Effects of Brief Early Exposure to Partially Hydrolyzed and Whole Cow’s Milk Proteins


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INTRODUCTION

The first weeks of life seem to be a critical period for the development of allergic manifestations to food antigens and particularly to cow’s milk proteins. Indeed, it has been shown that in infants with cow’s milk allergy supplements of cow’s milk formula are given significantly more often in the first 4 weeks than in control infants without cow’s milk allergy (1). Furthermore, in a recent prospective study of the incidence of cow’s milk allergy in healthy Danish infants, it was found retrospectively that all the “exclusively” breast-fed infants in whom allergy developed (n = 9 out of 39 cases) had indeed occasionally received cow’s milk formula in the newborn nursery; conversely, among the newborns who did not receive any supplement of formula (n = 210), none developed cow’s milk allergy compared to 39 among those who had received a supplement (n = 1539), a statistically significant difference (p < 0.05) (2).

However, the clinical and immunological consequences of a brief exposure to, or avoidance of, food antigens during the neonatal period in human infants have seldom been prospectively studied. To our knowledge, there are only two published reports addressing this question. One study is an open preliminary observational trial with a partially hydrolyzed hypoallergenic formula carried out in newborn infants from atopic families, showing that the hypoallergenic formula, first given as a supplement to breast milk during the first 5 days of life, was clinically well tolerated by all infants when given again 3 to 4 months later at weaning (3). However, the small number of children (n = 45) and the absence of a control group did not allow any conclusion about the possible preventive effects of the hypoallergenic formula against cow’s
milk allergy. The other study concerned 216 healthy term infants who were random-
ized either to receive early feeding with formula before breast-feeding or to be only
breast-fed. The infants were then followed up for 18 months. At the end of the follow-
up period, significantly fewer infants developed symptoms of allergy in the formula-
fed group (18%) than in the breast-fed group (33%, \( p < 0.05 \)). The difference was
most pronounced in infants with double heredity of atopy (11% versus 61%, \( p < 0.01 \)) (4). The latter results are in sharp contrast to those already discussed (1,2),
which, along with others (5–7), would imply antigen avoidance as a strategy in the
prevention of cow’s milk allergy. Unfortunately, none of these studies followed the
immune responsiveness [immunoglobulin G (IgG) and IgE, total and specific] to
cow’s milk proteins prospectively.

In this context, we decided to study whether preventing healthy newborn infants
from receiving cow’s milk proteins during the first days of life as a supplement to
breast milk (a) by feeding them a partially hydrolyzed hypoallergenic formula would
modify the immune response to cow’s milk proteins during the first year of life (sensi-
tization/memory) and (b) eventually decrease the frequency and/or severity of clinical
symptoms of intolerance to cow’s milk at the time of weaning. The results of this
prospective, double-blind randomized controlled study form the basis of the present
chapter (8,9).

STUDY DESIGN

Mothers from two maternity departments were asked during their last outpatient
visit before the birth of their child or during the first 36 hours after birth whether
they intended to breast-feed. In case of a positive answer, they were asked to partici-
pate and to give written consent. Family history of atopy was recorded. On isolated
occasions (less than ten), the pediatrician responsible for the inclusion decided not
to ask the mother to include her newborn when there was a highly atopic family
history (severe asthma and/or eczema in a parent, anaphylactic shock in a sibling).
Newborns were then included if healthy and as soon as they needed a supplement
to breast-feeding. Between November 1, 1988 and February 15, 1989, 256 newborns
were randomly assigned to receive either a standard adapted formula (AF Nidina®)
or the hypoallergenic formula (HF Nidal HA®) as supplement. The formulas were
blind. Daily volumes of any supplements needed were recorded. From day 5 to 7
onward, infants of both groups were exclusively breast-fed. Until day 90, breast-
feeding was supplemented by HF in both groups when necessary. During these 3
months the mothers were instructed not to give any food other than breast milk or
HF to their infant; they were not advised to modify their own dietary habits. During
the same period the pediatricians in charge of the infants at home received instructions
not to change the feeding regimen and to use only oral rehydration solutions in case
of diarrhea. At day 90, milk intake by the lactating mothers and, where this had
occurred, the dates of cessation of exclusive and later of mixed breast-feeding were
Intention To Breast Feed

