Old Herborn University Seminar Monograph 4:
Host-microflora interactions in the first years after birth.
Editors: Peter J. Heidt, Rial D. Rolfe, Volker Rusch, and Dirk van der Waaij.
Institute for Microbiology and Biochemistry, Herborn-Dill, Germany: 22-32 (2002).

BOVINE LACTOGENIC IMMUNITY: A CONCEPT WHOSE TIME HAS COME (?)

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SUMMARY

Since the pioneering studies of Paul Ehrlich, it has been widely accepted that breast-fed infants fare better than their non-breast-fed counterparts with regard to their resistance to infectious diseases. Indeed breast milk has many antimicrobial components, including antibodies, which could serve to protect the infant. Studies are reviewed which indicate that although the antimicrobial spectrum of human milk *in vitro* is quite diverse, not all pathogenic species are susceptible, susceptibility varies among strains of a species, and the potency of the antimicrobial activity varies from mother to mother. In addition, despite intensive efforts by national and international organisations, a significant proportion of infants are not breast-fed. This is of particular significance in lesser-developed countries where diarrhoeal disease is rampant. Studies with laboratory animal models, and a few studies with adult volunteers or in hospital nurseries, have shown that orally administered antibody of human or bovine origin can be markedly protective, if not therapeutic, against diarrhoeal disease caused by rotavirus and by enterotoxigenic *Escherichia coli*, and against necrotising enterocolitis in premature infants. Orally administered purified bovine colostral immunoglobulin, from newly parturient cows immunised with cholera enterotoxin, the cholera toxin related enterotoxin from *E. coli*, or outer membrane proteins of *Vibrio cholerae* protected infant rabbits against lethal direct intra-intestinal challenge with virulent *V. cholerae*. These and other experimental observations, published studies, and current analysis suggest that the concept of passive bovine lactogenic immunity, i.e., the oral administration of purified colostral or milk immunoglobulin from hyperimmunised cows, merits further controlled evaluation in field studies and could offer a means of protecting infants who are not breast-fed and of complementing and supplementing the immunity of infants.

Almost a century ago, *Paul Ehrlich* (1892) established unequivocally, in a series of brilliant experiments involving four or so mice apiece, that immunity - to abrin and ricin - was transmitted not by inheritance from immunised father mice but from immunised mother mice (Table 1). The latter immunity wanes with the age of the offspring. Furthermore, infant mice born of non-immune mice and nursed from mothers immunised against abrin or ricin were protected against toxin challenge, whereas infants born of immune mother mice and nursed by non-immune mice showed no protection

Table 1: Protection is transmitted by immune mothers but not by immune fathers[1]

Father: Mother:	Immune Normal	Normal Immune	Age at challenge (days)	Antigen (imm/chall)	Challenge (x lethal dose)
	5/6[2]	0/3	21-45	abrin/abrin	0.2-1.33
		0/3	21-45	ricin/ricin	4-10
		9/11	56-113[3]	abrin/abrin	0.25-4.00
		3/4	86-108[3]	ricin/ricin	1-2

[1]Data modified from *Ehrlich*, 1982
[2]Dead/Total
[3]After nursing period

(Table 2). "Wissen Sie ..... verstehen Sie," as Ehrlich would have said (*Marquardt*, 1951). [Coincidentally, Ehrlich studied abrin and ricin because he felt that these recently discovered toxic lectins were related to the bacterial toxins, diphtheria and tetanus toxins, which had also been recently described. *Ehrlich* (1891) was the first to show that abrin and ricin were immunologically different proteins].

Since their advent, the survival of mammals has depended upon the passive transfer of immunity from mother to offspring, whether transplacentally prior to birth, postnatally via breast milk, or both. This held true for humans as well until the domestication of animals made their milk available as a substitute source of nutrition. As early as 1900 B.C., Hammurabi's code regulated the practice of paid "wet nursing"; i.e., the nursing of another person's infant. Two centuries B.C., there began to appear evidence of feeding cups in graves of infants throughout Europe (*Lawrence*, 1989) and feeding horns (cow) from the twelfth century were found in the basement of St. Bartholomew's Hospital in London (*Walker-Smith*, 1975). In ancient Sparta, the wife of the king was obliged by law to nurse her oldest son. If he was nursed by a stranger, he lost his line of succession to the monarchy. It is said that Hippocrates wrote, "One's own milk is beneficial, other's harmful" (*Lawrence*, 1989).

