Milk antigen absorption in the preterm and term neonate

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SUMMARY  The concentration of β-lactoglobulin was measured in the sera of 47 preterm and term neonates during the first few days of life under standardised conditions after feeding with a cows' milk-based formula. Preterm neonates, particularly those of less than 33 weeks' gestation, had higher serum concentrations of β-lactoglobulin than term neonates given an equivalent milk feed. Prior feeding with breast milk did not diminish the amount of β-lactoglobulin absorbed. Our results suggest that the ability of the gastrointestinal tract to exclude antigenically intact food proteins increases with gestational age and that gut closure occurs normally before birth in man.

Gastrointestinal absorption of antigenically intact proteins has been shown to be increased in the neonatal period of several mammalian species. The calf and piglet, which acquire passive immunity almost exclusively from maternal milk, have a gut which is freely permeable to macromolecular proteins for the first few days post-partum. Feeding induces a rapid maturation of gut morphology and this results in functional gut closure to these proteins. Other species—such as the rat and mouse—have an extended period of selective absorption of immunoglobulin from maternal milk, which stops just before weaning. It is thought that certain factors in maternal milk enhance gut closure and indeed a species-specific effect of maternal milk on the maturation and antigen excluding function of the gut has been demonstrated in the piglet, beagle puppy, and neonatal rabbit.

The human fetus acquires maternal immunoglobulin via the placenta during the last trimester of pregnancy. Consequently it was thought that the neonatal human gut had little need for absorption of antigenically intact food proteins. However, the potential for increased absorption in early life exists, but the evidence for it occurring is circumstantial and is based on high levels of antibodies to food proteins in preterm infants at 3–6 months after birth and the fact that these levels decline with maturity.

This study was performed to determine whether absorption of the cows' milk protein, β-lactoglobulin, from the gastrointestinal tract of human neonates under standardised conditions differed according to gestational age, birth age, or prior feeding with breast milk.

Patients

Forty-seven neonates born in three maternity hospitals were studied. Ethical committee approval was obtained from each hospital. The neonates were divided into groups according to different feeding regimens.

Group 1. Twenty-six neonates of gestational ages 30 to 39 weeks were fed by nasogastric tube, blood samples being taken after at least 4 hours of hourly feeding in an attempt to achieve an equilibrium state. The median age since first feed at the time of sampling was 26 (range 21–52) hours and the median birth age was between 31 and 32 (range 23–168) hours.

Group 2. Twelve term neonates of gestational ages 37 to 41 weeks were receiving 3-hourly or 4-hourly bottle feeds. The median age since first feed at the time of sampling was 138 (range 109–161) hours and the median birth age was 142 (range 114–164) hours.

Group 3. Nine neonates had received some breast milk during their initial feeding regimen but had not received any breast milk for at least 9 hours before sampling. Five were fed hourly by nasogastric tube (gestational age between 32 and 37 weeks) and were sampled at feeding ages between 22 and 52 hours (birth ages 29 to 172 hours). Two of these neonates had received pooled heated expressed breast milk and 3 had received fresh expressed maternal milk before artificial feeding. Four babies were bottle fed after initially suckling at the breast (gestational ages between 37 and 41 weeks) and samples were obtained...
Negative chromatography of the washed to coat unknown samples was received in the theoretical Institute. In this manner when antigen was made available, specific antibody was quantitated. Standard 125I-labelled ultracentrifuged serum was assayed. Specific serum was incubated with the antigen with purification allowed to proceed. Antibody was then purified for incubation and antibody obtained was purified by ion-exchange chromatography followed by immunoabsorbent purification using β-lactoglobulin covalently linked to microcrystalline cellulose.

The IgG fraction of the rabbit antisera was used to coat polystyrene microfuge tubes (Sarstedt). After overnight incubation of the test serum in the coated tubes, 125I-labelled affinity purified rabbit anti-β-lactoglobulin was added to the washed tubes and was incubated for 18 hours. The tubes were then washed and counted in an LKB gamma counter. Standard solutions of β-lactoglobulin made up in known negative sera to allow quantitation of unknown samples were included in each assay. Serum samples were coded and assayed in triplicate. The sensitivity of the assay ranged from 0.1 to 100 ng/ml.

