Immunologic Benefits and Hazards of Milk in Maternal-Perinatal Relationship

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Aside from nutritional significance, milk affords infant mammals immunologic benefits. However, it is not without immunologically based hazards. These stem from its antigenicity and the fact that in certain species that receive their maternal immunologic endowment postpartum, hemolytic disease of the newborn may be mediated by colostral antibodies. Awareness that viable leukocytes are ingredients of colostrum and milk has stimulated interest in the significance of these cells. Skin grafting tests on foster-nursed rats and mice have given circumstantial evidence that, in these species, leukocytes may be transmitted naturally from the mother's blood stream to the suckling's blood stream through the milk, and that these cells may be beneficial (adoptive immunization) or, in some genetic contexts, harmful (initiating graft-versus-host disease). In man, too, studies on necrotizing enteritis and other diseases provide increasing support for the thesis that leukocytes in milk fulfill a protective function, possibly as a consequence of their "natural" transplantation.

For some years now, as students of Nature's activities in the field of transplantation, we have been interested in immunobiological aspects of the maternal-fetal relationship, particularly with respect to the cellular and humoral antibody exchanges that take place between mother and fetus (1). The possible consequences for the infant of maternal to fetal transfer of leukocytic cells are [a] induction of tolerance of those maternal tissue alloantigens that have not been inherited from the mother, that is, maternally induced tolerance; [b] the initiation of graft-versus-host or runt disease by reactivity of lymphocytes of maternal origin against paternally inherited alloantigens of the fetus (2); and [c] the evocation of a state of hypersensitivity to alien maternal tissue antigens. Laboratory experiments, conducted principally on inbred strains of animals of several mammalian species, have provided compelling circumstantial evidence that, with some strain combinations, the transfer of allogeneic hematopoietic or lymphoid cells to females gestating syngeneic fetuses may result in the delivery of offspring that will subsequently manifest specific hyporeactivity (tolerance) or hyperreactivity (immunity) toward test skin allografts from donors of the strain from which the maternal cellular inocula were derived (3, 4).

Runt disease, though very rare, occurs naturally in both rats and man (5-8). However, in several species, including rats, mice, guinea pigs, hamsters, and rabbits, it can be shown that carefully timed specific adoptive or active immunization of prepregnant or pregnant females may lead to the subsequent development of high incidences of graft-versus-host disease in the initially healthy progeny during the first month of life (4). These observations seem to reflect experimental heightening of a natural, two-way maternal-fetal exchange of lymphocytes that is normally without overt effect, although it may lead to a long-lasting low level of lymphocytic chimerism even in the mothers (9).

It must be emphasized that allogeneic fetuses have proved to be completely refractory to all attempts to procure their abortion or rejection from their union with maternal tissue at the level of the placenta by specific active or passive immunization of the mother against their alien, paternally inherited tissue antigens. Paradoxically, there is an impressive body of experimental evidence that the complex histocompatibility gene polymorphisms, which currently frustrate clinical transplantation, have a significant overall beneficial effect on fertilized eggs as potential free transplants on the endocrinologically prepared endometrium (10). Confrontation of its mother with alien tissue antigens not only improves the chances of a blastocyst's successful implantation over those of a genetically compatible blastocyst, but it also increases the growth rate of the resultant feto-placental unit, giving the infant a slight selective survival advantage.

It may well be that the risk of initiating runt disease affords a sufficient explanation of why, in those species that receive the principal portion of their maternal immunologic endowment before birth—for example, man and the guinea pig—in the form of ready-made antibodies, the lymphocytic mediators of cellular immunities that the

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mother has acquired are not included.

In most mammals, from the immunologic, nutritional, and other points of view, parturition represents a disconnection of the fetus from the two-way traffic exchange system with the mother through the placenta and represents substitution, usually on an intermittent basis, by the one-way system afforded by the breast.

Along with the immunologic benefits it confers on the dependent fetuses of some species, the placenta, as we have already seen, can also be a source of hazards of an immunologic nature such as runt disease. A much more familiar placentally mediated hazard in man is hemolytic disease of the newborn. This depends on the sensitization of women to certain paternally inherited antigens of the fetuses' erythrocytes, usually Rh_o (D), as a consequence of the natural "porosity" of the placenta to fetal erythrocytes and the facility with which it transmits immunoglobulins of the IgG class from the maternal to the fetal circulation.

