Fifth Nutricia Symposium

NUTRITION AND METABOLISM OF THE FETUS AND INFANT

Rotterdam 11–13 October 1978

EDITOR:

H.K.A. VISSER M.D.

1979

MARTINUS NIJHOFF PUBLISHERS

THE HAGUE BOSTON / LONDON

IMMUNE FACTORS IN HUMAN MILK

B. CARLSSON*, J.R. CRUZ**, B. GARCIA**, L.Å. HANSON*, and J.J. URRUTIA**

During recent years much information has accrued concerning factors in human milk which may help the breast-fed infant in its defense against infections. Some of these factors, especially those with specific immune functions, will be briefly reviewed.

LEUCOCYTES IN HUMAN MILK

Large numbers of leucocytes are present in human milk during late pregnancy and the first few weeks of lactation. These cells are primarily macrophages, in some samples up to 80% of the cells. The macrophages contain IgA antibodies in the cytoplasm (1) presumably originating from the mammary gland and therefore supposedly representing the local synthesis of such antibodies. No specific function has been demonstrated for the milk macrophages, but it has been suggested that they might protect the mammary gland against infection. It cannot be excluded that the macrophages or their content of IgA can play a role in the defense against microorganisms in the infant's gut.

T-lymphocytes are also recognized in the milk (2, 3). According to PARMELY et al. (4) and OGRA and OGRA (5) the T-lymphocytes in milk show a different reactivity against mitogens and antigens than do T-lymphocytes in peripheral blood, suggesting that they represent different populations. It is possible that the milk T-lymphocytes may be part of a cell-mediated local immune response and that they provide some form of immunity to the neonate; it has been demonstrated that tuberculin positivity can be transferred to the baby by breast-feeding (6, 7).

Human milk also contains B-lymphocytes which primarily produce

* Department of Immunology, Institute of Medical Microbiology and Department of Pediatrics, University of Göteborg, Göteborg, Sweden.
** Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala.

H.K.A. Visser (ed.), Nutrition and Metabolism of the Fetus and Infant, 263-271.

IgA antibodies (8). It is still unknown whether these cells represent the lymphoid cell population of the mammary gland or whether they reach the milk through the circulation. Observations in women with a Salmonella typhimurium infection during lactation have indicated that intestinal exposure may result in antibodies appearing in the milk (9). Experimental studies in rabbits (10) and in swine (11) have also suggested that intestinal exposure may be the initiator of milk antibodies. Intestinal colonization of women in late pregnancy with E.coli resulted in the appearance of leucocytes in milk a few days later forming plaques which could be recognized due to presence of secretory IgA antibodies against the O antigen of the colonizing strain (12). Experimental studies by Roux and coworkers (13) have strongly supported the notion that there is a homing of lymphoid cells from the gut after intestinal antigenic exposure, moving via the lymph and circulation to the mammary gland. The initiation of this homing seems to take place in the Peyer's patches in the gut and it is presumably directed by Ia-antigens on the epithelial cells of the mammary gland. Appearance of such Ia-antigens in the gland is under hormonal influence (14).

The homing to the mammary gland of specific IgA-producing cells after they have been exposed to antigens from the intestinal content, in the Peyer's patches, is probably part of a generalized homing process of IgA producing cells to the mucous membranes of exocrine glands (fig. 1). As a result the breast-fed baby is provided in the maternal milk with antibodies of the secretory type against a number of intestinal microorganisms to which the baby may be exposed after birth.

