Chapter 14

The Antimicrobial and Immunomodulating Actions of Milk Leukocytes

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1. Introduction

The ability of highly developed organisms to defend themselves against invading microorganisms depends on recognizing and destroying dangerous substances such as microbes and their toxins. Recognition and destruction are tasks of the immune system, primarily of the leukocytes. Subpopulations of leukocytes variously release antimicrobial substances such as immunoglobulins, lysozyme, complement factors and reactive oxygen metabolites into the cellular environment upon exposure to an antigenic stimulus, and they also ingest foreign materials or secrete cytokines which regulate the immune response. During lactation a mother can pass immunogenic substances to her offspring through her mammary secretions. These compounds in colostrum and milk are an important, if not decisive, part of the adoptive transfer of immunity from mother to offspring in many species. Most scientific investigations into the adoptive transfer of immunity through milk have focused on immunoglobulins rather than on leukocytes. However, adoptive transfer of the cells in milk also appears to occur. It is important to explain how immunocompetent maternal leukocytes can modulate immune responses in the newborn without recognizing and destroying the neonate's leukocytes and tissues as nonself elements that bear different, paternally-derived major histocompatibility complex (MHC) antigens. Conversely, why don't the immunocompetent leukocytes of the newborn attack and eliminate milk-derived cells that

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bear a maternal MHC? An important purpose of this chapter is to address these intriguing questions to the extent that current data permit. Another important question is whether mammary leukocytes have any protective role or whether the milk is simply a convenient route of excretion for these cells. A full understanding of the anti-microbial action of mammary gland leukocytes for all mammalian species must await more exhaustive study.

2. Cytology

Leukocytes, the main cellular component of mammary secretions, include mononuclear phagocytes (macrophages), polymorphonuclear neutrophils (PMN) and lymphocytes (Table 1). These cell types normally make up more than 90% of the somatic cells in colostrum and milk. The remaining fraction may contain a varying number of epithelial cells. Leukocytes and epithelial cells in milk are termed body, or somatic, cells to differentiate them from contaminant microbial cells. Milk somatic cell counts (MSCC) range from several hundred to several million cells per ml, depending on the species, the stage of lactation and, above all, the level of inflammation. The highest MSCC from non-infected glands of women (Ogra and Ogra, 1978; Crago *et al.*, 1979) and cows (Meriläinen *et al.*, 1979; Jensen and Eberhart, 1981) were observed immediately after delivery. Bovine colostrum contained concentrations of viable leukocytes similar to cell counts in the peripheral blood, i.e., from about 0.3 to 3.0×10^6 per ml (Riedel-Caspari, 1993).

The first role postulated for mammary leukocytes was to protect the gland itself against infection. This view was substantiated by Schalm *et al.* (1976), who turned a chronic *Staphylococcus aureus* mastitis into a gangrenous disease by treating the infected cows with an anti-bovine leukocyte serum. A second protective function was proposed by Newby and Bourne (1977) from the results of infusion experiments and by Sheldrake *et al.* (1985a), who observed an increase of immunoglobulin-positive cells after specific antigens were injected into the mammary gland. These authors concluded that mammary B lymphocytes may play a role in the local synthesis of immunoglobulins, thereby contributing to the adoptive transfer of immunity to the newborn. Other cell types also may play a role. The neonate ingests large numbers of macrophages, PMNs and T lymphocytes with its first meals. However, the significance of these cells in the adoptive transfer of immunity is still unclear.

Misinterpretations of cytological findings have been one reason for underestimating the protective potential of milk cells. In particular, based on light microscopic examination the predominant cell type was thought to be sloughed-off epithelial cells. This was due mainly to underestimation of macrophage numbers because, in milk, these cells differ morphologically from

