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Review article

Cellular components of mammary secretions and neonatal immunity: a review

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Summary — Mammary secretions contain viable maternal cells that are mainly lymphocytes, macrophages, neutrophils and epithelial cells. Their biological functions in the neonate, though not yet clearly established, are strongly suggested by experimental data. This paper reviews current knowledge of the cellular components of mammary secretions: their nature, *in vitro* properties, and demonstrated *in vivo* effects in the neonate, and discusses possible future experimental approaches. It is thought that the main role of the cellular components from mammary secretions is to interact with the development of local immunity in the newborn, and to modulate active immunization of the neonatal intestine during this critical period when the development of adapted responses to antigens (protection/tolerance/food allergy) is of crucial importance for the future of the young.

colostrum / milk / newborn / mucosal immunity

Résumé — **Composantes cellulaires des sécrétions mammaires et immunité néonatale. Synthèse.** *Les sécrétions mammaires contiennent des cellules maternelles vivantes, qui sont principalement des lymphocytes, des macrophages, des neutrophiles et des cellules épithéliales. Leur intérêt biologique pour le nouveau-né, s'il n'est pas encore clairement élucidé, est fortement suggéré par les données expérimentales. L'auteur présente les connaissances établies sur les composantes cellulaires des sécrétions mammaires : nature, propriétés *in vitro*, effets démontrés *in vivo* chez le nouveau-né, et discute les voies actuelles de recherche dans ce domaine. Le rôle majeur de ces composantes cellulaires pourrait être d'interagir sur le développement de l'immunité locale du nouveau-né, et de moduler l'immunisation active de l'intestin néonatal dans cette période critique où le développement de réponses adaptées aux antigènes (protection/tolérance/allergie alimentaire) est d'une importance cruciale pour le devenir du jeune.*

colostrum / lait / nouveau-né / cellules / immunité des muqueuses

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INTRODUCTION

Mammary secretions are a link between the mother and neonate after birth. Their functions extend beyond their nutritional aspects. The first days of life for a neonate correspond to a period of extremely intense organic growth, with high levels of cellular activity mostly relating to cell division and to the establishment of various physiological

functions. These activities occur in an environment where the first contacts with large quantities of foreign antigens are occurring and where it is necessary to quickly establish appropriately adapted responses. It has to be emphasized that the neonatal intestine, in addition to its role in nutrition physiology, is a major lymphoid organ and the principal site of antigenic contacts. An appropriate level of immediate defenses, genuine

or resulting from mammary secretions, is therefore essential for survival in the neonatal period. The development of local immunity during the neonatal period concerns not only the acquisition of immunity against pathogens, but also the maturation of the local immune system and the establishment of adapted responses (tolerance/active immunity). The transmission of passive immunity by mammary secretions is well known. In species such as swine, where the type of placenta prevents the passage of macromolecules from maternal blood to the foetus, the young is agammaglobulinemic at birth and must form a pool of seric immunoglobulins from the colostral maternal Ig. There is a continuous supply of secretory IgA in the milk that is induced by intestinal sensitization of the mother during pregnancy and mammogenesis, and which gives passive protection to neonatal intestine (Salmon, 1987; Berthon et al, 1993). Another aspect of lactogenic immunity is the link between mammary secretions and the active immunity of the neonate. The problem is complex, and at this time remains a field under investigation. Mammary secretions have been shown to contain a large variety of components that are immunological or that have the capacity to interact with the immune system: lymphoid and non-lymphoid cells, cytokines, immunomodulating substances such as casein derivatives, growth factors and hormones. All these components could contribute to the cellular defenses of the neonate, and could also interact with the development of the local immune system in the newborn and with the induction and orientation of active responses to foreign antigens (Bernard et al, 1983; Chernishov and Sluvkin, 1990). The interactions between the maternal components and the maturation of an active immunity in the neonate are as yet poorly understood, and many questions remain to be answered. This report, which focuses mainly on cells and cytokines (excluding growth factors, hormones and immunomod-

ulating substances), attempts to provide an overview of the nature and roles of cellular mammary secretion components for newborns, and the different approaches currently under investigation.

