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THE INTERPLAY BETWEEN THE IMMUNE DEFENCE IN THE NEONATE AND THE FLORA COLONISING THE GUT AFTER BIRTH

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SUMMARY

Specific and non-specific host defence in the new-born is of great importance for preventing many of the microbes, colonising the skin and various mucous membranes from causing infections. The passively transferred specific IgG antibodies from the mother as well as defence factors in the breast milk are important. On the other hand, the microbial flora exposing the mother and the infant will influence and direct the immune response of the foetus and new-born. This presentation will discuss the antibody response of the young to microbes with special reference to the possible effect of anti-idiotypic antibodies from the mother via the placenta or the milk. The differences in intestinal colonisation seen between Swedish and Pakistani infants is presented in relation to the differences in the mode of feeding.

A FOETAL AND NEONATAL ANTIBODY RESPONSE IN MAN POSSIBLY INDUCED BY ANTI-IDIOTYPIC ANTIBODIES FROM THE MOTHER

In some instances it has been noted that new-borns have antibodies that have not come from the mother. We have noticed secretory IgA and IgM (SIgA and SIgM) antibodies to appear in new-borns' saliva both against *E. coli* and poliovirus antigens (*Mellander* et al., 1984, 1986). These antibodies also appeared in new-borns of mothers with hypogammaglobulinaemia and IgA deficiency demonstrating that they must have been produced by the foetus (*Mellander* et al., 1986; *Hahn-Zoric* et al., 1992). The antibodies against the poliovirus in Swedish new-borns was of special interest since efficient vaccination using only inactivated poliovirus has eliminated poliomyelitis and there are, with very few exceptional imports, no wild or vaccine poliovirus in the country. Thus it is very unlikely that these foetuses can have been exposed to poliovirus antigens.

We have assumed that the antigenic stimulus of the foetus could be anti-idiotypic antibodies from the mother. In several assays we have found evidence for the presence of such anti-anti-poliovirus antibodies in the immunoglobulin preparation given to the

mothers with hypogammaglobulinaemia as a prophylaxis against infections (*Hanson* et al., 1989a; *Hahn-Zoric* et al., 1993). If our assumption is correct it means that the IgG antibodies passively received by the foetus from the mother may not only passively protect, but may even actively prime the immune system of the offspring. Such effects of anti-idiotypic antibodies given directly to new-born mice, or via the mother animal reaching the offspring through the milk have been demonstrated against bacterial as well as viral antigens (*Stein* and *Söderström*, 1984; *Okamoto* et al., 1989)

In a preliminary study we found some evidence that breast-fed infants responded better to parenteral diphtheria and tetanus toxoid and oral poliovirus vaccine than formula-fed infants (*Hahn-Zoric* et al., 1990). The SIgA responses in saliva and IgM in stool were higher at 3-4 months of age, whereas the serum IgG antibody responses to diphtheria toxoid and poliovirus were higher at 20-40 months of age. We have proposed that these enhanced responses might be the result of anti-idiotypic antibodies in milk priming the lymphoid system of the breast-fed infants. Some data indicate that human milk can contain anti-idiotypic antibodies (*Hanson* et al., 1989a; *Hahn-Zoric* et al., 1993).

Presumably the exposure of the mother to microbes will direct her immune response and her level of the corresponding idiotypes - anti-idiotypes, which will be transferred to the foetus. Little is presently known about whether stimulation or inhibition will ensue. We did not observe any obvious difference in the content in saliva from Swedish and Pakistani new-borns of SIgA and IgM antibodies to *Escherichia coli* O antigens (*Mellander* et al., 1985). Still the Pakistani new-borns and their mothers studied must be much more heavily exposed to such intestinal bacteria and those infants increase their salivary content of SIgA antibodies to *E. coli* O antigens quickly after birth (*Mellander* et al., 1985).