Randomisation

Birth D 5 D 90 D 150 D 365

HF Supplement Diversification & solids

AF Breast fed

Clinical Exam + + + + +
IgE (total) + + + + +
IgE (specific) + + + + +
IgG (specific) + + + + +

FIG. 1. Study protocol.

recorded. From day 90, HF was replaced in both groups by the adapted formula and the introduction of the other foods, delayed until then, was allowed and recorded (Fig. 1). The follow-up was first planned to end after a last visit at day 150; however, compliance to the protocol was so high (96%) that it was decided to modify it and to extend the follow-up to day 365. A new informed consent was proposed for this extension. From day 150 to day 365, infants did not have any dietary restrictions.

Infants were examined at days 6, 90, 150, and 365, and the occurrence of clinical symptoms (atopic dermatitis, wheezing, rhinitis, diarrhea, vomiting) was recorded by pediatricians without knowledge of the study group. Blood was drawn from the cord, from the heel at day 6, and by venipuncture at days 90, 150, and 365. Skin prick tests with β-lactoglobulin, casein, α-lactalbumin, serum albumin, ovalbumin, HF, histamine, and saline were performed at days 150 and 365.

Response Criteria

Since the aim of the protocol was to identify the effects of early formula introduction before establishing breast-feeding in a normal population, it was decided that the main criteria to assess the effects of the study would be biological ones: total and specific IgE and specific IgG for cow’s milk proteins. Indeed, in a general population, clinical signs and symptoms were expected to be poor outcome criteria because of (a) the low incidence of allergy to cow’s milk in a normal population and (b) the low probability that relatively small amounts of cow’s milk ingested by the newborn during the first days of life would modify the prevalence of clinical symptoms related to allergy during the following year. Even the protective effect of feeding breast milk for several months is at times difficult to prove (10).
METHODS

Total IgE levels were assayed using the high sensitivity radioimmunoassay (RIA) kit from Pharmacia (threshold 0.05 kU/liter). Serum levels of IgE specific for β-lactoglobulin, casein, α-lactalbumin, serum albumin, ovalbumin, and HF were estimated using RIA. Total IgG antibodies specific for the same antigens were estimated by enzyme-linked immuno-adsorbent assay (ELISA) according to standard methods. In short, 96-well flat-bottomed microtiter plates (Linbro EIA 1 Plus®) were used. The coating concentrations for the relevant antigens (obtained from Sigma, >95% purity except for lactalbumin) were determined by checkerboard titration and ranged from 0.3 to 50 μg/ml; coating was performed overnight at 4°C. Goat anti-human IgG biotinilated antibodies were used throughout. The assay was blocked with 1% hemoglobin solution and washed four times with Tween 20 in phosphate-buffered saline pH 7.4 (0.5%). The intra-assay (6% to 12%) and interassay (9% to 20%) coefficients of variation were considered acceptable.

Statistical Analysis

Analysis was performed using the SAS statistical software, applying nonparametric tests, namely, the Kruskall–Wallis test, for continuous data.

RESULTS

Characteristics of the Groups

Two hundred and fifty-six infants were enrolled, 128 in each group (AF/HF). During the study, three infants died between 3 weeks and 2 months of age from sudden infant death syndrome. Four infants were lost to follow-up before day 90 and two additional ones before day 150. Thus 247 children completed the study at day 150 and 189 infants were examined at day 365 (the rates of dropout were 96% and 74% at days 150 and 365, respectively).