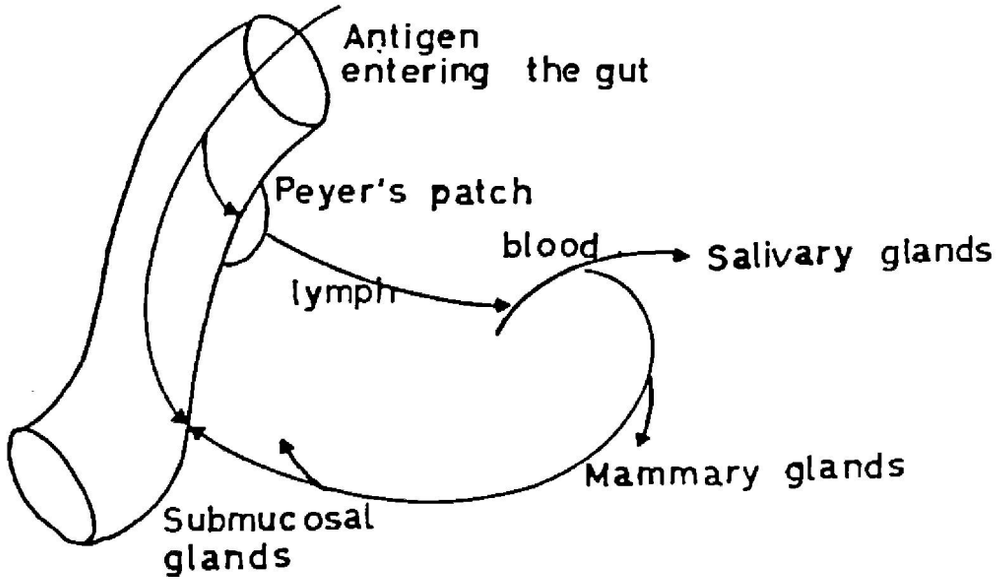

Fig. 1. Homing of IgA producing cells to exocrine glands after stimulation in the gut

THE SECRETORY IgA ANTIBODIES OF HUMAN MILK

As a consequence of the homing mechanism, human milk contains large amounts of secretory IgA (SIgA). The SIgA is composed of two IgA monomers stabilized by two polypeptide chains, the J-chain and the secretory component. The resulting antibody molecule is resistant against enzymic degradation and pH-changes (15, 16).

Using a new technique for specific quantitation of SIgA (17) it was found that the initial values in colostrum were as high as 18.5 g/l, rapidly diminishing to slightly less than 1 g/l. It was noted that, simultaneously with the decrease, there was an increase in volume which made the total output of secretory IgA rather constant, as has been indicated in earlier studies (18, 19). We found that the concentration of SIgA is not significantly different in women from privileged and under-privileged groups in Ethiopia (20) and Guatemala (table 1) (21). This agrees with earlier

Table 1. *Levels of secretory IgA (g/l) and specific IgA antibodies (% of reference) in milk samples from rural and urban Guatemalan women.*

		Socio-economic group		
		Rural (n = 10)	Urban poor (n = 10)	Urban elite (n = 10)
SIgA/l		0.626*	0.836	1.024
SIgA/24 h		0.309	0.357	0.456
anti E.coli	a	13.9	17.3	37.1
	b	6.9	6.7	20.2
anti Salmonella A	a	8.5	10.5	12.4
	b	4.2	4.1	6.7
anti Salmonella B	a	10.0	11.2	12.6
	b	1.7	3.8	6.6
anti Salmonella C_1	a	12.0	21.3	31.5
	b	5.6	9.8	18.5
anti Salmonella C_2	a	9.2	12.9	11.8
	b	4.3	4.5	6.4
anti Salmonella D	a	8.1	9.9	12.8
	b	4.0	3.7	6.9
anti Salmonella E	a	15.9	21.6	26.8
	b	7.9	8.1	15.8
anti cow's milk	a	10.3	11.2	36.7
	b	5.5	5.1	19.6

* mean

a: antibody level/unit of volume

b: antibody level corrected for 24 h milk volume

studies on milk from Pakistani women where the levels of E.coli antibodies were the same as in healthy Swedish women although the Pakistani women were severely under-nourished (22). Preliminary observations in the Pakistani women suggested that their milk volumes were exceedingly low, however, diminishing their total output of secretory IgA. They were under heavy stress, with their children admitted to hospital for severe disease, so further studies are required to define whether or not lactation failure will result in decreased volumes but not lowered concentrations of milk components.

Human milk contains antibodies against a number of O and K antigens of E.coli. Such antigens are virulence factors of E.coli, especially the K1 antigen found on about 80% of strains causing neonatal sepsis and/or meningitis (23). This may be one reason why such infections are more common in infants which have had little breast feeding than in matched controls who get milk SIgA anti-K1 (24). In milk from exposed mothers high levels of antibodies of enteropathogenic E.coli are found (20). They also have SIgA antibodies against Shigella (25) and against E.coli and Vibrio cholerae enterotoxin (26). SIgA antibodies against Salmonella antigens are also recognized in milk (21).

In investigations of milk samples from urban elite, urban poor and rural women in Guatemala it was found that the antibody levels against E.coli enterotoxin, against E.coli O antigens and certain Salmonella O antigens were often higher in the urban elite women (table 1). The explanation for this is not yet obvious, but it may be related to the fact that the urban elite women use processed food which is not available to the poor women. The differences in titres can not be explained by differences in concentrations of SIgA or differences in volumes of milk which are similar in the three groups (21).