NATURE AND IN VITRO PROPERTIES OF CELLULAR MAMMARY SECRETION COMPONENTS

Nature of mammary secretion cells

For any mammalian species, there are more than 1×10^6 cells mL^{-1} in colostrum and milk. Global cellularity and cell types vary according to species, time of lactation and physiological and individual conditions, but the point is that the newborn receives a significant number of cells through mammary secretions. For example, piglets ingest 500–700 million viable maternal cells daily. Four cell types characterize mammary secretions: lymphocytes, macrophages, neutrophils and epithelial cells. Lymphocytes represent 5–9% of mammary secretion cells in humans, 20–25% in sheep, more than 30% in cows and 40% in rodents and, during the colostral phase, 15–25% in swine (Crago et al, 1979; Ouzrout et al, 1991; Park et al, 1992; Na et al, 1992; Evans et al, 1982; Schollenberger et al, 1986a; Magnusson et al, 1991; Le Jan, 1993). Based on the results obtained so far, it is accepted that most mammary gland and mammary secretion lymphocytes originate from the mesenteric lymph nodes (Parmely and Beer, 1977; Kumar et al, 1989). Macrophages and neutrophils predominate in the mammary secretions of most species, except in swine where they are outnumbered by epithelial cells. Epithelial cells are present in low numbers during the colostral and involution phases, when the mammary gland is undergoing architectural reconstruction (Taylor-Papadimitriou and

Stampfer, 1991). In swine, however, these epithelial cells represent 20–40% of the total number of colostrum cells, and 60–90% of milk cells. All these cell types are potentially interesting from a biological point of view.

T lymphocytes

T lymphocytes outnumber B lymphocytes in mammary secretions. Early studies have indicated that they may represent a population of functional cells that selectively migrate in mammary secretions. Richie et al (1980) suggested that human colostrum T lymphocytes are mostly activated lymphocytes, as 50% of them form thermostable rosettes with sheep red blood cells, as compared with 3% of peripheral blood T cells. A wide range of functional properties was established for these cells: the proliferative response of sow colostrum T cells to ovalbumin (Evans et al, 1982), of rat and human colostrum T cells to mitogens, allogeneic cells, bacterial and viral antigens (Ogra et al, 1983; Parmely et al, 1976), the response to transplantation antigens and the production of chemotactic factors (Nair et al, 1985). However some contradictory observations, such as the lower response of these cells to phytohaemagglutinin (PHA) and their reduced capacity to mediate antibody-dependent cell cytotoxicity (ADCC) (Kohl et al, 1980), have made a clear-cut consensus on their functions difficult: are they functional cells, or are they simply being eliminated in the mammary secretions?

It is now clearly established that T lymphocytes from mammary secretions are selected cells, with phenotypes differing from those encountered in peripheral blood, and that they are able to express *in vitro* functional capacities. In human mammary secretions, 83% of the secreted lymphocytes are T lymphocytes, with a CD4/CD8 ratio significantly lower than that found in

peripheral blood. The main characteristic of this group of cells is the predominance of memory-activated T cell phenotypes. The markers of activation CDw29+, UCHL1+, LFA1+, 2H4CD45R-. HLA-DR and CD25 (IL2-receptor) are expressed on 85% of milk T cells. This proportion is only 10% in peripheral blood. Almost all CD4+ and CD8+ milk cells are CD45RO+RA-, indicating that they range from unprimed CD45RA+RO- T cells to antigen-primed, memory T cells. The expression of CD2 (T11) and CD54 (ICAM-1) is twice as high in colostrum lymphocytes as in blood lymphocytes (Bertotto et al, 1990a, 1991; Eglinton et al, 1994; Wirt et al, 1992). There are twice as many lymphocytes expressing the γ - δ T cell receptor (TCR) in human milk as in blood. These γ - δ T cells in milk express non covalently bound γ - δ chains, while in the blood most γ - δ T cells express the disulphide-linked form of TCR (Bertotto et al, 1991). The expression of non covalently bound chains has been shown to be associated with actin rearrangement and motility capacity after lymphocyte activation (Grossi et al, 1989). These phenotypic characteristics are in correlation with the *in vitro* established functions of colostrum T lymphocytes. They present a low proliferative response to stimulation by PHA, respond to a wide range of bacterial and viral antigens and produce significant amounts of gamma interferon when stimulated by mitogens or Newcastle disease virus. Similar to blood lymphocytes, milk lymphocytes proliferate and produce γ interferon in response to anti-CD2 and anti-CD3. The phenotype and functional repertoire of milk T lymphocytes are the same as those of peripheral blood memory T cells (Bertotto et al, 1990b, 1991). It is clearly established that T lymphocytes are antigen-pulsed cells, able to develop a secondary immune response, and that a directed and not a random passage of T lymphocytes exists in human mammary secretions. The same conclusions have been obtained in other species.