During the Middle Ages, well-to-do English mothers did not nurse their infants. Although this was already recognised as a means of birth control, they preferred to have as many as 12-20 children rather than "spoil their figures and make them old before their time" (*Fildes*, 1986). In Eighteenth-Century-France around the time of the Revolution, breast-feeding was not customary and children were either given to wet nurses or fed artificially (*Lawrence*, 1989). Until the last several decades, women were urged to raise their children "scientifically" with a diet comprised of cod liver oil, orange juice, and artificial feeding (*Apple*, 1987; *Lawrence*, 1989).

Following the observations of Ehrlich on the importance in mice of the passive immunity provided by milk and with the emergence of the field of immunology, comparisons began to be made (as early as 1895 in Berlin) on the mortality rate differences between breast-fed and artificially fed infants (*Knodel*, 1977). The campaign to promote breast-feeding began. Since then, it has become increasingly evident in many studies world-wide (*Jelliffe* and *Jelliffe*, 1988) that breast-feeding the infant for at least 6 months (preferably

Table 2: Immunity is transmitted to infant mice from normal mothers by nursing immune mothers[1]

Foster mothers		Challenge	Challenge
Immune	Normal	antigen	(x lethal dose)
1/5[2]		abrin	1.25-40
0/6		ricin	2.25-40
	6/6	abrin	1.25-40

[1]Data modified from *Ehrlich*, 1892
[2]Dead//Total

for 1 year) until his immune system becomes fully operational is perhaps the one of the most important things a mother can give her child. This passive immunity, in the form of immunoglobulins, immuno-important cell populations, and non-specific antimicrobial agents, along with the nearly perfectly evolved nutrition, affords an infant a relatively protected state in which to grow relatively unimpeded by constant bouts with severe life-threatening diseases, particularly diarrhoeal diseases. In addition to the antimicrobial substances found in breast milk, the exposure of the infant to entero- and other pathogens, in diet and in environment, is reduced.

Although the vast majority of studies which have demonstrated that breast-feeding reduces infant morbidity and mortality have been flawed in one way or another - because of understandable lack of appropriate controls or other variables - the volume of the evidence in favour of breast-feeding is convincing (*Feachem* and *Koblinsky*, 1984; *Jason* et al., 1984; *Kovar* et al., 1984; *Mata*, 1978, 1986). Thus, it has become universally accepted that breast-fed babies fare better than formula-fed babies with regard to resistance to infectious diseases and especially to diarrhoeal diseases. Each year, diarrhoeal diseases affect over 150 million and kill more than 4 million children under the age of 5 in the lesser-developed countries of the world (*Cleason* and *Merson*, 1990; *Snyder* and *Merson*, 1982).

If we accept that breast-feeding is indeed beneficial in terms of protection against infectious diseases, in addition to the reduction of exposure to pathogens in the environment - in contaminated food and water - what are the protective mechanisms of breast-feeding? Table 3 lists many of an ever-increasing number of antimicrobial components, which have been observed in human milk. Although many have been shown to be active in *in vitro* tests, their potential clinical importance remains to be evaluated in experimental animal models or in human beings. Of the components listed, the immunoglobulins are the most likely to be of practical significance. They have been demonstrated to neutralise bacterial toxins, to inactivate viruses, to prevent bacterial adherence to host cells, and, in some instances, to have direct antibacterial effects - sometimes in combination with other factors such as lactoferrin, lysozyme, and perhaps complement components of the alternative pathway.

As summarised in Table 4, which includes studies from our own (*Boesman-Finkelstein* and *Finkelstein*, 1985; *Dolan* et al., 1986, 1989) as well as other laboratories, mothers' milk has a broad spectrum of antimicrobial activity which ranges from the upper

Table 3: Antimicrobial components of human milk*

Immunoglobulins
1. SIgA
2. Other Ig Classes
Bifidobacterium bifidus growth factor
Lactoferrin
Lysozyme
Lactoperoxidase
Alpha-2 macroglobulin
Alpha-1 antitrypsin
Ribonuclease
Lipid
1. Free unsaturated fatty acids and monoglycerides
2. Gangliosides (GM1)
3. Glycolipid receptor analogues
Carbohydrate
1. Oligosaccharide receptor analogues
2. Non-lactose carbohydrates
Cells
1. T and B lymphocytes
2. Neutrophils
3. Macrophages

*Table derived from *Goldman* et al. (1985); *Hanson* et al. (1988); *Lawrence* (1989); and *May* (1988).