Table 1. Differential cell counts of mammary secretions*

Species	Stage of lactation (time post partum)**	PMN	Macro-phages	Lympho-cytes	Epithelial cells	Authors
Human	colostrum (d1–3)	5	77	11	n.d. ***	Diaz-Jouanen and Williams, 1974
Human	prepartum	21	66	11	n.d.	Ho *et al.*, 1979
	colostrum	42	48	7	n.d.	
	milk (d8)	20	72	5	n.d.	
Human	colostrum (d1–4)	54	37	7	0	Crago *et al.*, 1979
Porcine	prepartum	72	1	26	n.d.	Evans *et al.*, 1982
	colostrum (d3)	55	15	23	6	
	milk (d10)	39	15	14	31	
Porcine	colostrum (d1–2)	61	7	11	20	Schollenberger
	milk (d3–31)	47	10	12	31	*et al.*, 1986a
Porcine	prepartum	93	3	2	41	Lee *et al.*, 1983
	colostrum (d1)	62	21	8	7	
	milk (d2–21)	37	20	12	41	
Porcine	colostrum (d1)	70	2	n.d.	1	Williams, 1993
Porcine	colostrum	50	n.d.	n.d.	n.d.	Persson *et al.*, 1996
	milk (d22)	43	n.d.	n.d.	n.d.	
Bovine	colostrum (d1–2)	9	79	12	n.d.	Meriläinen *et al.*, 1979
	milk (d6)	12	74	.14	n.d.	
Bovine	dry period	3	89	7	1	Lee *et al.*, 1980
	colostrum (d1,4)	62	35	4	0	
	milk	3	80	16	2	
Bovine	dry period	22	48	30	n.d.	Jensen and
	colostrum (d2)	30	42	28	n.d.	Eberhart, 1981
	milk (d3–6)	35	35	30	n.d.	
Bovine	colostrum	38	40	23	n.d.	Duhamel *et al.*, 1987
Bovine	colostrum fm (d2)	23	70	7	n.d.	Östensson, 1993
	colostrum rm (d2)	39	51	11	n.d.	
	milk fm (m3)	17	79	4	n.d.	
	milk rm (m3)	32	45	23	n.d.	
Ovine	involution	40	51	9	0	Lee and
	colostrum (d1)	66	24	34	<1	Outteridge, 1981
	milk	2	84	13	2	

* Means expressed as percent of identified leukocytes and epithelial cells; data partially converted to enhance inter-study comparisons.

** Approximate period during which the mammary secretions were collected, expressed in days (d) or months (m) post partum; foremilk (fm) or residual milk (rm).

*** Not determined.

the mononuclear phagocytes found in the blood (monocytes) and most other organs. The reason for this morphologic dissimilarity has been revealed by electron microscopy to be a result of uptake by milk macrophages of cell fragments, fat, casein micelles and lipid droplets from the glandular environment. It is now generally accepted that most of the somatic cells in mammary secretions are viable leukocytes and that milk macrophages form part of the mononuclear phagocyte system (MPS), formerly called the reticulo-endothelial system (Lee *et al.*, 1980; Mielke and Koblenz, 1980a). Nevertheless, epithelial cells also may be present in appreciable numbers, e.g., in mammary secretions of women (Ho *et al.*, 1979) and sows (Lee *et al.*, 1983; Schollenberger *et al.*, 1986a).

The MSCC and the milk differential cell counts (MDCC) are influenced by species, breed, variations between individuals, period of lactation or gravidity, milk fraction (foremilk or residual milk) and, above all, the presence of pathogenic or non-pathogenic microorganisms in the gland (Smith and Schultz, 1977; Ogra *et al.*, 1978; Crago *et al.*, 1979; Lee *et al.*, 1983; Harmon and Heald, 1982; Mielke and Koblenz, 1981; Östensson 1993). Even a weak inflammatory stimulus, such as the instillation of phosphate-buffered saline into the gland, can provoke an alteration in the number and activity of mammary leukocytes (Heyermann and Senft, 1985). Another expeimental procedure used to produce a sterile intramammary inflammation has been to insert plastic devices into the gland cisterns of the bovine udder. Raising the MSCC by this procedure provided protection against experimental challenge with *S. aureus* (Schultze and Paape, 1984) and *S. uberis* (Paape *et al.*, 1988), but failed to reduce natural infections of the udder in field conditions (Corlett *et al.*, 1984). In other studies, clinical symptoms of acute mastitis were produced by infusion of endotoxins from Gram-negative bacteria into the bovine udder. The symptoms were comprised of the general signs of acute inflammation, including release of chemotactic factors by macrophages and massive migration of neutrophils from the blood to sub-epithelial sites. Within a few hours the neutrophils penetrated the basal lamina, migrated through the epithelium into the milk and dominated the MDCC (Nickerson and Pankey, 1984; Guidry *et al.*, 1983; Mattila and Frost, 1989).

The most significant physiological changes in MSCC and MDCC occur at parturition when leukocytes accumulate in the mammary gland. This is possibly due to hormonal changes. With the onset of lactation, leukocytes leave perivascular areas of the connective tissue and settle at sites that permit easy passage into alveolar areas and the milk. In most of the species investigated, the absolute somatic cells counts declined from parturition until the end of lactation, at which time the counts rose dramatically (Seelig, 1980; Campbell *et al.*, 1950).