The two feeding groups did not differ from one another in terms of several baseline characteristics including family history of atopy (32% versus 25% in the HF compared to the AF group), the number of infants with cord blood IgE levels greater than 0.7 kU/liter (14 versus 16 in the HF compared to the AF group), and the presence of household pets. The groups did not differ either in birthweights, total volume of supplement ingested during the first days (427 ± 443 versus 459 ± 417 ml in the HF compared to the AF group), duration of breast-feeding, and amounts of milk drunk by the mothers during pregnancy and lactation.

Clinical Responses

Prevalences of clinical symptoms and positive skin prick tests were not significantly different in the two groups at day 90, day 150, and day 365 (data not shown).
TABLE 1. Specific IgG values (median) at days 90 and 150 for infants fed standard adapted formula (AF) or hypoallergenic formula (HF) as supplement to breast-feeding during the first days of life.

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Day 90</th>
<th></th>
<th>Day 150</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HF</td>
<td>AF</td>
<td>p</td>
<td>HF</td>
<td>AF</td>
</tr>
<tr>
<td>β-Lactoglobulin</td>
<td>177.5</td>
<td>195</td>
<td>0.46</td>
<td>407.5</td>
<td>507.5</td>
</tr>
<tr>
<td>α-Lactalbumin</td>
<td>334</td>
<td>428.5</td>
<td>0.005</td>
<td>347</td>
<td>420</td>
</tr>
<tr>
<td>Casein</td>
<td>0</td>
<td>0</td>
<td>0.11</td>
<td>292.5</td>
<td>355</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>0</td>
<td>180.5</td>
<td>0.16</td>
<td>730</td>
<td>924.5</td>
</tr>
<tr>
<td>HA formula</td>
<td>386</td>
<td>532</td>
<td>0.19</td>
<td>367</td>
<td>543</td>
</tr>
</tbody>
</table>

* p = 0.03 for median values in the HF group, being lower than in the AF group for all antigens.

Biological (Immune) Response Criteria

Median titers of specific IgG were higher in the AF than in the HF group at day 90 and day 150 for all antigens tested, but not at day 365. The differences reached significance for α-lactalbumin at day 90 (p < 0.005) and at day 150 (p < 0.05) and for casein (p < 0.05) and HA (p < 0.01) at day 150. The probability that these results occurred by chance was 3% at day 150, a significant trend (p = 0.03) (Table 1).

Median titers of total serum IgE were higher in the AF group than in the HF group at days 90, 150, and 365, although the differences never reached significance (Table 2). The numbers of positive (>0.1 kU/liter) specific IgE assays were similar in both groups at the three times (days 90, 150, and 365) for β-lactoglobulin, α-lactalbumin, casein, bovine serum albumin, and HA.

When the effect of early feeding was analyzed according to neonatal (cord blood) IgE levels, the median titers of total IgE in the AF and HF groups were not different in the infants whose neonatal IgE values were less than 0.7 kU/liter (n = 128). However, in infants whose neonatal IgE values were greater than 0.7 kU/liter, the total IgE level on days 150 and 365 was twofold higher in the AF group than in the HF group. However, significance was not reached because of the small numbers of newborns with raised IgE (n = 30) (Table 3).

TABLE 2. Median values of total IgE for normal term infants fed standard adapted formula (AF) or hypoallergenic formula (HF) as supplement to breast feeding during the first days of life.

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>HF</th>
<th>AF</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord</td>
<td>0.1</td>
<td>0.1</td>
<td>0.53</td>
</tr>
<tr>
<td>Day 5</td>
<td>0.1</td>
<td>0.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Day 90</td>
<td>1.4</td>
<td>1.8</td>
<td>0.39</td>
</tr>
<tr>
<td>Day 150</td>
<td>4.2</td>
<td>4.95</td>
<td>0.21</td>
</tr>
<tr>
<td>Day 365</td>
<td>8.8</td>
<td>10.4</td>
<td>0.48</td>
</tr>
</tbody>
</table>
When the effect of early feeding was analyzed according to the volume of supplement, it appeared that median titers of total IgE in the HF group were not affected by this variable (<200 ml, 200 to 500 ml, >500 ml); in the AF group, however, median titers of total IgE were highest at 90, 150, and 365 days in infants who had received 200 to 500 ml. Thus in this group of infants median titers of total IgE were significantly higher in those who had received AF compared to those who had received HF ($p < 0.01$ at day 90, $p = 0.03$ at day 150, $p = 0.06$ at day 365) (Table 4).