The milk also contains antibodies against viruses such as rota-virus (27) and polio-virus (28). The mode of function of the milk SIgA antibodies to various microbial antigens may simply be binding and neutralizing viruses and binding bacteria blocking their attachment to the epithelium of the gut preventing further steps of the infection process. In fact such an anti-adherence activity of milk antibodies to E.coli has been demonstrated (20, 29, 30).

INTESTINAL EXPOSURE AND VACCINATION INFLUENCING THE MAMMARY GLAND IMMUNE RESPONSE

Exposure of mucous membranes is known to induce a local secretory IgA production (16). From the above-mentioned observations of the homing process it is, however, obvious that exposure of the intestinal mucosa involving Peyer's patches will result in a transfer of the secretory IgA response also to distant exocrine glands like the mammary gland. It can therefore be expected that repeated exposure of the intestinal mucosa should result in a continuous production of milk IgA antibodies. Such repeated exposures may explain the fact that the milk contains antibodies against such a large number of different antigens from enterobacteria. It would otherwise be difficult to understand how the reportedly short lived SIgA response could be present against such an array of antigens. Still it seems unlikely that the mothers have been exposed within months to so many various antigens.

In a recent study we noticed that it was possible to boost an already existent local immune response by parenteral vaccination. Thus, Pakistani women who had been naturally exposed to V.cholerae had antibodies in the saliva as well as in the milk against the lipopolysaccharide of V.cholerae. A parenteral vaccination not only increased the serum antibodies, as expected, but also the milk and saliva antibodies with a response that was dominated by SIgA (31). In contrast, Swedish women who had no local response in the form of SIgA antibodies against the V.cholerae in the saliva or milk did not show any booster effect on SIgA of parenteral vaccination (21). It therefore seems possible to boost the milk SIgA antibody titres by parenteral vaccination if a local response already exists. In the absence of such a response the parenteral vaccination will have no effect on the SIgA levels.

More recently, it has been noted that in lactating women who already have secretory IgA antibodies in their milk against polio-virus, peroral vaccination with live polio-virus lowers these titres. This was especially evident if cholera vaccination was performed simultaneously, but peroral administration of polio vaccine alone was found to significantly diminish the titres in 6 out of 10 women as well (21). The interpretation of the finding is not yet apparent, but may be related to a local consumption of the virus SIgA antibodies, or possible induction of tolerance in the gut. Further studies will have to investigate this possibility and also determine whether or not it is possible to boost the milk antibody

response, instead by using an inactivated polio vaccine given parenterally. Anyhow, these studies indicate that it is possible to modulate the local antibody response of the mammary gland occurring as SIgA antibodies in the milk.

FOOD ANTIBODIES IN HUMAN MILK

As a consequence of the assumption that the Peyer's patches sample antigens from the intestinal content exposing lymphoid cells that home to exocrine glands, it can be expected that milk contains antibodies against food proteins also and not only against antigens from more or less invasive microorganisms. Antibodies against cow's milk have been demonstrated in human milk [32, 33]. Significantly lower levels of SIgA milk antibodies to cow's milk were found in groups of under-privileged mothers from Guatemala, where cow's milk is not included in the diet, than in an urban elite group (table 1).

The role of food antibodies in the gut is not clear, but since Walker et al. [34] found that antibodies can diminish the uptake of native undegraded food proteins in the gut and increase the amount of degraded material absorbed, it is possible that the cow's milk antibodies in the human milk play a similar role in the infant. This was analysed in a comparative study of infants which were directly transferred from breast feeding to artificial feeds and infants which were kept on mixed feeding, that is breast feeding and artificial feeds, for more than 3 weeks. It was seen that the infants on mixed feeding had significantly lower titres of serum antibodies against cow's milk proteins than the others [32]. This suggests that the milk SIgA antibodies may diminish the systemic exposure to potentially allergenic food components. This may be of relevance till the time when the infants own SIgA production in the gut can take over such an activity. On this basis it may be suggested that breast feeding should be prolonged, and a period of mixed feeding, including breast milk, should be inserted before weaning of infants with atopic heredity.

NON-SPECIFIC HOST-DEFENSE FACTORS IN HUMAN MILK

The difference in pH and consistency of the stool of the breast fed baby compared to that of the artificially fed is striking. The reason for this difference is multifactorial. Presumably the SIgA antibodies may add to

the reduction in bacterial numbers and prevention of the attachment of the bacteria to the epithelium of the intestine. Many other components may, however, take part in this like lactoferrin which has been shown to be bacteriostatic by binding iron which is required as a growth factor for bacteria (35). Obviously, the bacteria produce chelating agents which bind iron in competition with lactoferrin. In the presence of antibodies the production and or release of these chelating agents is interfered with and as a result lactoferrin binds the iron and the bacteriostatic effect becomes more evident than with antibodies or lactoferrin alone (36). Other components such as lysozyme and bifidus factors may add to the conditions that seem to make lactobacilli thrive in contrast to E.coli which are found in lower numbers in breast-fed infants than in non-breast-fed (37).