2.1. Macrophages

The differential cell counts show significantly fewer lymphocytes and many more mononuclear phagocytes in mammary secretions than in the peripheral blood. The mere presence of macrophages in milk and colostrum is not surprising because the mammary gland, like many other organs, harbors a large number of mononuclear phagocytes. However, the extent to which these cells dominate the somatic cell count of secretions from healthy glands is surprising. In contrast to the macrophages of most other tissues, milk mononuclear phagocytes exhibit a pronounced vacuolization. Electron microscopic analysis reveals that the vacuoles contain lipid droplets, casein micelles, debris and degraded neutrophils (Smith and Goldman, 1968; Mielke and Koblenz, 1980b; Lee *et al.* 1983). Vacuolization of milk macrophages is intensified during the colostral period, ebbs during lactation and increases again at the beginning of the dry period (Lee *et al.*, 1969, 1980). It has been postulated that macrophages (as well as PMN) contribute to the reabsorption of milk lipids during the involution of the mammary gland and that these phagocytes serve as "street cleaners" in the gland during lactation. Nevertheless, it remains unclear why their numbers rise at the beginning of lactation and what the importance is of their large scale phagocytosis of non-pathogenic particles, which could also be removed mechanically with the flow of milk. In fact, the ingested material may compromise the ability of the milk macrophage to ingest and destroy microorganisms. Thus, at first glance, it seems doubtful that these garbage-laden macrophages would be of benefit either to the gland or to the suckling neonate.

In vitro studies on the macrophages of mammary secretions have revealed a wide range of activities similar to those of other members of the MPS. These activities include an enhanced random migration by mammary macrophages exposed to a soluble factor in milk (Mushtaha *et al.*, 1989), the presence of Fc and C3b surface receptors and at least some capacity for ingestion and destruction of opsonized and non-opsonized microorganisms (Lascelles *et al.*, 1969; Mohr *et al.*, 1970; Smith and Goldman, 1968; Jensen and Eberhart, 1975; Robinson *et al.*, 1978; Pitt, 1979). In addition, milk macrophages can present antigen to lymphocytes (Smith and Goldman, 1970; Smith *et al.*, 1971; Crago *et al.*, 1979; Mielke and Koblenz, 1981) and produce cytokines such as MIF and LIF (Mohr *et al.*, 1970) as well as antibacterial substances including lysozyme, lactoferrin and the complement factors C3 and C4 (Murillo and Goldman, 1970; Pitt, 1979). It may be assumed that mononuclear phagocytes are an important, if not the major, source of these anti-microbial factors in colostrum and milk. Perhaps these cells depend on the stimulus of exposure to particles such as debris or micelles to generate and release antimicrobial products into the milk.

Another feature of mammary phagocytes renders them active contributors to the adoptive transfer of immunity to the neonate (Pittard *et al.*, 1977). Milk phagocytes, both macrophages and neutrophils, take up lactoferrin and immunoglobulins from the surrounding fluid (Moro *et al.*, 1983; Laven *et al.*, 1981). As IgA dominates the immunoglobulins in the milk of most species, this immunoglobulin class usually predominates in the vacuoles of mammary phagocytes. Moreover, these cells avidly retain ingested immunoglobulin (Pitt, 1979; Weaver *et al.*, 1981). Even after days in culture, the intracellular IgA concentration of human colostral macrophages remained five-fold higher than that in the culture medium. In contrast, addition of ingestible particles to the cultures, e.g., Candida, *E. coli* or latex beads, induced a significant release of IgA into the culture medium within 15 to 30 minutes. Colostral immunoglobulins are essential for all mammalian species with an epitheliochorial placenta, which precludes the intrauterine transfer of antibodies to the fetus. Even in species such as humans, clinical evidence clearly demonstrates that under suboptimal hygienic conditions colostrum, and particularly its secretory IgA, prevent enteropathogenic microorganisms from adhering to the intestinal mucosa and provoking diarrhea. Hence the attachment of immunoglobulin-laden leukocytes to the mucosa of the intestinal tract, followed by the release of the ingested antibody, is an important adjunct in support of the neonate's intestinal defense.

2.2. Neutrophils

Mammary phagocytic cells also include varying numbers of PMN. In contrast to the long-lived, antigen-presenting and immuno-regulatory macrophages, neutrophils are short-lived and have only one purpose, which is to invade sites of acute inflammation and perform phagocytic microbicide, after which the phagocyte dies. PMN may approach 100% of the somatic cell count in the secretions of severely inflamed glands (Schalm,1977). In nearly all species investigated, PMN comprise a higher proportion of the somatic cell count in colostrum than in mature milk. This may reflect an inflammation-like condition in the gland at parturition or simply an easy passage through the vascular wall at this stage. As a short-lived and non-dividing leukocyte population, PMN enter the mammary gland directly from the blood and do not undergo morphological changes in the manner of the mononuclear phagocyte. From electron microscopic studies it is apparent that neutrophils are also able to concentrate globules and debris in cytoplasmic vacuoles, thereby contributing to mammary involution, although PMN seem to be less effective in phagocytosis than macrophages (Lee *et al.*, 1969, 1983; Ho *et al.*, 1979). However, in contrast to macrophages, PMN are able to release large quantities of oxygen radicals into mammary secretions. Thus the PMN may be the main source of reactive oxygen metabolites for the extracellular lactoperoxidase system, one of the most important humoral defense

mechanisms against bacteria, at least in the bovine udder (Hallén Sandgren *et al.*, 1991).