INTESTINAL COLONISATION OF PAKISTANI AND SWEDISH NEW-BORNS WITH GRAM-NEGATIVE AEROBES

Pakistani infants were significantly earlier colonised in the gut with Gram-negative aerobes than Swedish infants (*Adlerberth* et al., 1990). This was true whether they were delivered at home or at a hospital, whether through vaginal delivery or through caesarean section. The Pakistani infants had a more variable flora than the Swedish infants, who mainly had *E. coli*, whereas *Enterobacter*, *Citrobacter* and *Klebsiella* were more common in the Pakistani infants, although less so if they were breast-fed than if given others foods (*Adlerberth* et al., 1990).

The binding to a colon epithelial cell line of some colonising *E. coli* could occasionally be inhibited by a meconium extract. This was seen for strains with adhesins which agglutinated red blood cells of blood group p (-) without being inhibited by mannose (*Hanson* et al., 1989b). It is possible that this mechanism can play a role for the capacity of the new-born to handle some of the bacteria colonising the gut directly after birth. It is likely that the effect is due to the presence of receptor analogues in the meconium, just as we have shown receptor analogues in human milk which can prevent adhesion of pneumococci and *Haemophilus influenzae* to retropharyngeal cells (*Andersson* et al., 1986).

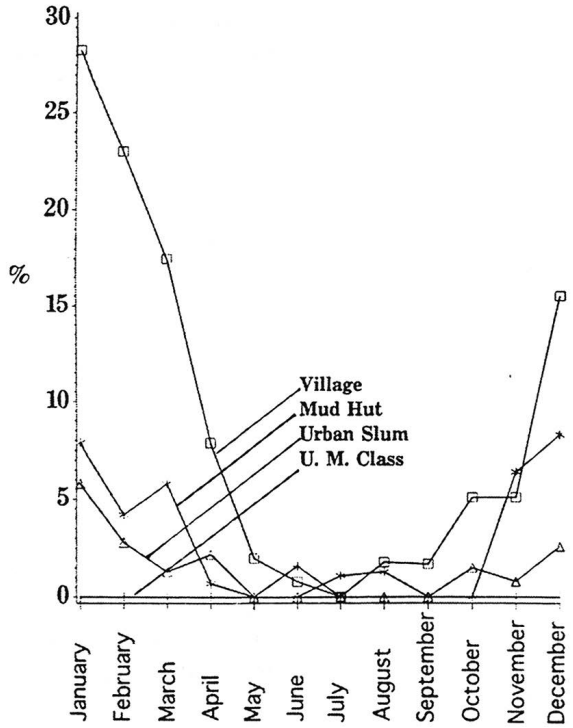

Figure 1: Exclusive breastfeeding in a prospective follow up of four different population groups (ages from 1 to 6 months) in and around Lahore, Pakistan, in relation to the month of the year.

We have proposed that human milk supports the health of the breast-fed infant not only by the anti-adhesive effect of antibodies and receptor analogues, but also via a number of components in milk which are anti-inflammatogenic (*Goldman* et al., 1986). Thus lactoferrin inhibits complement, the secretory IgA antibodies do not activate complement and the lysozyme limits chemotaxis and inflammatogenic activity of neutrophils. Milk lipids inhibit neutrophil superoxide production. The superoxide is also counteracted by the antioxidants in milk, alfa-tocopherol, cysteine and ascorbic acid and is destroyed by the milk catalase and glutathione peroxidase, etc. These capacities may be useful for instance for dampening the effect of endotoxin from the gut flora on the neonate's intestine.

In a recent study in Pakistan we found that even partial breastfeeding could protect against neonatal sepsis compared to non-breastfeeding with an odds ratio of 18 (*Ashraf* et al., 1990). Only one infant out of the 42 cases of neonatal sepsis and 269 controls was exclusively breast-fed. Comparison showed that partial breastfeeding was much more common among the controls than among the cases ($p<0.001$). Thus partial breastfeeding, protected against neonatal sepsis although the infants had been very early colonised in the gut (*Adlerberth* et al., 1990), presumably via the various fluids and materials given instead of colostrum during the first 1-3 days of life in this region (*Hanson* et al., 1986). It is obvious that the early feeding patterns may play an important role for the outcome of the neonate.