The proportions of T lymphocytes among mammary secretion cells and the T4/T8 ratio in secretions and in blood are respectively 23, 1.0 and 2.3% in rat (Na and Seelig, 1993; Na et al, 1992). In sheep, 67% of milk lymphocytes are T lymphocytes with a T4/T8 ratio of 0.36 (Ouzrout et al, 1991). In bovines, the proportion of T lymphocytes varies from 16% in the colostrum to 62% in late lactation, with a T4/T8 ratio of 0.85 in comparison to 1.5 in blood (Park et al, 1992). T Lymphocytes of bovine mammary secretions express predominantly TCR α - β and have a twofold higher level of CD2 and fivefold lower level of CD45R+ expression in comparison with peripheral blood lymphocytes (Taylor et al, 1994). In swine, 10–25% of colostrum cells are lymphocytes, of which 70–90% are T cells, with a T4/T8 ratio (0.57) significantly lower than that of blood (0.80). In sow milk, less than 1% of total cells are T lymphocytes (Evans et al, 1982; Schollenberger et al, 1986b; Le Jan, 1993, 1994). Sow colostrum T lymphocytes do not express interleukin 2 receptors (IL2-R), but respond to mitogenic stimulation by proliferating and expressing IL2-R (Le Jan, 1994).

B lymphocytes

Initial *in vitro* studies on the B lymphocytes in mammary secretions gave contradictory results in different experimental systems. For example, Crago and Mestecky (1984) did not obtain immunoglobulin production after stimulation of human colostrum B lymphocytes with pokeweed mitogen (PWM) or the Epstein–Barr virus, and suggested that, unlike T lymphocytes, the colostrum B lymphocytes were non functional cells that were being eliminated in mammary secretions. These negative results could have been related to background contamination by macrophages, which could have masked the *in vitro* production of small quantities of IgA by the B cells. Slade and Schwartz (1989), inducing autolysis of macrophages using a leucine-

O-methyl ester, demonstrated that human milk lymphocytes respond to *in vitro* stimulation by producing IgA. The *in vitro* functional capacities of B lymphocytes are established (Mestecky et al, 1991). After being transformed by the Epstein–Barr virus, cell lines producing IgA, IgG and IgM have been established from human milk B lymphocytes (Alvital et al, 1985; Shinmoto et al, 1992).

Neutrophils and macrophages

The functional capacity of neutrophils isolated from mammary secretions is uncertain. The normal functions of phagocytosis, motility, respiratory burst and polymorphonuclear (PMN)-mediated killing are decreased in mammary secretion neutrophils, in comparison with those of the peripheral blood. They contain IgA, but do not release it during the phagocytosis of latex particles (Kohl et al, 1980; Nair et al, 1985; Schollenberger et al, 1986c; Ozkaragoz et al, 1988). In contrast, mammary secretion macrophages have established *in vitro* functional activities. They have enhanced levels of motility and a greater capacity to invade collagen gels in comparison with blood monocytes (Okaragoz et al, 1988). They contain IgA, which they liberate during the phagocytosis of latex particles or opsonized bacteria by a mechanism that can be blocked by actin filament inhibitors (Weaver et al, 1982, 1984). They express Class II antigens, and this expression is enhanced by γ interferon (Rivas et al, 1994). Mammary secretion macrophages express *in vitro* characteristics of functional phagocytic and antigen presenting cells.