Neutrophils from milk have been subjected to a variety of functional tests and comparisons have been made with their counterparts in the blood. It is generally agreed that the phagocytic activity of milk neutrophils is low in comparison with that of blood neutrophils. Moreover, milk PMN contain fewer primary granules and less glycogen than blood neutrophils, ingest fewer bacteria than blood PMN *in vitro* and kill ingested organisms less effectively (Smith and Goldman, 1968; Paape *et al.*, 1975). The reduced microbicidal activity of milk PMN in these studies can be attributed, at least in part, to a reduced oxidative burst.

The uptake of milk lipids, casein and antibody-antigen complexes is considered to be the reason for the low phagocytic activity of milk PMN. These cells appear to become "exhausted" by the massive uptake of particulate material. This view is supported by *in vitro* findings with blood neutrophils, which show similarly depressed phagocytic activity when they are cultivated in a medium that contains milk (Russel and Reiter, 1975; Russel *et al.*, 1976; Ho and Lawton, 1978; Csorba *et al.*, 1979; Pickering *et al.*, 1980; Heyermann and Senft, 1985; Schollenberger *et al.*, 1986c; Targowski and Niemialtowski, 1986). Moreover, two additional experiments support the hypothesis that the milk medium, rather than the advanced age of milk PMN, is responsible for the low phagocytic activity of these cells. Firstly, neutrophils from residual bovine milk, i.e., cells that spent less time in the gland, ingested more opsonized yeast particles *in vitro* and showed higher chemiluminescence than stripping milk neutrophils which had been present in the gland for at least half a day (Hallén Sandgren *et al.*, 1991). Secondly, PMN harvested directly from the bovine teat cistern by a surgical method that precluded contact with milk ingested more particles and released more myeloperoxidase (determined by luminol-dependent chemiluminescence) than blood neutrophils from the same cow. Therefore, it appears that neutrophils entering the mammary gland constitute a cell population that is highly active, with a permanently activated NADPH oxidase as well as a continuous release of myeloperoxidase (Hallén Sandgren *et al.*, 1992). As in the case of the macrophage, some functions of the PMN in the mammary gland appear to become down-regulated in a process that has the main purpose of enriching mammary secretions with soluble antimicrobial substances. Such substances may offer more immediate antimicrobial protection than phagocytic processes both to the mammary gland and to the suckling neonate.

2.3. Lymphocytes

Lymphocytes represent the phylogenetically modern part of the immune system. Only a few primed lymphocytes are required to modulate immune

responses decisively through clonal expansion, antibody formation or lymphokines. Thus maternal lymphocytes may be a means of transferring immunologic experience from mother to offspring. It is therefore worthwhile to examine mammary gland lymphocyte reactions.

Several lymphocyte subpopulations, particularly T and B lymphocytes, have been identified in mammary secretions. However, a rigorous interpretation of these results requires critical assessment of methodology. For example, surface immunoglobulins do not conclusively identify B lymphocytes because T lymphocytes, colostral macrophages, PMN and even non-cellular elements can stain positively for immunoglobulins.

Between 50% and 90% of lymphocytes from mammary secretions of different species are T cells, as determined by spontaneous rosette formation with heterologous erythrocytes (Diaz-Jouanen and Williams, 1974; Ogra *et al.*, 1978; Schore *et al.*, 1981), reaction with Helix pomatia lectin (Concha *et al.*, 1978a,b, 1980) or reaction with monoclonal antibodies (Duhamel *et al.*, 1987; Jain *et al.*, 1991). Generally, only about 4% of milk lymphocytes have been identified as B cells using the method of erythrocyte-antibody-complement (EAC) rosettes (Bush and Beer, 1979; Schore *et al.*, 1981; Salmon and Delouis, 1982; Williams, 1993). An exception is the sheep, in which 30% of milk lymphocytes are reported to be B cells on the basis of EAC rosetting (Lee and Outteridge, 1981). Using B cell-specific monoclonal antibodies, 3% to 30% of milk lymphocytes stained positive in human (Jain *et al.*, 1991) and bovine (Duhamel, 1987) colostra. Despite the postulated local synthesis of immunoglobulins in the mammary gland, B lymphocytes constitute a minority of milk somatic cells and plasma cells have not been detected in milk (Crago *et al.*, 1979) except for one report on sheep milk (Lee and Outteridge, 1981). The absence of plasma cells in milk may be due to a low requirement for these highly productive cells in the synthesis of immunoglobulins or to their location at sites from which they are not readily mobilized.