It has been known for some years now that the breast-dependent phase of mammalian life is not entirely without immunologic risk for the infant. A classic example is jaundice of newborn mule foals, a fatal disease closely resembling hemolytic disease of the newborn in man in its symptomatology and pathogenesis (11). The subsequent progeny of mares that have given birth to an affected mule foal consistently succumb to the disease if sired by donkeys, but not if sired by horses. The cause of this disease is, first, immunization of the mare against certain donkey-specific erythrocyte antigens of her mule fetus, and, second, transfer of the resultant antibodies to the infant mule after birth. It will be recalled that in ungulates maternal antibodies are unable to cross the placenta. The young acquire their maternal immunologic endowment in its entirety by absorption across the intestinal epithelium of the highly concentrated IgG immunoglobulins present in the colostrum. Their ability to absorb these antibodies from the gut in an undegraded form is restricted to a very short time interval. Consequently, mules born of sensitized mares remain perfectly healthy if prevented from receiving milk from their mothers for a few days. Hemolytic disease of the newborn of similar cause sometimes occurs following intraspecies matings in horses and pigs, where erythrocyte isoantigens are involved.

Antigenic Status of Milk and its Possible Consequences

By virtue of its various complex protein constituents, it is scarcely surprising that many different antigenic specificities are recognized when colostrum or milk of one species is injected into a host of a different species, or that cow's milk ranks high on the list of food allergens, particularly in children. A strong prima facie case exists on the basis of both clinical and experimental observations that crib death, or the Sudden Infant Death syndrome, which is the leading cause of death of infants during the first year of life in the United States, is frequently due to anaphylactic reactivity in the lungs to aspirated milk by formula-fed infants (12, 13).

Since mammary glands do not produce their unique secretion until relatively late in life and it does not normally leave the lactiferous ducts to gain access to the tissues, it is not surprising that some of its ingredients, including casein, have been shown on the basis of animal experiments to be autoantigenic, or that lactating women and cows may develop immediate-type hypersensitivity to their own milk (14, 15). It is conceivable, therefore, that, very infrequently, babies may become allergic to their mothers' milk, just as a few unfortunate women have been shown to have become highly allergic to seminal plasma from their husbands (16).

In the sections that follow, we shall describe briefly an unexpected experimental finding in rats and its preliminary analysis (17), which first alerted us to the possibility that birth does not necessarily preclude further transmission of viable leukocytes from maternal to infant tissues and that this may present an immunologic hazard in certain genetic contexts. It appears that the alveolar epithelium of the lactating unit transmits leukocytes much more readily than does the trophoblast.

Skin Lesions Associated with Suckling in Infant Rats

In the course of a study on the influence of allogeneic or interstrain pregnancies on maternal reactivity to paternal strain tissue transplantation antigens in rats, it was noted that a small percentage of (FI X DA)F_1 hybrid infants, whose Fischer (FI)-strain mothers had received skin allografts from one of their babies on the fifth day postpartum, developed spreading exfoliative lesions of their perianal and lower abdominal skin 7 to 10 days later (17). None of the affected individuals looked sick nor was their growth retarded, and the lesions resolved within about a week of onset, although the fur retained a sparse, ruffled appearance for much longer. Neonates born of untreated mothers never displayed these lesions.

Skin lesions of this type are the familiar harbingers of graft-versus-host disease in infant mice and rats inoculated soon after birth with immunocompetent cells from an unrelated adult donor (2). Thus, one possible explanation of the disease is that it was caused by the natural transmission, through the milk, of specifically immune lymphocytes from the postpartum sensitized mothers to their DA-antigen-bearing F_1 hybrid offspring and the mounting of graft-versus-host reactions by these cells against the host's lymphohematopoietic tissue systems and skin. Recovery of the affected subjects might have resulted from transfer of insufficient numbers of putative attacking cells to cause the fatal form of the disease.

The observations now to be presented were obtained from experiments designed to test this rather unlikely, although attractive, hypothesis.

Timing of the maternal exposure to the offspring's alien alloantigens seemed to be important, since a higher incidence of the disease resulted if the mothers were grafted with DA skin 7 to 10 days rather than 5 days postpartum. The fact that the disease failed to develop among the progeny of females grafted 21 days postpartum is scarcely surprising, since suckling has almost terminated by then (*see* Table 1).