Epithelial cells

In most species, epithelial cells represent a minor component of the colostrum cell population. The capacity of these cells to grow

in vitro has been demonstrated in humans and bovines, where colostrum is a source of cells for mammary gland epithelial cell cultures (Taylor-Papadimitriou and Strampfer, 1991; Buehring, 1990). Genomic DNA and mRNA for β -casein have been isolated and amplified from human colostrum epithelial cells (Lindquist et al, 1994). In contrast, epithelial cells represent a major component of the cells from mammary secretions in swine. In sow milk, large epithelial cells, laden with fatty globules, secretory components and secretory IgA, represent more than 60% of the total cell contents (Evans et al, 1982; Schollenberger et al, 1986a; Le Jan, 1993). These cells do not grow in vitro, and have the characteristics of totally differentiated alveolar epithelial cells. Swine colostrum contains more than 20% epithelial cells; these cells are small in size, with little or no cytoplasmic IgA, and have low levels of secretory component expression. They are able to grow in vitro for more than three passages. When cultivated in the presence of lactating sow serum or an aqueous extract of sow colostrum, these cells undergo differentiation and produce α -lactalbumin. In culture, these cells are sensitive to the porcine coronavirus transmissible gastroenteritis (TGE). They produce infectious virus particles and express viral antigens (Le Jan, 1993; Le Jan and Chevaleyre, 1996b). They may also express Class II antigens, depending on their degree of differentiation. Their in vitro capacity to process antigens is under investigation. We hypothesize that the epithelial cells from sow colostrum can produce cytokines and can act as antigen-presenting cells.

Cytokines

It appears that free cytokines form an integral part of mammary secretions, but to date only a limited amount of data is available. These data relate to some species and some cytokines. However, whenever a particularly

biologically important cytokine was looked for, it was found in the mammary secretions in a biologically active form. In vitro, the capacity of colostrum, milk and mammary gland epithelial cells to produce cytokines has been established. Human milk contains γ interferon at levels four times higher than maternal blood (Eglinton et al, 1994; Bocci et al, 1993), and significant amounts of interleukin 6 (IL-6) (Rudloff et al, 1993; Bocci et al, 1993). Tumour necrosis factor (TNF) is also present in human milk (Rudloff et al, 1992). In bovine mammary secretions, TNF- α is not present in the colostrum but is present in the milk throughout the lactation period, with a decrease in quantity four to six weeks before parturition (Rewinski and Yang, 1994; Sordillo et al, 1991). Transforming growth factor (TGF)- β has been found in high concentrations in early bovine colostrum, and its level decreases from the 12th hour after parturition to reach a negligible level at the 30th (Tokuyama and Tokuyama, 1993). In humans, TGF- β is present at a concentration near 1 300 ng/mL in colostrum and 900 ng/mL in milk (Saito et al, 1993). Human colostrum contains high levels of interleukin 1 (IL 1) β , but no IL-1 α (Munoz et al, 1990). Epithelial cells from human and bovine mammary glands have been shown to produce, during in vitro cultures, IL-6, interleukin 8 (IL-8) and TNF (Palkowetz et al, 1994; Basolo et al, 1993). Mononuclear cells from human milk produce in vitro IL1 α and β , IL-6, IL-8 and TNF α (Skansen-Saphir et al, 1993). Cells from rat milk, after in vitro non-specific stimulation by concanavalin A (ConA) or specific stimulation by an antigen, produce TNF, interleukin 2 (IL-2) and IL-6 (Na and Seelig, 1993).

DEMONSTRATIONS OF IN VIVO FUNCTIONS OF MAMMARY SECRETION CELLS

We present here the established information concerning the interactions between

mammary secretion cells and the newborn: the fate of colostral cells after ingestion, the effects of cellular components on immune defenses and immunological parameters.