Monoclonal antibodies directed to differentiation antigens of the main immunoregulatory lymphocyte, the T cell, reveal several distinct subsets in mammary secretions, including OKT4(CD4), OKT8(CD8) and γ/δ positive cells (Crago *et al.*, 1979; Richie *et al.*, 1982; Moro *et al.*, 1985; Bertotto *et al.*, 1990). The numbers of OKT4 and OKT8 positive T cells were more nearly equal in colostrum than in the peripheral blood of humans (Richie *et al.*, 1982), an observation that remains unexplained. Bertotto *et al.* (1990) demonstrated that non-sensitized "naive" T lymphocytes, identified by monoclonal antibodies against a series of differentiation antigens, were less frequent in human breast milk than in blood. Thus, antigen-primed memory T lymphocytes appear to prevail in human milk, and from these results it may be concluded that memory and effector T cells preferentially pass into the gland.

In vitro, mammary lymphocytes exhibit a proliferative response to a variety of stimuli. A high proportion of human milk cells exhibited transformation when they were stimulated with a polyclonal phytomitogen (PHA), but a small proportion also responded to specific antigens such as diphtheria antigen, tetanus toxoid or tuberculin (Smith and Goldman, 1968). The antigen-specific blastogenic response of milk lymphocytes to tuberculosis antigens (purified protein derivative) *in vitro* was shown to correlate with that of peripheral lymphocytes and with the skin reactivity of the donor to the same antigen (Mohr *et al.*, 1970). These early findings that sensitized T cells are simultaneously present in blood and milk have been confirmed in a series of publications on mammary secretions in humans (Diaz-Jouanen and Williams, 1974; Parmely *et al.*, 1976; Ogra and Ogra, 1978; Meggs and Beer, 1979) and other species (Smith and Schultz, 1977; Concha *et al.*, 1978a,b,1980; Schore *et al.*, 1981; Salmon, 1987). It was generally noted that milk lymphocytes were less responsive in terms of cellular proliferation than blood lymphocytes. However, the converse also has been reported, e.g., in response to the K1-antigen of *E. coli* (Parmely *et al.*, 1976), the cytomegalovirus (Meggs and Beer, 1979) and endotoxin (Nonnecke and Kehrli, 1985). An enhanced responsiveness to "gut-specific" antigens on the part of milk lymphocytes, together with some clinical observations (Allardyce *et al.*, 1974; Goldblum *et al.*, 1975) and lymphocyte homing experiments in mice (Roux *et al.*, 1977; Lamm *et al.*, 1978; Weisz-Carrington *et al.*, 1978; McDermott and Bienenstock, 1979), gave rise to speculations that certain clones of gut-derived lymphocytes might have preferential access to the mammary gland and should, therefore, be regarded as part of the common mucosal immune system (Bienenstock *et al.*, 1978; Parmely and Beer, 1977). This hypothesis remains controversial.

The results of a series of experiments on rats, swine, cows and sheep have indicated that sensitized lymphocytes from gut-associated lymphoid tissue (GALT) may colonize the mammary gland pre-partum, but without any clearcut preferential access comparable to that of other peripheral blood lymphocytes (Manning and Parmely, 1980; Sheldrake *et al.* 1985a; Salmon 1987; Harp and Moon, 1987; Harp *et al.*, 1988). Two studies in lactating and non-lactating ewes (Sheldrake and Husband, 1985; Sheldrake *et al.*, 1985b) confirmed that, without an intramammary antigenic stimulus, only a marginal migration of intestinally primed GALT B lymphocytes to the mammary gland can be expected. Furthemore, following immunization of ewes an increase in specific antibody-containing cells of intestinal origin was apparent in the respiratory tract but not in the mammary gland (Scicchitano *et al.*, 1984), indicating that the lymphoid tissue of the lung forms part of the common mucosal immune system whereas that of the mammary gland does not. It is probable that the mammary gland provides offspring with both GALT-specific and systemic lymphocytes without any preference regarding the origin of the lymphocytes.

Very small numbers of cytotoxic T cells and natural killer (NK) cells have also been reported in mammary secretions, the latter on the basis of the surface marker HNK-1 (Leu 7/CD56) (Moro *et al.*, 1985). Functional tests of antibody-independent cytotoxicity or the mixed leukocyte reaction have demonstrated that, in general, the activity of the responsible leukocyte populations from milk was lower than that of the corresponding blood leukocytes (Kohl *et al.*, 1978a,b, 1980; Parmely *et al.*, 1976; Parmely and Williams, 1979).

