gland, supporting an entero-mammary link.

2. Materials and methods

2.1. Animals, blood serum and milk samples

C57BL/6J *M. m. domesticus* and PRM/Alf mice (Aubin-Houzelstein et al., 2003) were housed at INRA, Jouy-en-Josas, France. Milk was collected at early lactation (4 days postpartum; 4 dpp), mid-lactation (12 dpp) and late lactation (18 dpp) (as described in Boumahrou et al., 2011) and blood was extracted from the retro-orbital sinus.