Fate of colostral cells in the newborn

It is clearly established in many species including mice (Weiler et al, 1983), ovines (Schnorr and Pearson, 1984; Tuboly et al, 1995), primates (Jain et al, 1989) and swine (Tuboly et al, 1988; Williams, 1993; Le Jan et al, 1995) that colostral cells are able to cross the neonatal intestinal barrier. All the above studies, using cell labelling by fluorochromes or radioactive markers, were performed during a short period (up to 60 h after ingestion). This is too short a time period for an evaluation of the duration of the persistence of maternal cells in the newborn; however, semi-allogeneic maternal cells are accepted by neonatal intestinal mucosa. After ingestion, maternal cells have been detected in intestinal mucosa, mesenteric lymph nodes, blood, lungs, liver and spleen. In pigs this absorption is restricted to the duodenum and jejunum, with preferential passage in the duodenum (Le Jan et al, 1995). The mechanism of entry of colostral cells into the intestinal mucosa is not established. However, specific recognition of maternal cells has been established in the pig during *in vivo* and *ex vivo* experiments (Tuboly et al, 1988; Williams, 1993). Only maternal colostral cells can pass through the intestinal barrier; maternal peripheral blood leukocytes (PBL) and colostral cells from a different sow cannot. No similar donor restriction exists in primates, as Jain et al (1989) have shown in the trans-intestinal absorption of human colostral cells in newborn baboons. The duodenal epithelium of piglets does not express Swine leukocyte antigens (SLA) Class 1 antigens during the first four days following birth. The level of expression is lower on the jejunal epithe-

lium of piglets than in adult pigs (Le Jan and Chevalere, 1996a). This reduced expression of SLA Class 1 relates to its ability to accept maternal cells, but is more likely an indication of the immaturity of the neonatal intestinal epithelium. In humans, major histocompatibility complex (MHC) Class 1 antigens are fully expressed by the neonatal intestinal epithelium (Rognum et al, 1992).

Established protection in experimental models

There are few experimental demonstrations of *in vivo* protective effects of cells from mammary secretions, but those that do exist are clearly conclusive. There are many difficulties in evaluating the effects of cellular components on neonatal diseases. In lethal diseases of newborns, such as transmissible gastroenteritis in piglets, the evolution time of the disease is short, and it is believed that in order for a defence to be effective it must act quickly. The immune exclusion by secretory IgA is likely to be more efficient than a specific cellular immunity. In order for a disease to be an effective model for assessing the contribution of cellular defence, one must be able to quantify this defensive response. Up to now, established models have concerned parasitic and viral diseases. The reference model demonstrating cellular protection by cells from mammary secretions is the infestation of newborn rats by *Trichinella spiralis*. In this disease, it is established that the inflammatory events leading to the expulsion of the parasites from the intestine is due to specific T lymphocytes. Female rats immunized against *Trichinella spiralis* are able to transfer immunity to the suckling newborns. The degree of protection is quantified by the number of parasites harboured in the intestine after a challenge. Cross-fostering experiments have established that the protection is conferred by the milk of the immu-

nized mother; non-immune mothers receiving intravenously mesenteric T lymphocytes from immunized syngeneic rats, can protect their sucklings through their milk, as well as the direct per os administration of sensitized T lymphocytes to the newborn (Kumar et al, 1989, 1990; Na et al, 1992). In another parasitic model, infestation of mice by *Hymenolepis nana*, suckling neonates are protected after the inoculation of lactating mothers with lymph-node cells from immunized mice, or by direct ingestion of these cells. This transfer of protection is abrogated by pre-treatment of the cells with anti-Thy-1.2 monoclonal antibody and complement (Asano and Okamoto, 1992). In newborn calves, colostrum lymphocytes contribute to the protection against an experimental infection by a rotavirus (Archambault et al, 1988). Protection after the challenge is quantified by the duration of the virus excretion in the feces. The protection is complete, that is, there is no virus excretion, after the administration of immune colostrum without cells. There is no difference between the control calves and calves receiving colostrum lymphocytes from non-immunized mothers. There is, however, a significant decrease in the duration of the viral excretion and the level of virus titers in feces, after a challenge between calves receiving immune-mother colostrum lymphocytes and control calves or calves receiving colostrum lymphocytes from non-immunized mothers. This establishes the fact that colostrum immunized lymphocytes reduce the disease course.

Effects on immunological parameters of newborn

Cases where cellular immunity has been transmitted by mammary secretions have been observed. In humans, delayed-type reactions can be transmitted by breast-feeding against schistosomal antigens (Eissa et al, 1989) and tuberculin (Mohr, 1972). Ogra et al (1977) have shown a transient sensi-

tivity of blood lymphocytes from infants breast-fed from an immunized mother to the purified protein derivative of *Mycobacterium tuberculosis*; however, their conclusion that 'at the moment, it is difficult to determine whether such reactivity represents uptake of antigen-sensitized intact cells or a simple absorption of pharmacological mediators released by T lymphocytes during their passage through the intestinal tract of the newborn' remains valid and illustrates the need for caution in the interpretation of in vivo-measured effects of colostrum cellular components.