DA strain infants transferred to FI mothers, which after giving birth to (FI X DA)F_1 litters were grafted with

Table 1. Influence of Postpartum Sensitization of Fischer (FI) Female Rats Against the Alien Tissue Antigens of Their Nursing Infants on Development of Skin Lesions in the Latter

Experiment	Sensitizing Skin Allograft	Day of Grafting Postpartum	Type and Percentage of Infants at Risk Developing Skin Lesions*	
			Natural (FI X DA)F_1	Foster-Nursed DA
1	5-Day-old F_1 donor	+ 5	18 (40)	0 (10)
2	10-Day-old F_1 donor	+ 10	68 (40)	100 (10)
3	10-Day-old DA donor	+ 10	20 (5)	83 (6)
4	21-Day-old F_1 donor	+ 21	0 (50)	...
5	Adult DA donor	+ 7	96 (23)	100 (5)

* The figures in parentheses indicate the actual numbers of infants studied.

F_1 hybrid or DA skin, also developed the disease. But similar DA infants foster-nursed by normal ungrafted FI mothers that were nursing their own (FI X DA)F_1 hybrid progeny failed to develop skin lesions. When FI mothers whose (FI X DA)F_1 infants had skin lesions as a consequence of grafting were subsequently remated to DA males and regrafted with DA antigen-bearing skin 10 days after the birth of their second litters, the incidence and severity of the lesions were significantly greater than in the case of the first litters, lending support to the thesis that the disease under study was indeed immunologically based (Table 2, experiment 4).

Evidence was sought about the capacity of viable lymphoid cells, introduced directly into the gastrointestinal tract of allogeneic neonatal hosts, to instigate the disease. A panel of (FI X DA)F_1 hybrid infants born of normal FI mothers were fed daily with the aid of a cannula from the tenth day postpartum during a 5-day period with aliquots of 10×10^6 viable lymphoid cells dispensed in 0.2 ml of Hanks' solution from adult FI donors that had been sensitized against DA tissue antigens. More than 50% of the subjects developed skin lesions. Feeding similar animals with serum from the immunized donors was ineffective.

If there was a causal relation between sensitization of lactating mothers against alien histocompatibility antigens of their sucklings and the development of skin lesions in the latter, then sensitization of FI rats nursing 10-day-old (FI X DA)F_1 infants with lymph node or spleen cells from hybrid donors should have an effect similar to that of grafting them with skin of the same genetic make-up. Contrary to expectation, intraperitoneal inoculation of the mothers with 40×10^6 cells proved to be completely ineffective, raising the question whether histocompatibility antigens were involved (Table 2, experiments 2 and 3).

Two additional observations that further jeopardized the initial assumption that transplantation antigens had anything to do with the phenomenon were that (a) the disease developed in 42% of the (FI X DA)F_1 progeny of FI mothers that received skin grafts from syngeneic (that is, FI) donors 10 days postpartum (Table 3, experiment 1); and (b) grafting similar mothers with skin from unrelated, third party, BN strain donors also resulted in the development of a fairly high incidence of skin lesions among the (FI X DA)F_1 progeny at risk, as well as among foster-nursed BN strain infants (Table 3, experiment 2).

The theory that transplantation antigens have anything at all to do with the phenomenon under study was utterly refuted when it was found that particularly extensive and striking lesions developed in the skins of a high proportion of DA sucklings whose DA mothers had been grafted with skin from 10-day-old or adult DA donors on the tenth day postpartum (Table 3, experiments 3 and 4). If the disease is immunologically based, the only antigen(s) that could possibly be involved is an autoantigen associated with some component of skin. Preliminary support for this view was forthcoming from the observation that sham-grafting, that is, the mere preparation of graft beds, in the integuments of DA rats nursing DA sucklings also resulted in the development of definite, though mild, skin lesions in the latter (Table 3, experiment 5). Traumatization of the skin and the ensuing natural repair process may have resulted in the release of whatever autoantigen was involved. Numerous reports in the literature suggest that in both man and animals skin lesions develop naturally or can be caused to develop on an autoimmune basis (18, 19), and we are now testing the premise that this is the basis of our rat skin lesions.