respiratory through the enteric microflora. In our laboratory, the following potential pathogens have been shown to be inhibited by pooled human whey: Groups B and D streptococci (markedly bactericidal); coagulase-positive and coagulase-negative staphylococci (bacteriostatic); *Hemophilus influenzae* (markedly bactericidal); *E. coli* (enteric isolates were markedly inhibited, whereas systemic isolates were not); *Shigellae* (8/10 strains inhibited); *Salmonella typhimurium* (all inhibited by whey with variation in degree from strain to strain); blood isolates of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Serratia marcescens* (inhibition varied from strain to strain); and *Vibrio cholerae* (marked inhibition). *Campylobacter* strains, in our study were not markedly inhibited in whey under the micro-aerophilic culture conditions used but, interestingly, they were killed in commercial infant formula used as a control. On the other hand, *Ruiz-Palacios* et al. (1990) have just demonstrated an association between *Campylobacter* antibodies in human milk and prevention of diarrhoea caused by *Campylobacter*. This apparent contradiction indicates that caution should be exercised when extrapolating from results obtained *in vitro*.

It should be noted, however, that not all strains of a given species of pathogenic bacteria are equally susceptible to the antimicrobial activity of, in this case, pooled whey from Missouri mothers (*Dolan* et al., 1986, 1989). Furthermore, the potency of the antimicrobial effects of whey varies from individual to individual - probably in large measure dependent on the prior immunologic experience of each mother (*Boesman-Finkelstein* and *Finkelstein*, 1985).

Thus, acknowledging that the health of breast-fed babies will generally be better than those who are not, one might predict that some individual mothers' breast-feeding might not be as

Table 4: Microorganisms inhibited by human breast milk *in vitro* or by breast feeding

Bacteria	
Campylobacter	Klebsiellae
Chlamydia	Pasteurellae
Clostridium botulinum	Pneumococci
Clostridium difficile	Pseudomonas
Corynebacterium diphtheriae	Salmonellae
Escherichia coli	Serratia
enterotoxigenic	Shigellae
enteropathogenic	Staphylococci
enterohemoragic	Streptococci
enteroadherent	Vibrio cholerae
Hemophilus	
Viruses	
Coxsackie	Polio
Cytomegalovirus	Parainfluenza
Dengue	Respiratory syncytial
Herpes simplex	Rotavirus
Influenza	Rubella
Japanese B encephalitis	Semliki forest
Mumps	
Parasites	
Ascaris lumbricoides	Schistosoma mansoni
Cryptosporidium	Trichomonas vaginalis
Entamoeba histolytica	Trypanosoma rhodesiense
Giardia lamblia	
Fungi	
Candida albicans	

protective as breast-feeding by other individual mothers and that some pathogens or strains of pathogens might be more or less responsive than others.

In addition, despite the weight of evidence and the global emphasis by UNICEF and the World Health Organisation on the importance of breast-feeding, the vast majority of mothers, particularly in the lesser-developed countries of the Third World, are not breast-feeding and, if they are, not long enough. Indeed, UNICEF has recently acknowledged that its efforts to promote breast-feeding have been less than successful:

> "Breast-feeding appears to be on the decline in many developing nations as commercial pressures, the use of milk powder and feeding bottles in hospitals...., and the increased participation of women in the labour force, all conspire to make bottle feeding seem the attractive option. The continuation of this trend would be disastrous (*Grant*, 1990)."
>
> If the trend continues, what then can be done to protect the infants who are not breast-fed (and to complement and supplement the immunity of those who are)?

Among the antimicrobial components in milk listed previously (Table 3), the antibody-containing immunoglobulins head the list of potentially useful agents. *Peterson* and *Campbell* (1955) first conceived of the use of passive oral immunotherapy. Subsequently, *Hanson* (1961) described a secretion-

unique immunoglobulin, now known as secretory immunoglobulin A (SIgA) as a major protein in human milk. Beginning in 1958, a number of studies have been performed to evaluate the effects of orally administered antibodies, in one form or another. These attempts may be divided into those directed toward therapy of existing disease and those directed to prophylaxis against disease in the present or future.