Table 1: Infants not breast-fed (%) in the four population groups in relation to age

Age (months)	1	6	12	18	24
Village	2.4	3.5	4.3	11.1	80.2
(n)	(332)	(289)	(281)	(279)	(262)
Mud hut	5.6	7.4	21.9	50.3	75.4
(n)	(270)	(204)	(196)	(183)	(167)
Urban slum	9.5	27.6	42.9	64.4	87.2
(n)	(264)	(243)	(203)	(177)	(180)
Upper middle class	13.6	59.6	75.2	81.5	95.6
(n)	(162)	(141)	(133)	(130)	(113)

FEEDING PATTERNS OF INFANTS IN PAKISTAN

Whereas breastfeeding with an early onset after delivery is now the rule among Swedish infants, the pattern is different for Pakistani infants. This is obvious from the follow-up of 1473 children in four socio-economically different populations in Pakistan; a village, a mud hut area, a city slum area and an upper middle class control group. First, there was no difference in feeding patterns between boys and girls. Very few infants were exclusively breast-fed, but this number varied with the area of living and with the season of the year (Figure 1). Exclusive breastfeeding was more frequent in the village than in the other three areas. During the hot season April-September exclusive breastfeeding was almost not practised because the mothers gave more extra fluid during this period (*Jalil* et al., 1990). The number of infants given breast milk and water increased strikingly both in the village and the mud hut area during the hot season. The number of only breast-fed decreased simultaneously.

Exclusive as well as partial breastfeeding became continuously less common with age, especially during the second half of the first year. The non breast-fed were more than twice as common in the upper middle class group than in the village or mud hut area during the first 2 years of life (Table 1). During the first month of life the non breast-fed infants were only 2.4% in the village, 5.6% in the mud hut area, 9.5% in the urban slum and 13.6% in the elite group.

Studying the onset of breastfeeding, it was obvious that most started within the first 96 hours of life (*Hanson* et al., 1986). The onset was somewhat earlier in the urban slum and the city elite than in the village and mud hut area where a delay of 48-72 hours was common. Before the onset of breastfeeding the neonates are given other fluids and material including honey, laxatives and herb extracts by spoon, bottle or hand (*Hanson* et al., 1986). The bottle is most common in the upper middle class group, the spoon in the mud hut area and the village, the hand in the urban slum (Table 2). This brings a major risk for exposure to microbial pathogens.

These observations taken together, i.e. the rather late onset of breastfeeding after birth and the rarity of exclusive breastfeeding have consequences for the bacterial colonisation of these new-borns, such as a more hetero-

Table 2: The mode of administration (in %) of extra fluid prior to the onset of breastfeeding in the four population groups

	Spoon	Hand	Bottle	Other
Village	39.5	12.1	5.0	43.4
(n)	(180)	(55)	(23)	(198)
Mud hut	60.3	11.2	13.7	14.8
(n)	(216)	(40)	(49)	(53)
Urban slum	20.3	61.2	4.3	14.2
(66)	(199)	(14)	(46)	
Upper middle class	7.3	14.6	76.4	1.7
(n)	(17)	(34)	(178)	(4)

genous intestinal flora with more potential pathogens, possibly also in higher numbers. This may be one explanation of the high morbidity and mortality of these infants in sepsis/meningitis and early acute and prolonged gastro-enteritis. It is most likely that it would be advantageous to initiate breastfeeding directly after birth and to avoid other often contaminated fluids and foods altogether.

CONCLUSIONS

The foetus responds to microbial antigens, possibly as a result of exposure to transplacental maternal anti-idiotypes against microbial antigens.

Pakistani infants produce higher levels of mucosal IgA antibodies to the intestinal flora during the first weeks of life than Swedish infants. The Pakistani new-borns are also earlier colonised in the gut with Gram-negative aerobes than Swedish infants. The intestinal colonisation of the Pakistani infants is equally early whether they are delivered vaginally or by caesarean section, whether they are delivered at home or in hospital.

The mode of feeding influences the colonisation. Late onset of breast-feeding and giving other foods and fluids than human milk presumably relates to the early intestinal colonisation of the Pakistani infants.

During the hot season of the year the breast-fed babies are given more extra fluid and exclusive breastfeeding becomes non-existent during the period they need the protection of breastfeeding the most.

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