The Neglected Cellular Component of Milk

It is a long-established, though poorly known, fact that normal colostrum and milk are essentially suspensions of viable cells in highly nutritive medium (17, 20). Apart from a small proportion of alveolar and ductal epithelial

Table 2. Influence of Postpartum Sensitization of Fischer (FI) Females Against the Alien Tissue Antigens of Their Nursing (FI X DA)F_1 Hybrid Progeny on Development of Skin Lesions in the Latter

Experiment	Antigenic Stimulus	Day of Sensitization Postpartum	Infants at Risk Developing Skin Lesions*
			%
1	Skin allograft from adult F_1 donor	+ 10	44 (25)
2	40×10^6 Lymph node cells intraperitoneally from adult F_1 donor	+ 10	0 (17)
3	40×10^6 Spleen cells from 10-day-old F_1 donor	+ 10	14† (35)
4	Skin allograft from 10-day-old F_1 donor (second set experiment)	+ 10	91 (33)

* The figures in parentheses indicate the actual number of infants studied.
† Very trivial skin lesions.

Table 3. The Specificity of the Antigenic Stimulus That, Administered to Lactating Rats 10 Days Postpartum, Causes the Development of Skin Lesions in Their Sucklings

Experiment	Strain of Mother	Antigenic Stimulus (Skin Graft)	Natural Infants at Risk*		Foster-Nursed Infants at Risk	
			Type	With Lesions	Type	With Lesions
				%		%
1	FI†	FI isograft, 10-day-old donor	(FI X DA)F_1	42 (26)	DA FI	67 (6) 20 (5)
2	FI	BN allograft, 10-day-old donor	(FI X DA)F_1	43 (21)	BN	67 (3)
3	DA	DA isograft, 10-day-old donor	DA	93 (15)	(FI X DA)F_1	6 (16)
4	DA	DA isograft, adult donor	DA	50 (36)	—	—
5	DA	Sham-grafted only	DA	56 (43)	—	—

* The figures in parentheses indicate the actual number of infants studied.
† FI = Fischer.

cells in various stages of disintegration from the mammary gland, the other cellular elements are almost entirely of hematogenous origin (Figure 1). The latter conclusion is consonant with the familiar heavy infiltration of both the interstitial connective tissue and the hypertrophied ductal and alveolar epithelium of the breast with mononuclear cells during lactation. This cellular infiltration is maximal shortly after parturition when colostrum is being secreted, declining thereafter as milk becomes the product. Aside from epithelial elements, milk-born cells include abundant lipid droplet-laden macrophages sometimes referred to as colostrum corpuscles, which can attach to glass, show amoeboid movement and phagocytic activity, polymorphonuclear neutrophils, lymphocytes of various sizes and occasionally eosinophils.

Although milk cell counts vary within wide limits over short time intervals in samples from the same individual, in samples from different individuals of the same species, and between members of different species, they are usually not greatly inferior to the leukocyte count in peripheral blood. Moreover, the differential counts are of the same orders of magnitude as those of peripheral blood. Recent work has shown that human milk contains T and B lymphocytes in approximately equal proportions, and that on the basis of in-vitro tests including mixed leukocyte culture reactions done in our laboratory, their functional capacity is not inferior to their counterparts obtained from peripheral blood. Colostral mononuclear cells are responsive to mitogens in vitro and have also been shown to express in vitro correlates of some of the delayed hypersensitivities that are part of the immunologic experience of the donor. According to Murillo (21) colostral lymphocytes can synthesize IgA and β1C/β1A, but not IgM or IgG.

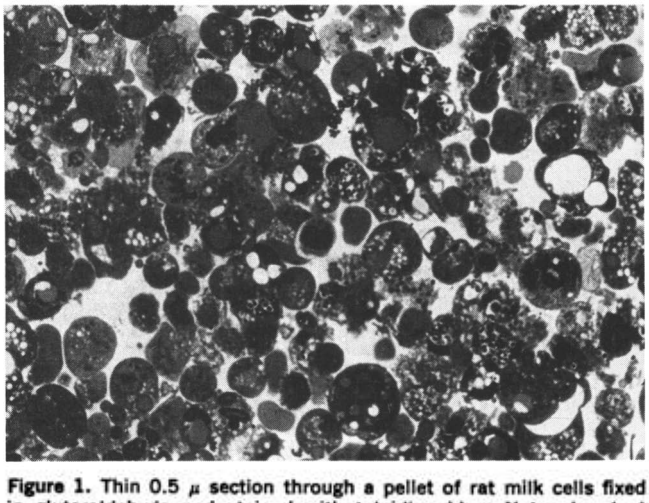

Figure 1. Thin 0.5 μ section through a pellet of rat milk cells fixed in glutaraldehyde and stained with toluidine blue. Note abundant lipid-laden macrophages, neutrophils, and a few lymphocytes, as well as anucleate, lipid-containing milk corpuscles. (Magnification, × 1100.)