Results

Neonates in group 1 of 33 weeks' gestation or less had significantly higher serum β-lactoglobulin concentrations than the term neonates of group 1 (P<0.05) and group 2 (P<0.001) (Fig. 1). Group 1 neonates between 33 and 37 weeks' gestation also had higher values than the group 2 neonates (P<0.05).

The 12 bottle-fed term neonates of group 2 did not differ significantly from the 5 group 1 neonates of similar gestational age who had been fed by nasogastric tube.

When groups 1 and 2 were considered together, preterm neonates had significantly higher serum concentrations than did term neonates (P<0.002).

There was no correlation between serum β-lactoglobulin concentrations and birth age or feeding age within groups 1 and 2 or within the combined groups.

Earlier exposure to breast milk in the few studied

![Fig. 1 Serum β-lactoglobulin concentrations per ml of milk given per kg during the preceding 4 hours (groups 1 and 2).](http://adc.bmj.com/10.1136/adc.57.5.369)
in group 3 did not significantly alter the β-lactoglobulin values compared with neonates of similar gestational ages from groups 1 and 2 who had been fed by the same route at similar birth ages and had not received breast milk (Fig. 2).

Eleven of the study infants had pre-feeding samples or cord blood assayed for β-lactoglobulin and in none of these could any be detected.

Discussion

Beta-lactoglobulin (MW 36 000) is the major whey protein of cows' milk, but is not found in human breast milk. This study shows that preterm neonates given an equivalent load of a cows’ milk-based formula have significantly higher serum β-lactoglobulin concentrations than term neonates. In fact, the total amount of β-lactoglobulin present in the circulation of the preterm infants may be grossly underestimated since there is evidence that plasma volume with respect to weight is greater in the preterm than the term infant.

The amount of antigenically intact protein absorbed by the term neonate is of the order of 10-5 of that ingested. The term neonate absorbs similar amounts to non-atopic adults given equivalent antigen loads according to body weight and our results indicate that absorption in the very preterm infant may be up to 100-fold greater.

The results are therefore compatible with either increased absorption of antigenically intact proteins by the preterm infant’s gut, or less efficient clearance from the circulation. Several factors may contribute to increased absorption and higher serum concentrations of β-lactoglobulin in preterm infants. Gastrointestinal motility increases and transit times decrease with advancing fetal age. Protein digestion in the gut lumen and at the epithelial brush border may be less efficient since stomach peptic and duodenal enteropeptidase activities are known to increase with gestation during the last trimester of pregnancy. Epithelial cell morphology, endocytosomal transport, and proteolytic mechanisms may also differ at different gestational ages.

It is possible that some of the diminution in β-lactoglobulin levels with increasing gestational age is due to more effective clearance mechanisms in the more mature neonate. The antigen may be cleared by complexing with maternally derived immunoglobulin and in this respect the relationship of the lower serum β-lactoglobulin concentrations after 33 weeks’ gestation found in the study and the accelerated rate of increase of total and subclass IgG concentrations known to occur at 32–33 weeks’ gestation is noteworthy. Liver filtration may be important in the clearance of ingested antigens or antigenic fragments in the newborn and also may be less efficient in maturity.

Although feeding breast milk in other species has been shown to enhance gut closure in terms of morphology and function, no alteration in β-lactoglobulin levels was seen in those neonates who initially received breast milk. In the human neonate there may not be any functional enhancement of antigen exclusion attributable to breast milk factors, but the numbers studied were small and this needs confirmation. Similarly, although birth age and feeding age had no apparent effect over the narrow ranges studied in this investigation, sequential studies during longer periods of time may demonstrate lower serum values with increasing time. Xylose absorption, which is lower in preterm neonates suggesting immature absorptive cell function, has been shown recently in serial studies to rise with birth age, supporting the concept of major changes in gastrointestinal function at a cellular level in the perinatal period.

It appears therefore, that gut closure in man is not an abrupt event but occurs gradually with fetal maturation and is normally complete by birth in the term neonate. The amounts of antigenically intact protein absorbed are insignificant nutritionally even in the preterm infant. They are however sufficient to immunise, as antibodies to food proteins may be demonstrated in most healthy individuals. Despite this, most people do not develop hypersensitivity responses to food proteins and a state of partial
References


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