3. Adoptive Transfer of Cellular Immunity Through the Colostrum

Pitt *et al.* (1977) demonstrated that, in a *Klebsiella pneumoniae* infection model, colostral leukocytes provided effective protection for newborn rats against necrotizing enterocolitis. When isolated colostral leukocytes (albeit not in defined numbers) were added to milk formula or to milk subjected to freezing and thawing, the infected neonatal rats survived the experimental infection, as did control rat pups given complete fresh rat milk. In contrast, frozen/thawed rat milk or the formula alone resulted in 90% and 100% mortality, respectively. *In vitro* experiments confirmed the antibacterial potential of leukocytes in the milk of rats as well as the inadequacy of milk formula and frozen milk in this regard.

Beneficial properties of colostral leukocytes were also apparent in a trial involving twenty colostrum-fed calves (Riedel-Caspari, 1993). The animals were experimentally infected with an hemolytic "attaching and effacing" strain of *E. coli* three hours post partum. Immediately after infection, as well as on three consecutive feedings, the calves received either cell-depleted pooled colostrum or the same pooled colostrum supplemented with the colostral cells from their own dams. During the first week of life the calves that had received the cell-supplemented colostrum excreted significantly fewer infectious bacteria in their feces than the calves fed the cell-depleted colostrum. Also, complete elimination of the infectious organisms was accelerated by brief supplementation with colostral leukocytes immediately after birth. It is noteworthy that cells from two glands that developed clinical mastitis seemed to be the most effective in promoting elimination of the *E. coli* infection.

Unfortunately, the impressive results with experimental *Klebsiella* and *E. coli* infections could not be confirmed in colostrum-deprived calves which were infected with Rota-Corona virus and pathogenic *E. coli* under natural conditions (Riedel-Caspari and Schmidt, 1991a; Riedel-Caspari *et al.*, 1991). In these studies, calves deprived of colostrum were fed twice daily either milk substitute or substitute supplemented with the complete cell harvest from 2 liters of their own dam's colostra. Two of seven calves that received colostral leukocytes sur-

vived the first four weeks of life, and three of six non-cell-supplemented calves survived. In addition, one of seven supplemented calves, but three of five non-supplemented calves, developed a significant interferon-α concentration in the blood serum on day 3 of life, the presumptive peak of viral infection. Moreover, the bactericidal activity of whole blood against *E. coli* was low and the beginning of immunoglobulin synthesis (as judged by the rise of blood immunoglobulin concentration during the first four weeks of life) was delayed in the supplemented animals compared to the non-supplemented animals. Contrary to the colostrum-deprived animals, none of thirty-three calves in two additional experimental groups kept under the same conditions and fed either cell-supplemented or cell-depleted colostrum died or even developed significant diarrhea. Clearly colostral immunoglobulin is of vital significance for an agammaglobulinemic neonate such as the calf and cannot be replaced by leukocytes. Moreover, the modestly reduced survival rate and the impaired immune competence of cell-supplemented calves given milk substitute indicate that isolated leukocytes could even have detrimental effects if they are administered to an animal without concomitant adoptive transfer of immunoglobulins.

3.1. Materno-Fetal Transfer of Systemic Immune Functions

Apart from the possibility that cells in milk may support local defense in the neonatal intestine, the more exciting question is whether the maternal cells in colostrum are able to cross the intestinal wall and modulate systemic immune responses of the neonate. Early studies suggested that T cell functions could be transferred from mother to offspring via the milk. Type 4 delayed hypersensitivity reactions, such as the tuberculin (PPD) skin reaction, are known to depend on sensitized T lymphocytes and adoptive transfer is possible only by passage of sensitized cells. Mohr (1973) and Schlesinger and Covelli (1977) compared the PPD skin sensitivity of twenty breast-fed and fifteen non-breast-fed children of mothers who were PPD-positive but clinically free of tuberculosis. More than half of the breast-fed children exhibited anti-PPD skin sensitivity, whereas only one of the non-breast-fed infants (and none of more than eighty breast-fed children of PPD-negative mothers) did so. However, the conclusion that cell-mediated immunity to PPD was transferred to the infants through colostral T lymphocytes is questionable because reinfusion of fluorescently labeled maternal leukocytes showed that granulocytes and lymphocytes crossed the placental barrier and could be detected in the cord blood of more than 50% of infants at delivery (Desai and Creger 1963; Field and Caspary, 1971). Sensitized T cells could therefore have crossed the easily penetrable hemochorial human placenta, although epidemiological evidence for this hypothesis was weak. However, support for the postulated colostral transfer of sensitized T lymphocytes has come from studies on other species. Duhamel (1986) showed that PPD sensitivity was passed to calves

consuming colostrum from PPD-positive cows. This is noteworthy because the impermeable bovine epitheliochorial placenta excludes not only leukocyte traffic but even the transfer of immunoglobulins.