Opinions have varied concerning the possible significance of these milk-borne cells. Early views that their presence reflected mastitis or that they fulfill a nutritive role are difficult to sustain. That they protect the ductal and glandular components of the breast against invading microorganisms by phagocytosis, secretion of IgA antibodies, and possibly by release of nonspecific antimicrobial agents is more plausible.

Suckling-Induced, Altered Reactivity to Skin Allografts and Runt Disease in Rats

Our work on the previously described lactation-associated skin lesions, in conjunction with the information that milk represents a potential source of viable donor lymphocytes for a suckling rat, encouraged us to apply some simple procedures of transplantation immunology, in conjunction with foster-nursing, to determine whether these cells can possibly gain access to the tissues of sucklings (22).

Four panels of newborn FI rats born of normal mothers were treated as follows: [1] raised by their normal FI mothers to provide controls; [2] transferred immediately after birth to Lewis (LE) strain foster mothers before they had received any milk from their own FI mothers; [3] allowed to nurse on their own FI mothers for the first 24 hours of life and then transferred to LE foster mothers; and [4] transferred immediately after birth to (FI X LE)F_1 hybrid foster mothers.

When they were 21 days old, the animals in all four groups were challenged with LE strain skin allografts to

Table 4. Influence of Foster-Nursing Newborn Fischer (FI) Rats on FI, Lewis (LE), or (FI X LE)F_1 Hybrid Mothers on Their Subsequent Reactivity to LE Test Skin Allografts*

Experiment	Nursing Mother	Infants		Test Skin Allografts						Mortality Between 5 to 90 Days
		Age When Transferred to Foster Mother	Grafted	Day 8	Day 10	Day 12	Day 14	>Day 16	MST ± SD†	
			no.	←		*no. (%)*		→	*days*	*%*
A	FI	Not transferred	37	37 (100)	17 (46)	2 (15)	0	0	9.9 ± 1.10	0
B	LE	0	30	25 (83)	21 (70)	12 (40)	5 (17)	3 (10)	11.3 ± 1.23	38
C	LE	24 h	15	10 (67)	3 (20)	1 (7)	1 (7)	0	8.4 ± 1.25	12
D	(FI X LE)F_1	0	26	20 (77)	15 (58)	8 (31)	2 (8)	2 (8)	11.0 ± 1.2	4

* All hosts grafted with LE skin when 21 days old.
† Median survival time ± standard deviation.

compare the reactivity of the recipients of allogeneic milk cells with that of recipients of syngeneic milk cells.

As the findings summarized in Table 4 show, both the distribution of actual graft survival times and the median survival times differed significantly in the first three groups. Grafts on the control animals had a median survival time of 9.9 ± 1.10 days and none lived beyond 13 days, whereas the median survival time of grafts on the exclusively LE strain milk-fed rats was significantly longer (11.3 ± 1.23 days), and a few of the grafts survived well beyond 16 days. This suggested that a feeble degree of tolerance or unresponsiveness of the alloantigens concerned had been induced. One of the reasons for selecting the present strain combination for these experiments was the high degree of tolerance-responsiveness that neonatal subjects of the one strain have to cellular inocula from an adult donor of the other strain. For example, the intravenous injection of as few as 250 000 LE lymphomyeloid cells into neonatal FI hosts induces some degree of tolerance of subsequent LE skin grafts in a high proportion of the subjects (23).

None of the group C animals, which had received 24 hours' sustenance by their own FI mothers before transfer to LE foster mothers, gave any indication of being tolerant. Indeed, they rejected their grafts more rapidly than the controls (median survival time, 8.4 ± 1.25 days versus 9.9 ± 1.10 days). We attribute their immune status to the subsequent activity of the population of mature syngeneic immunocompetent cells that they acquired initially through their mothers' milk. In effect, they had been adoptively immunized!