Svirsky-Gross (1958) and *Tassovatz* and *Kotsitch* (1961) successfully showed that passive oral administration of human milk successfully stopped epidemics of *Escherichia coli* O111:B4 in their new-born nurseries. Recognising that the immunoglobulin concentration in colostrum is higher than that in milk, *Larguia* et al. (1977) controlled an outbreak of enteropathogenic *E. coli* diarrhoea in a premature nursery by oral administration of 5 ml/kg/day of a pool of colostrum from several mothers. *Narayanan* et al. (1980) showed significant protection against infection in 32 high-risk low-birth-weight infants by partially feeding human breast-milk (breast-milk during the day, formula at night) compared with 38 infants receiving formula alone. *Barnes* et al. (1982) evaluated the protective effect of orally administered commercial pooled human serum gammaglobulin in a group of 75 low-birth-weight infants in a nursery where rotavirus was known to be endemic. In the placebo group, 6 of 11 babies developed severe rotavirus diarrhoea whereas only 1 of 14 given Ig was affected. In a recent study by *Eibl* et al. (1988), the development of necrotising enterocolitis in low-birth-weight new-borns was prevented by oral administration pooled human serum immunoglobulins (Cohn Fraction II containing 75% IgG and 25% IgA). There were no cases in the 88 infants receiving Ig compared with 6 in the control group of 91. In that study, oral Ig administration was completely protective.

The newly parturient cow may be regarded as an immunoglobulin-producing factory that secretes into its colostrum kilogram amounts of IgG1, the bovine milk immunoglobulin counterpart of the human secretory IgA (*Lascelles* and *McDowell*, 1974). As with human SIgA (*Kenny* et al., 1967; *Lindh*, 1975), bovine IgG1 has been shown to be relatively protease-resistant and immunologic reactivity is retained after passage through the intestinal tract (*Hilpert* et al., 1974, 1975; *McClead* and *Gregory*, 1984). A number of studies have examined the therapeutic/protective effects of feeding hyperimmune bovine immunoglobulin. In this laboratory (*Boesman-Finkelstein* et al., 1989), hyperimmune bovine IgG1 was purified from the colostrum of newly parturient cows who had been immunised with cholera toxin (CT), *E. coli* heat-labile toxin (LT) and *V. cholerae* outer membrane proteins. Administered by gastric feeding tube in infant feeding formula, all were shown to protect 6-day-old infant rabbits from diarrhoea following intra-intestinal challenge with virulent cholera vibrios. Both protection studies, as well as immunologic analysis by checkerboard immunoblotting (*Kazemi* and *Finkelstein*, 1990), a technique recently developed in our laboratory, indicated that homologous preparations are more reactive (unpublished results).

Mietens et al. (1979) evaluated the therapeutic effect of orally administered hyperimmune bovine milk immunoglobulin concentrate (MIC, containing about 40% Ig), containing antibodies to 14 serologically different strains of enteropathogenic *E. coli*, on 60 infants (ages 10 days to 18 months) suffering from *E. coli* diarrhoea. No therapeutic effect was demonstrated although the period of excretion of the

homologous serotypes of *E. coli* was reduced. A similar attempt to treat infants with rotavirus diarrhoea with an MIC containing antibodies to 4 different human rotavirus serotypes was also unsuccessful although the higher titred anti-rotavirus preparation did reduce the excretion of virus (*Brussow* et al., 1987; *Hilpert* et al., 1987). Recently, *McClead* et al. (1988) gave purified bovine immunoglobulin containing anti-cholera toxin antibodies to patients with active cholera. Although toxin neutralising activity was found in the stools of most of the patients, orally administered antibody did not alter the course of active cholera diarrhoea.

Although feeding antibody-containing Ig preparations has quite clearly not been successful therapeutically, administration of such Ig prophylactically would seem to be more logical since the disease may not be reversible by antibody whereas the prophylactic effect of feeding human milk against diarrhoeal disease in infants has long been known. Recently, *Tacket* et al. (1988) demonstrated that a MIC containing antibodies against CT, LT, and a variety of enteropathogenic strains of *E. coli* was protective in studies using adult American volunteers who were challenged with enterotoxigenic *E. coli*. None of the 10 volunteers receiving the immune colostrum had diarrhoea when challenged with 10^9 colony forming units of enterotoxigenic *E. coli* H10407, whereas, 9 of the 10 receiving control colostrum did. All excreted the challenge *E. coli* strain. *Ebina* et al. (1985) orally administered 20 ml of hyperimmune bovine anti-rotavirus colostrum daily to 6 infants in an orphanage while 7 control infants received 20 ml of commercial milk. After a period of 1 month, 6 of the 7 control infants had developed rotavirus diarrhoea, whereas 5 of the 6 colostrum-fed infants were free of diarrhoea. Two of these 5, interestingly, developed demonstrable complement-fixing antibodies to rotavirus during this period. In another group in the same study, hyperimmune colostrum administration had no therapeutic effect on rotavirus infection. *Davidson* et al. (1989) showed that infants fed bovine colostrum from cows immunised with 4 serotypes of human rotavirus were protected against nosocomial rotavirus infection. In that study, 9 of 65 control children, but none of the 55 colostrum-fed infants developed rotavirus diarrhoea. Although it was not altogether clear that antibody, rather than other components of the colostrum, was responsible (*Boesman-Finkelstein* and *Finkelstein*, 1989), it was clear that the colostrum preparation protected against rotavirus diarrhoea.