Colostral transfer of T helper cell functions has been demonstrated in the nude mouse (Hale *et al.*, 1976). B lymphocytes of homozygous nude mice (nu/nu) are unable to produce antibodies against antigens that need the mediation of T lymphocytes, e.g., heterologous erythrocytes. In contrast, heterozygous mice (nu/+) can generate T-dependent antibody responses. When homozygous (nu/nu) offspring were suckled on heterozygous (nu/+) foster mothers, they developed the ability to form antibodies against sheep erythrocytes. The results of this experiment, as well as from studies on the transfer or suppression of graft-versus-host reactions in rodents (Beer *et al.*, 1972; Beer and Billingham, 1973; Head *et al.*, 1977; Uphoff, 1977), are consistent with the concept of the passage of antigen-reactive cells from mother to offspring via colostrum and milk. Opposite findings (Silvers and Poole, 1975) may be due to unidentified variables in the experiments.

In addition to the foregoing, Head and Beer (1979) demonstrated transfer of tumor resistance via colostrum to susceptible newborn mice. The different susceptibility of two strains of mice, C57BL/6 and A, to a Leydig cell tumor is explained by strain-specific differences in the activities of cytotoxic T cells, NK cells and macrophages in the elimination of neoplastic cells. When fostered on dams of the resistant strain, about half of newborn mice of the susceptible C58BL/6 strain resisted experimental tumor induction following inoculation with tumor cells. This finding is in contrast to the 100% mortality observed when newborns of the susceptible strain were suckled on dams of the same strain. Cross-fostering to the resistant strain clearly provided the adopted offspring with leukocytes that modulated tumor resistance. However, it cannot be determined from this experiment whether the enhanced resistance observed resulted from the transfer of maternal cells to the neonate or from the absorption of milk cytokines by the gut. Milk leukocytes release cytokines such as LIF and MIF (Mohr *et al.*, 1970), interferon (Emodi and Just, 1974; Lawton *et al.*, 1979) and tumor necrosis factor-α (Mushtaha *et al.*, 1989) in vitro. It is possible that these molecules can modulate immune responses of the newborn by way of absorption through the gut, although clear evidence for this is lacking.

3.2. Passage of Milk Cells Across the Intestinal Wall

Adoptive transfer of immune functions by way of the milk would be substantially explained if viable maternal leukocytes were demonstrated in the neonate. Demonstrating passage of milk leukocytes across the intestinal wall, however, has been a challenge because of the small numbers of maternal cells

that locate in the tissues of the newborn. In three studies, leukocytes from various sources were injected directly into intestinal segments after laparotomy and were detected in the intestinal wall and lymphoid tissue close to the sites of application within a short time after administration. Radio-labeled lymph node cells from Fisher (Fi) × Dark Agouti (DA) F1 hybrids or from purebred Fisher rat donors were detected by autoradiography in the epithelium and the lamina propria of adult Fi × DA hybrid recipients 24 to 48 hours after infusion (Seelig and Billingham, 1981). Sheldrake and Husband (1985) used cells from mesenteric lymph nodes of syngeneic donors and confirmed such translocation of leukocytes into the intestine of the neonatal rat. These authors also infused radio-labeled allogeneic blood leukocytes from ewes into different gut segments of recipient lambs. Within two hours the labeled cells were detected in the lacteals and the mesenterial lymph nodes of the recipients. Finally, allogeneic colostral cells with a fluorescein isothyocyanate (FITC) label were injected into intestinal loops of calves and were subsequently detected using fluorescent antibodies applied to sections of the epithelium and lamina propria of the recipients (Liebler-Tenorio and Riedel-Caspari, unpublished data).

In apparent contrast to the foregoing studies, Miller (1981) failed to detect orally administered colostral leukocytes in a morphological study of the intestinal mucosa of suckling mice. However, additional studies have shown that orally administered leukocytes can, indeed, locate in the recipient intestine. Tuboly *et al.* (1988) fed technetium-labelled porcine colostral cells to piglets and detected these cells by autoradiography in intestinal sections of the recipients. Likewise, Tuboly *et al.* (1995) demonstrated intestinal uptake of colostral cells by lambs, whether the cells were administered orally or intraduodenally. Williams (1993) demonstrated that FITC-labeled colostral leukocytes were present in the peripheral blood of colostrum-deprived newborn piglets from two hours to twenty-four hours after oral administration. Colostral cells were also detected in the spleen, liver, lungs, and lymph nodes. In contrast, leukocytes prepared from the peripheral blood of the dams did not penetrate the intestinal barrier. In addition, use of intestinal explant cultures from colostrum-deprived neonatal piglets indicated intercellular passage of colostral leukocytes, but not of peripheral blood mononuclear cells, between epithelial cells of the duodenal and jejunal mucosa. At present, this finding appears inconsistent with the results of studies on the passage of leukocytes from the mesenteric lymph nodes of rats and the peripheral blood of lambs (Sheldrake and Husband, 1985).