Taken together, human milk contains many components which may with combined efforts diminish the number of potentially pathogenic microorganisms in the infants gut. With the lower numbers of such pathogens the chances for the SIgA antibodies to prevent attachment of the microorganisms to the epithelial membranes of the gut may be better (30). The efficiency of these various host-defense factors in human milk may be an important reason why repeated studies have indicated that breast-fed infants have less frequent infections, especially in the gastro-intestinal tract, than artificially fed (38, 39, 40, 41).

CONCLUSIONS

Human milk contains in early lactation considerable numbers of macrophages as well as T- and B-lymphocytes. The biological significance of these cells has not been settled.

The dominating immunoglobulin in milk is secretory IgA which presumably is locally produced in the mammary gland. The special structure of these SIgA antibodies make them resistant against proteolysis, so they can function in the gastrointestinal tract. It seems that this local antibody response is closely connected with exposure to antigens in the intestine. As a result the human milk contains antibodies of the secretory IgA type against a large number of antigens of various microorganisms appearing in the gut, such as E.coli, V.cholerae, Shigella, Salmonella and polio- and rota-virus. These antibodies may primarily function by binding the microorganisms, hindering them from attaching to and infecting via the mucous membrane of the gut.

Recent findings have indicated that it is possible to boost an already existent mammary gland antibody response by parenteral vaccination.

Human milk also contains antibodies against food proteins which may be of importance to prevent the development of food allergy in atopic infants. Through a prolonged period of mixed feeding with human milk and artificial feeds while the infant develops its own secretory IgA system, a diminished exposure to the potential allergens of the artificial feeds may result, decreasing the risk for atopic allergic reactions.

Human milk contains several non-specific host-defense factors including lactoferrin which may aid in lowering the number of potential pathogens in the infants' gut, aiding the secretory IgA antibodies in their role as preventors of contact between microorganisms and host tissue.

ACKNOWLEDGEMENTS

This work has been supported by SAREC, the Swedish Medical Research Council (No. 215), the Wenner-Gren and Arla Foundations and Ellen, Walter and Lennart Hesselman Foundation for Scientific Research. J.R. Cruz was receiving a WHO grant.

REFERENCES

1. Pittard, W.B., S.H. Polmar and A.A. Fanaroff. 1977) J. Reticuloend. Soc. 22:597.
2. Smith, C.W. and A.S. Goldman. (1968 Pediat. Res. 2:103.
3. Díaz-Jouanen, E.P. and R.C. Williams. 1974 Clin. Immunol. Immunopathol. 3:248.
4. Parmely, M.J., D.B. Reath, A.E. Beer and R.E. Billingham. 1977 Transpl. Proc. 9:1477.
5. Ogra, S.S. and P.L. Ogra. 1978) J. Pediat. 92:550.
6. Schlesinger, J.J. and H.D. Covelli. (1977) Lancet 2:529.
7. Mohr, J.A., R. Lee and W. Mabry. 1970) J. Surg. Onc. 2:163.
8. Murillo, G.J. and A.S. Goldman. 1970) Pediat. Res. 4:71.
9. Allardyce, R.A., D.J.C. Shearman, D.B.L. McClelland, K. Marwick, A.J. Simpson and R.B. Laidlaw. 1974) Brit. Med. J. 3:307.
10. Montgomery, P.C., B.R. Rosner and J. Cohn. (1974) Immun. Common. 3:143.
11. Bohl, E.H. and L.J. Saif. 1975 Infect. Immun. 11:23.
12. Goldblum, R.M., S. Ahlstedt, B. Carlsson, L.A. Hanson, U. Jodal, G. Lidin-Janson and A. Sohl-Åkerlund. (1975) Nature 257:797.
13. Roux, M.E., M. McWilliams, J.M. Phillips-Quagliata, P. Weisz-Carrington and M.E. Lamm. 1978) J. Exp. Med. 147:934.
14. Klareskog, L., U. Forsum and P.A. Peterson. In manuscript.
15. Tomasi, T.B. and J.M. Bienenstock. (1968) Adv. Immunol. 9:1.
16. Hanson, L.A and P. Brandtzaeg. (1979) In: Immunologic Disorders in Infants and Children, Stiehm, E.R. and V.A. Fulginiti eds., W.B. Saunders, Philadelphia, Pa., In press.