Mycobacterium-reactive γ/δ T cells are present in the colostrum of tuberculin-positive mothers (Bertotto et al, 1993). This correlates well with the presence of sensitized lymphocytes in the newborn, but does not imply that delayed-type skin sensitivity is necessarily due to these maternal cells. Pabst et al (1989) have shown an enhanced lymphoblastic response to BCG vaccination in breast-fed infants, in comparison with infants reared on milk formulae, and this was not related to the immune status of the mother. This result indicates that a non-specific enhancement of cellular responses to certain antigens by mammary secretions can exist. This could be due to the cellular or acellular components of mammary secretions.

In mice (Zhang and Miller, 1993), there is a cellular and humoral hypimmune response to non-inherited maternal MHC antigens. After the ingestion of maternal milk, maternal cells are present in the lymph nodes of newborn. The observation that there is a prolonged survival of maternal skin grafts suggests that the transferred maternal T cells selectively inactivate host T cells capable of recognizing them. Cross-fostering experiments in mice have also shown that milk-feeding modifies the recognition of MHC, even though the mechanism for this (cells or other factors) is not yet established (Nepomnaschy et al, 1988).

In calves (Riedel-Caspari, 1993), comparisons of the effects of different diets (colostrum with or without cells, milk substitutes with or without a colostrum cell supplement) have shown that following colostrum cell ingestion there is a higher response of blood lymphocytes to non-specific mitogens and a higher level of seric antibody formation. This is in agreement with observations by Brenner et al (1986), that breast-fed infants have a higher level of lymphocyte response in the early weeks after birth. In piglets (Williams, 1993) there is a significant augmentation of the PBL response to PHA, ConA and PWM after colostrum cells have been ingested by the newborn. Tuboly et al (1995), using tetanus anatoxin as an antigen, demonstrated that maternal colostrum lymphocytes remain immunologically active after trans-intestinal absorption across the newborn intestinal mucosa, and that these cells transfer an immunological memory.

RESEARCH NEEDS AND DEVELOPMENTS

It is clearly established that mammary secretions contain a wide range of factors potentially able to interact with the immature neonatal local immune system, at a time when this system is confronted with contact with various antigens simultaneously. It is therefore probable that the newborn's intestinal immune system must be capable of recognizing the type of response to develop (humoral or cellular immune exclusion, or tolerance), and that the maternal cells, cytokines, growth factors, hormones and immunomodulatory substances of colostrum and milk may influence the stages in the process of recognition of self and of foreign antigens during this critical neonatal period. It is also clear that the topic of neonatal active immunity is poorly understood. Transmission of passive immunity by lactogenic

immunity and active immunity of the young after weaning have been much more clearly elucidated in their fundamental and practical aspects.

The questions can be defined in very simple terms: How do colostrum and milk reduce neonatal pathology? What are the roles of the immune components of colostrum and milk in the establishment of the local immunity of neonate? How could these effects be modulated? The answers to these questions would involve: (i) improving the understanding and control of atopic diseases and alimentary allergies, particularly in humans; (ii) reducing neonatal pathology in animal production; (iii) improving zootechnical potentialities in intensive production systems where losses due to pathology are mostly due to the effect of an inadequate balance between opportunistic, or occasionally pathogenic, ubiquitous germs and organisms; and (iv) elaborating neonatal vaccination strategies and manufacturing improved milk substitutes. This implies an integrated experimental approach, which should include immunology, physiology and developmental biology.