The studies described above have shown that either bovine or human antibodies are efficacious prophylactically, but they were generally not effective therapeutically. Immunologically compromised subjects, however, may be an exception to this rule. Hyperimmune bovine colostrum from cows immunised with *Cryptosporidium* oocysts administered via a naso-gastric tube was reported to be effective in the treatment of 4 immunodeficient patients – two of whom had AIDS - with chronic diarrhoea caused by *Cryptosporidium*: 1) a 3-year-old hypogammaglobulinaemic was treated with 200 ml hyperimmune colostrum/day for 12 days (*Tzipori* et al. 1986, 1987); 2) a 38-year-old AIDS patient received 500 ml/day colostrum for 21 days (*Tzipori* et al., 1987); and 3) a 4-year-old on immunosuppressive therapy for acute lymphoblastic leukaemia received 500 ml colostrum/day for 10 days (*Tzipori* et al., 1987). They recovered from their diarrhoea within 3-5 days. Another recent report (*Ungar* et al., 1990) also showed therapeutic/prophylactic effi-

cacy of using hyperimmune bovine anti-*Cryptosporidium* oocysts in the treatment of AIDS patients. Colostrum was administered continuously (20 ml/hour) via a naso-duodenal tube for 60 hours. Within 48 hours of cessation of therapy, stools were fully formed and no *Cryptosporidium* oocysts were detected. The mechanism of the colostrum action appears to depend on its antibody content, perhaps by interfering with oocyst re-attachment thus breaking the pathogen's life cycle. But, the observations raise the possibility that orally administered hyperimmune bovine immunoglobulin may be useful in immunologically compromised patients including those with hereditary immune deficiency states or those with acquired immunodeficiencies such as patients with AIDS.

If we accept that hyperimmune bovine immunoglobulin can be protective (and perhaps in some instances therapeutic), the next question is whether or not it is a practical option to be applied on a broad scale, i.e., is it feasible and can it be sufficiently economical to be useful in lesser developed countries. There is no direct information available to answer this question. However, some assumptions can be made based upon published information. For example, the study of *Tacket* et al. (1988), cited above, reported that adult American volunteers were protected against challenge with 10^9 viable enterotoxigenic *E. coli* which was a 100% infective dose in control subjects. The protected volunteers were given 3.55 g of milk immunoglobulin concentrate (MIC) three times a day for seven days. The MIC used contained 40% immunoglobulin and a mixture of antibodies, of which many may be assumed to be irrelevant. If we assume, e.g., that 10% of the antibodies were effective then the dose of effective immunoglobulin per volunteer per day was of the order of 400 mg. We may also assume that this dose was excessive in view of the fact that it resulted in 100% protection against an unnaturally high challenge. Can we assume that 10-fold less, i.e., 40 mg per day, would be sufficient? Although that remains to be shown by appropriate experimental studies, it seems to us to be a potentially acceptable assumption. In as much as a kilogram or more of immunoglobulin can be harvested from a newly parturient cow, based on the above assumptions, a single cow could provide 25,000 daily doses for adults and perhaps 100,000 doses for children. These assumptions are related to using colostrum from newly parturient cows. It is also possible that there could be sufficient antibody present in mature milk from immunised cows to be protective. Further, herds of cows could potentially be immunised with multiple antigens to provide milk which could be protective against a variety of pathogens.

Admittedly, the problem of providing antibody-containing infant feeding formulae or the antibody itself in a stable and sanitary form, remains to be resolved. But, in as much as infants who are not breast-fed are presently being fed breast-milk substitutes or formulae in an unsanitary way, it seems that the provision of protective formulae would be an improvement.

We conclude that bovine lactogenic immunity is indeed a concept whose time has come. Or, at least, the time has come for further evaluation of the concept.

ACKNOWLEDGEMENTS

Studies in the authors' laboratory, reported herein, were supported in part by grants AI 16776 and AI 17312 from the National Institute of Allergy and Infectious Diseases. The authors are grateful to Mohammad Kazemi for the checkerboard immunoblotting of the bovine colostral antitoxic immunoglobulin.

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