Strong corroborative evidence of the natural transfer of viable lymphocytes through milk was forthcoming from the careful long-term follow-up of the animals. Whereas there was no mortality beyond the first 5 days postpartum in the control series, 38% of group B animals died, the majority after developing skin lesions and what seemed to be graft-versus-host disease. Few of the group C animals died, presumably as a consequence of the protection given them by the reactivity of the syngeneic adult lymphocytes that they had acquired initially from their natural FI mothers against the potentially harmful cells. In addition to the results of postmortem examinations on nearly all the young rats that died, the observation that FI rats nursed from birth onwards by (FI X LE)F_1 foster mothers (group D) suffered negligible mortality is in complete accord with the diagnosis of runt disease in the group B animals, since lymphocytes from F_1 hybrids are genetically incapable of reacting against infant hosts of either parental strain. When subsequently challenged with skin allografts from LE donors, some of the group D animals also manifested impaired reactivity.

Studies with Other Donor/Host Rat Strain Combinations

Essentially similar evidence of milk-cell induced tolerance and runt disease was obtained in experiments in which LE strain neonates were foster-nursed from delivery onwards by FI females. However, as might have been anticipated, when these experiments were repeated with an Ag-B locus incompatible strain combination by foster-nursing FI (Ag-B^1) neonates on DA (Ag-B^4) females and vice versa, only a very equivocal degree of hyporeactivity to DA skin allografts resulted. This was probably because of the advanced state of responsiveness of the young at birth to the "strong" antigens involved, resulting in a high cell dosage requirement for tolerogenesis.

Nevertheless, 48% of FI infants that had been nursed exclusively by DA foster mothers succumbed to runt disease. Again, some measure of protection was afforded by allowing newborn FI rats to suckle on their own mothers for the first 24 hours of their life, the mortality being reduced to 28%.

The apparent capacity of mothers of at least some species to "donate" lymphocytes naturally to their young through the milk has two implications for experimental immunologists, which are under current investigation in our laboratory.

1. Since, in the progeny of intra-strain matings, suckling seems to result in equipping an immunologically immature host with a variable number of syngeneic mature immunocytes, it may contribute, in some donor/host strain combinations, to refractoriness of the neonates to tolerance-induction by intravenous inoculation of allogeneic cells (23). It may also explain the variability in results frequently obtained with more favorable strain combinations. If this reasoning is correct, foster nursing homozygous strain infants from birth onwards by appropriate F_1 hybrid females may facilitate tolerogenesis with regard to transplantation antigens. Supportive evidence for this premise has been obtained from experiments involving the induc-

tion of tolerance of FI tissue antigens in DA strain host rats by neonatal intravenous inoculation of (FI X DA)F_1 hybrid bone marrow cells. With this combination an inoculum of 10 X 10^6 cells induces a high degree of tolerance of FI strain skin allografts in less than 50% of DA rats nursed by DA mothers. However, a similar inoculum induces a high degree of tolerance in 85% of DA mothers foster-nursed from birth onward by (FI X DA)F_1 hybrid mothers. This finding suggests an explanation for reports that some tumor allografts that normally fail to thrive after inoculation into "resistant" strains of mice will sometimes flourish in hosts of the resistant strain if the latter have been foster-nursed on mothers of a susceptible strain (24).

2. Milk may be a neglected source of T lymphocytes in neonatally thymectomized or congenitally athymic "nude" mice (25). Experiments are currently in progress in which nude mice are either allowed to suckle on their own BALB/c/(nu/−) mothers or are transferred to unrelated C57BL/6 foster mothers. So far, it has been noted that all the latter animals have developed a conspicuous erythrodermia within 10 to 12 days and succumbed to a wasting syndrome a few days later, whereas the former animals have developed normally and have survived to adulthood.

Discussion

Consonant with the circumstantial evidence presented that, in the rat, transmission of lymphocytes can occur naturally from mothers or foster-mothers to sucklings through the milk are reports that indicate that neither the pH nor the enzyme contents of the infant rat's stomach are likely to be inimical to ingested cells (26, 27). Furthermore, the high buffering capacity of milk may be important in protecting its cell moiety, even in older animals.

Also consistent with the apparent ability of milk-borne lymphocytes to enter host tissues from the gastrointestinal tract is the demonstration that, after the inoculation of allogeneic lymphocyte suspensions into the uterine lumens of rats and mice, these cells can traverse the intact endometrial epithelium and, depending on the genetic relationship between donor and hosts, sensitize the latter or instigate severe local graft-versus-host reactions in the uterus (28). It is also pertinent to mention that if newborn rabbits are "fed" labeled, viable, bovine leukocytes, these cells are subsequently able to be seen beneath the epithelium of the mucous membranes of the esophagus and stomach (29).