Migration patterns of colostrum cells in newborn

Semi-allogeneic maternal colostrum cells are accepted by the newborn intestinal mucosa, and the experimental data suggest that these cells persist in newborn organisms long enough to exert their functions. Many questions remain to be resolved about this migration; regarding the mechanisms of transepithelial crossing, their persistence in the newborn, and the phenotype and localization of migrating cells. It is established that in mice primates, ruminants, rabbits and pigs, colostrum cells cross the intestinal barrier and persist during the time period of the experimental surveys, which have not

exceeded 60 h up until now. The migration mechanism has not been elucidated. Specific recognition of colostrals maternal cells has been established in swine (Tuboly et al, 1988; Williams, 1993), during both in vivo and ex vivo experiments. The results indicate that an active process, which presumably implies colostrals cells and mucosal epithelium, occurs. The enhanced mobility of human colostrals macrophages (Ozkaragoz et al, 1988), and the predominance of human colostrals γ - δ T cells expressing non-covalently linked TCR chains (Bertotto et al, 1991) support the hypothesis of an active migration of colostrals cells in humans. The degree of maturation of the neonatal intestine could also be a determining factor, as suggested by the decreased levels of SLA Class 1 antigens expressed by the intestinal epithelium of newborn piglets (Le Jan and Chevalleyre, 1996a). It can be hypothesized that colostrals lymphocytes migrate to the lymph nodes and the macrophages and epithelial cells to the mucosal surfaces, as suggested by the fact that the same membrane determinants are shared by colostrals and pulmonary human macrophages (Biondi et al, 1984). The model of in vitro interaction between colostrals cells and neonatal intestine explants established by Williams (1993) in pigs offers a useful methodological approach for the study of colostrals cell migrations. A technique is being developed in our laboratory which labels pig-blood lymphocytes and colostrals cells with PKH26, an aliphatic fluorochrom (Horan et al, 1990). Maternal blood lymphocytes intravenously transferred to histocompatible piglets were detected in the recipient blood with this new technique during a 15-day survey, and the labelling did not impair the in vitro functions of the colostrals cells (transformation of T lymphocytes in response to mitogenic stimulation, and in vitro growth of epithelial cells). This method could be used for tracking colostrals cells in piglets and for phenotypic characterization.

Functions of mammary secretion cells

Lymphocytes

T lymphocytes are both the most prevalent and the most active of the lymphocytes present in mammary secretions. B lymphocytes selectively accumulate in the mammary gland during gestation (Chabaudie et al, 1993). Their primary established function is to synthesize dimeric IgA, which will then be translocated and excreted in the milk throughout lactation. Since the B lymphocytes would secrete the same IgA in the newborn as they already have been shown to secrete in the mammary gland, the newborn does not really need these maternal B lymphocytes. Depending on the phenotypic and in vitro functional characteristics of colostrals T lymphocytes, these T cells probably transfer immune functions to the neonate and also secrete cytokines. The established immunoprotective functions of mammary secretions, such as the transfer of cellular immunity and protection against infectious disease, suggest mediation assumed by CD4 and CD8 cells. Based on the in vitro results it could be expected that CD4 cells would produce INF- γ , TNF- β and IL-2 cytokines in the newborns. In fact, interferon γ levels are increased in human milk (Eglinton et al, 1994; Wirt et al, 1992). It is possible that immunoregulatory factors in mammary secretions maintain activated T lymphocytes in a latent phase and delay the secretion of soluble factors by these cells. Since the CD8/CD4 ratio is higher in mammary secretions than in the blood and since the CD8 have been shown to be activated in humans, it is expected that they produce IL-4 and TGF β , which is known to be an immunosuppressor factor. This role is suspected in TNF β knock-out mice, who remain free of spontaneous inflammation during the suckling period (Kulkarni and Karlsson, 1993). The enhanced proportion of T8 lymphocytes in mammary secretions could contribute to the natural immunosup-

pressive properties of breast milk, allowing a controlled activation of the immune system during weaning. Two research directions need to be explored: the analysis of the cellular immunity transfer and helper functions of T4 cells, and the study of the control of immune activation by T8 cells.

Phagocytes and epithelial cells

Since polymorphonuclear neutrophils isolated from mammary secretions have reduced functional capacities such as motility and phagocytosis, it seems likely that their role is essentially devoted to the defense of the mammary gland. This supposition is supported by the author's observations that most neutrophils from sow colostrum are destroyed during the washing steps used for cell preparations, and further supported by the fact that in instances of subclinical mammary gland inflammation, colostrum and milk cell preparations are contaminated by high numbers of live neutrophils. Macrophages from mammary secretions are active *in vitro* and could contribute to neonatal immune defenses by phagocytosis and IgA excretion (Weaver et al, 1982, 1984). As proposed by Ozkaragoz et al (1988), they could invade the intestinal mucosa and interact with intestinal epithelium. Their expression of Class II antigens is enhanced by γ interferon (Rivas et al, 1994), and the internalization of the antigens present in the mammary gland by the macrophages could in fact be enhanced by IgA. Their principal role in the newborn should be to act as cytokine-producing (Politis et al, 1991) and antigen-presenting cells.