17. SOHL-ÅKERLUND, A., L.Å. HANSON, S. AHLSTEDT and B. CARLSSON. (1977) Scand. J. Immunol. 6:1275.
18. MCCLELLAND, D.B.L., J. MCGRATH and R.R. SAMSON. (1978) Acta Paediatr. Scand. suppl. 271:1.
19. SCHUBERT, J. and A. GRUNBERG. 1949) Schweiz. Med. Wochenschr. 79:1007.
20. HANSON, L.A., S. AHLSTEDT, B. CARLSSON, S.P. FÄLLSTROM, B. KAIJSER, B.S. LINDBLAD, A. SOHL- ÅKERLUND and C. SVANBORG- EDEN. (1978) Acta Paediatr. Scand. 67:577.
21. HANSON, L.Å., B. CARLSSON, J.R. CRUZ, B. GARCIA, J. HOLMGREN, S.R. KHAN, B.S. LINDBLAD, A.-M. SVENNERHOLM, B. SVENNERHOLM and J. URRUTIA. 1978) National Institute of Child Health and Human Development Conference on the Immunology of Breast Milk. In press.
22. CARLSSON, B., S. AHLSTEDT, L.Å. HANSON, G. LININ-JANSON, B.S. LINDBLAD and R. SULTANA. (1976) Acta Paediatr. Scand. 65:216.
23. ROBBINS, J.B., G.H. MCCRACKEN, E.C. GOTSCHLICH, F. ØRSKOV, I. ØRSKOV and L.A. HANSON. 1974) New Engl. J. Med. 290:1216.
24. WINBERG, J. and G. WESSNER. 1971) Lancet 1:1091.
25. CARLSSON, B., T. MEITERT, E. GARON, L. COGULESCU and L.Å. HANSON. In manuscript.
26. HOLMGREN, J., L.A. HANSON, B. CARLSSON, B.S. LINDBLAD and J. RAHIMTOOLA. (1976) Scand. J. Immunol. 5:867.
27. SIMHON, A. and L.J. MATA. (1977) Lancet 1:39.
28. HODES, H.L., R. BERGER, E. AINBENDER, M.M. HEVIZY, H.D. ZEPP and S. KOCHWA. (1964) J. Pediat. 65:1017.
29. SVANBORG- EDÉN, C. and A.-M. SVENNERHOLM. (1978) Infect. Immun. 22:790.
30. HANSON, L.A., S. AHLSTEDT, B. CARLSSON, B. KAIJSER, P. LARSSON, I. MATTSBY BALTZER, Å. SOHL-ÅKERLUND, C. SVANBORG-EDÉN and A.-M SVENNERHOLM. (1978) In: Secretory immunity and Infection, Mc Ghee J.R., J. Mestecky and J.L. Babb eds., Plenum Publishing Corporation, p. 165.
31. SVENNERHOLM, A.-M., J. HOLMGREN, L.Å. HANSON, B.S. LINDBLAD, F. QUERESHI and J. RAHIMTOOLA. (1977) Scand. J. Immunol 6:1345.
32. HANSON, L.A., S. AHLSTEDT, B. CARLSSON and S.P. FÄLLSTRÖM. (1977) Int. Arch. Allergy appl. Immunol. 54:457.
33. MCCLELLAND, D.B.L. and T.T. MCDONALD. (1976) Lancet 2:1251.
34. WALKER, W.A., M. WU, K.J. ISSELBACHER and K.J. BLOCH. (1975) J. Immunol 115:854.
35. BULLEN, J.J. (1976) In: Acute Diarrhea in Childhood (Ciba Foundation Symposium 42). Amsterdam. North-Holland. p. 149.
36. GRIFFITHS, E., J. HUMPHREYS, A. LEACH and L. SCANLON. (1978) Infect. Immun. 22:312.
37. HAENEL, H. (1970) Am. J. Clin. Nutr. 23:1433.
38. TASSOVATZ, B. and A. KOTSITCH. (1961) Ann. Pediat. 8:285.
39. GERRARD, J.W. (1974) Pediatrics 54:757.
40. HANSON, L.A and J. WINBERG. (1972) Arch. Dis. Childh. 47:845.
41. GOLDMAN, A.S. and C.W. SMITH. (1973) J. Pediat. 82:1082.