The results of several clinical or clinically oriented studies are beginning to mount a cogent argument that milk-dispensed cells of maternal origin fulfill an important immunologically protective role in the human breast-fed infant, while lacking in the formula-fed infant. Mohr (30) tested the cutaneous reactivity to tuberculin of children who were breast-fed either by tuberculin-negative or by tuberculin-positive mothers. The results suggested acquisition of a transient specific cellular immunity through nursing. Whereas breast-fed children of tuberculin-negative mothers were always tuberculin negative, breast-fed offspring of tuberculin positive mothers reacted positively if less than 3 years old, suggesting transfer of delayed hypersensitivity through breast milk.

A significant number of human infants succumb to necrotizing enterocolitis, a disease that is characterized by exfoliation of the intestinal mucosa, subsequent perforation, and peritonitis (31). The disease is primarily seen in premature, low birth-weight infants who have suffered some severe perinatal stress. Aside from prematurity, predisposing factors include transient asphyxia, disturbance of the splanchnic circulation, and infection. Three conditions apparently have to be met for a susceptible infant to develop the disease: formula feeding, injury to the intestinal mucosa, and bacterial infection. This disorder is rarely, if ever, seen in countries where the use of formula feeding is rare and where high-risk patients are fed fresh breast milk. Barlow and her associates (32) have shown that subjection of newborn rats to transient daily hypoxia produces a faithful model of this disease. All succumbed to it if formula-fed, but not if they were breast-fed. Protection was shown to be dependent on the presence of viable macrophages in the milk. Protection was also afforded by viable macrophages from blood or peritoneal exudates, and milk lost its capacity to protect if it was treated to kill the cells.

In man, the infant acquires its maternal immunologic endowment of humoral antibodies almost exclusively across the placenta before birth, and only IgG antibody molecules are transmitted by this organ (11). Although antibodies are present in both colostrum and milk, essentially none of them are absorbed from the infant's gut. For example, in no case have Rh antibodies been detected in serums of infants fed with milk or serum containing high titers of them. Likewise, no significant absorption of ABO isoagglutinins occurred from the gut. As this seemingly paradoxical situation became well substantiated in the later 1950s, the advocates of breast feeding seemed to have lost considerable ground.

In a review of 1956, Vahlquist (33) asserted that ". . . available data refute the concept that human milk is of any appreciable importance as a source of protective antibodies for the child." The conclusion seems to be that if maternal antibodies fail to enter the infant's blood stream, they can do it no good. Here the situation remained for a few years until the important discovery was made that secretory IgA is the predominant immunoglobulin in milk and that its unique biological properties enable it to give significant protection against enteric microorganisms, probably by preventing their attachment to epithelial cells of the mucous membrane. Outbreaks of *Escherichia coli* enteritis are controllable by breast milk. Goldblum and co-workers (34) have recently found that deliberate colonization of the gastrointestinal tract of "volunteer" pregnant women with defined strains of *E. coli* to which they had no previous antibodies may result in IgA synthesis by B lymphocytes in their Peyer's patches. These investigators believe that some of these cells may enter the breast from the blood stream on a selective basis and actually be secreted in the milk and so transmitted to the suckling.

For the past 2 decades *E. coli* has been one of the most common causes of meningitis in newborn infants in the United States. In Sweden, where breast-feeding is still

common, the incidence of neonatal *E. coli* meningitis is much lower in breast-fed than in formula-fed infants. One recent study (35) suggests that gastrointestinal colonization by *E. coli* is considerably diminished in breast-fed as compared with bottle-fed babies and that anticapsular antibodies in the breast milk probably prevent adherence and penetration of the mucosal surface of the gastrointestinal tract by the organism.

Conclusions

Reconsideration of the immunologic benefits and hazards of milk in the light of various recent findings certainly affords a sound basis for the old notion that the mother or a "wet" nurse could influence an infant's health, both adversely as well as favorably, through the milk. It also places us in a strong position to defend the thesis that, on the basis of millions of years' experience as she evolved the mammals, Nature has developed for each species a far better diet for the newborn than have the manufacturers of formula diets.

Perhaps the time has come for *Homo sapiens* to take a new look at the biological significance of the female breast and to de-emphasize his current tendency to regard it solely as a symbol of femininity.

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