Epithelial cells are a major component of mammary secretions in swine. In sow milk, they represent 60–90% of the total number of cells, and it has been concluded (Le Jan, 1993) that they are exfoliated from the alveolar epithelium and that they contribute to the transfer of secretory IgA to the neonatal intestine. The IgA that is normally

present in mammary secretions has passed through a classic trans-mammary epithelium translocation. If, however, the IgA is liberated from the epithelial cells by cell lysis in the gastric or intestinal lumen, it will have a non-cleaved secretory component, and, as a result, different properties (mucus adhesion, resistance to proteolysis). In sow colostrum, epithelial cells can grow and differentiate *in vitro*, and can express viral antigens after *in vitro* infection. This capacity suggests that they could interact with the local immune system of the piglet by cytokine secretion and antigen presentation after passage through the intestinal barrier.

It may be of significant interest for the neonate immune system that maternal cells, which have already processed foreign antigens, can pass through the neonate intestinal barrier. These processed antigens would induce an immunity in the neonate that it might not otherwise have been able to mount for itself, because for example: (i) the antigen had been captured by IgA; (ii) a local immune response was rendered difficult by the rapid cell division occurring in the neonate intestine or the lack of receptors on neonate cells; or (iii) epitopes might be presented that are different from those that the newborn antigen-presenting cells would have processed. Mammary secretions could, by way of antigen presentation, direct and modulate the newborn response to specific antigens, and could act on the balance between immune response, tolerance and allergy. This aspect is being studied in our laboratory. A model of *in vitro* culture of colostrum epithelial cells is being developed to analyse the processing of antigens by secretory component and IgA. Antigens of TGE coronavirus are being used. Possible further developments are the analysis of local piglet immune response after the administration of antigen-presenting colostrum cells. Another suggested role for macrophages and epithelial cells is a local

secretion of cytokines, which would interact with the local immunity of the newborn. This can now be studied through the detection of secreted cytokines and also the expression of specific mRNA using labelled colostrum cells in newborn intestinal mucosa.

CONCLUSION

It is established that mammary secretions constitute a cellular link between the mother and newborn. The information presented here seems to indicate that these cells exert biological functions. Experimental models have demonstrated the protective properties of these cells. It can be considered that, under natural conditions, specific cellular protection is not the major role of colostrum and milk lymphocytes. If, for a given pathogen, there is specific T lymphocyte in the colostrum or milk, it can be assumed that a specific secretory IgA will also be present, and that the immune exclusion mechanisms acting during lactation will be more efficient than cellular cytotoxicity. Transmission of immune memory has been demonstrated by mammary secretion cells and could be of primary importance to the newborn. Many factors other than specific memory T lymphocytes could enhance primary antigenic stimulation, for example the presence of immunomodulatory substances in mammary secretions and the possible effects of anti-idiotypic antibodies (Telemo et al, 1991). The immunoregulatory effect of cells from mammary secretions is strongly supported by the predominance and characteristics of T8 lymphocytes in the colostrum and milk. It is clearly established that breast-feeding reduces atopic diseases in humans (Saarinen and Kajoosaari, 1995), although the intervention of cellular components has not yet been demonstrated.

Antigenic stimulation and orientation of immune responses of the neonatal intestine may be one of the most important func-

tions of mammary secretion cells. The neonatal intestine represents a unique system. It consists of an immature mucosa, untouched by previous antigen contacts, that receives adult antigen-presenting cells which are not recognized as foreign cells. This is the case for macrophages, and presumably for colostrum epithelial cells in swine. Further investigations are necessary to assess the capacity of mammary secretion macrophages, and colostrum epithelial cells in swine, to induce local immune responses in the newborn. This is a critical period for the neonate and the orientation of its adapted responses to various antigens is of crucial importance for the survival and development of the young.

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