Apart from the anatomic origin of maternal leukocytes, the genetic relationship between the donor and recipient may play a role in the passage of maternal cells across the intestinal wall. Studying outbred piglets, Tuboly *et al.* (1988) observed that colostral leukocytes only from a recipient piglet's own dam passed through the intestinal wall; cells from other dams remained trapped in the

intestinal mucosa. A similar observation was reported by Kmetz *et al.* (1970), who administered virus-infected bovine lymphocytes to newborn rabbits and detected these xenogeneic cells in the intestinal wall, but not in the mesenteric lymph nodes, of the recipients. From the present literature it is hard to determine how close the genetic relationship must be to permit the passage of colostral leukocytes into or through the neonatal intestine. Passage between two closely related inbred mouse strains, as reported by Hale *et al.* (1976), is not surprising. However, transfer of leukocytes from purebred foster mothers of Dark Agouti and Fisher rats to the reciprocal offspring seems to occur at an early stage after birth, despite the fact that the strains are highly histoincompatible and develop runting disease and a graft-versus-host reaction when given reciprocal skin allografts (Beer *et al.*, 1975; Head *et al.*, 1977). Moreover, prolonged survival of skin grafts from the reciprocal strain was observed when the recipient rat pups were suckled on foster mothers of the reciprocal strain immediately after birth (Beer *et al.*, 1975). The latter experiments, although not confirmed by Silvers and Poole (1975), provide evidence that maternal immunocompetent cells not only gain access to the immune system of the neonate but influence immune functions despite MHC incompatibility. This phenomenon currently can be explained only in terms of tolerance induction in the neonate.

Maternal cells may persist in offspring for a considerable period of time. For example, various authors have described leukocyte chimerism on the part of male children bearing cells with a 46 XX karyotype in their blood. In one case, such chimerism was found to persist in a male infant up to 39 weeks after birth (Loke, 1978). Such apparent tolerance may also explain the classic observation of Peer (1958) that maternal skin grafts given to human infants survive for several months, whereas paternal grafts are rejected within days. Tolerance depends on the stage of immunologic maturation of the recipient and can still be induced after birth. This is critical for the neonates of all species with a placental barrier that is impermeable to maternal cells.

4. Transfer of Pathogenic Microorganisms Via Milk Leukocytes

Colostral leukocytes have occasionally been implicated as vehicles for pathogenic microorganisms, although the evidence for this mechanism is slight. Mitsuda *et al.* (1989) investigated the hypothesis that sporadic vaccination failure against hepatitis B virus (HBV) in children of HBV-positive mothers results from the transfer of virus-laden colostrum and colostral leukocytes. HBV-specific DNA was detected by polymerase chain reaction in eight samples of whey and colostral cells from 10 antigen-positive mothers. Only one infant developed HBV carrier status as indicated by detection of cord blood HBV DNA. These results are

consistent with the report of Beasley *et al.* (1975) that the transfer of virus-containing colostrum and colostral leukocytes is a minor factor in infection of newborn infants with HBV. Kmetz *et al.* (1970) likewise failed to demonstrate the transmission of bovine leukemia virus when cultured bovine leukocytes were fed to newborn rabbits. However, the reason for the negative outcome of this experiment might have been the fact that the xenogeneic leukocytes were unable to cross the intestinal mucosa of the recipients.

Transmission of intracellular blood parasites such as *Cowdria ruminantium* which transmits heartwater disease to ruminants in East Africa, generally depends on the cooperation of blood-sucking arthropod vectors that transfer the parasitized erthrocytes from a carrier to another suscetible organism. However, in light of new findings, colostral cells may provide a mode of vertical transmission of this parasite. When isolated colostral cells from cows living in a heartwater-endemic area were injected intravenously into five susceptible goats, three of the recipient animals developed clinical signs of the disease (Deem *et al.*, 1996).

5. Colostral Leukocytes and the Neonate—Friends or Foes?

It is obvious that colostral leukocytes may support antimicrobial defense and immune responses of the offspring, including functions such as the synthesis of T cell-dependent antibodies and clonal expansion of lymphocytes. Subpopulations of mammary leukocytes produce soluble factors, including lysozyme, complement components, reactive oxygen metabolites and immunoglobulins, which impart anti-microbial resistance locally and systemically. Even resistance to certain tumors may be transferred via colostrum. On the other hand, colostral leukocytes have been shown to contribute to the transmission of graft-versus-host reactions, and apparently they can impair antimicrobial resistance if not integrated appropriately into the immune system of the neonate. Finally, it remains possible that intracellular pathogens can be transferred from mother to offspring by colostral leukocytes. Thus, whether colostral and milk leukocytes act as friends or foes to the suckling offspring depends on a variety of factors which have not yet been fully identified. The relationship between the donor and recipient, however, seems to play a decisive role.

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