**DISCUSSION**

During the first year of life, no clinical effect of the early feeding regimen could be observed. This was probably due to the fact that the infants were not selected for parental history of atopy. Indeed, it was decided not to select the infants, in order to investigate the possible immune modulation triggered by early artificial feeds in a normal population. It was also considered inappropriate at this stage to randomly assign high-risk infants to receive cow’s milk proteins only.

The humoral immune response can be modulated in a normal population by the early mode of feeding. It has been shown that the later cow’s milk was first given...
EXPOSURE TO COW'S MILK PROTEINS

to healthy infants, the later and the milder was the rise of cow's-milk-specific IgG antibodies (11). Similarly, total IgE levels have been found to be lower during the first 4 months of life in infants breast-fed for more than 6 months than in infants receiving cow's-milk-based formulas (7,12).

Our study shows that extremely brief (a few days) and early (before establishment of breast-feeding) modifications of the diet have a modulating effect on the immune response to oral antigens. Feeding a partially hydrolyzed formula during the first days of life as supplement to breast milk decreased the specific IgG responses to cow's milk at weaning and later, compared to a group that received an adapted formula. Analysis of total serum IgE levels indicated that the IgE response might be modified by the neonatal feeding regimen (a) in infants with cord blood IgE levels greater than 0.7 kU/liter and (b) when the total volume of supplement was between 200 and 500 ml. An effect of milk volumes on IgE production has already been mentioned in a retrospective study (13).

Clearly, the infants kept an "immunological memory" of the type of supplement they received during their first days of life: HF was less immunogenic than whole cow's milk proteins. This finding was observed despite the fact that breast-feeding happened to be shorter than expected, a vast majority of infants being already bottle-fed at day 90.

Our observations are difficult to compare with published reports: we studied normal infants exposed early to formulas for a few days, whereas most recent studies concerned at-risk infants fed the tested formulas for several months after birth (5-7). However, although we did not observe any clinical differences between the groups, our results are in broad agreement with the hypothesis, based on these and other (1,2) human studies, that a decrease in oral antigen load in infancy may reduce the subsequent incidence of atopy. On the other hand, our observations and those of several other investigators (1,2,5) are difficult to reconcile with those of Lindfors and Enocksson (4), whose protocol was similar and also involved normal infants, but which was limited to clinical symptoms. A possible explanation for the discrepancy between these two studies may be the fact that differences in volumes or in timing of supplements may induce different immune responses in normal neonates or in those with an atopic history. A similar age- and volume-dependent effect of ingested proteins has been shown in mice (14).

CONCLUSION

Although animal experiments point to the importance of the first days of life in modulating the immune response to oral antigens, the clinical and immunological consequences of a brief exposure to, or avoidance of, food antigens during the neonatal period in human infants are contradictory and poorly documented.

A study that we recently completed by modulating effect of feeding in the neonatal period indicated that the prevalence of clinical symptoms at weaning and up to 1 year of age was similar in two groups of normal breast-fed infants randomly assigned
to receive blindly either an adapted formula (AF) or a partially hydrolyzed, hypoallergenic formula (HF) as supplement to breast milk when necessary. Preliminary results showed that in AF-fed compared to HF-fed infants, (a) median titers of specific IgG were higher at 3 and 5 months for all antigens and (b) median titers of total IgE were two times higher at 5 and 12 months (although not significantly) in infants with cord blood IgE levels greater than 0.7 kU/liter and significantly higher at 3, 5, and 12 months when the total volume of supplement was between 200 and 500 ml.

It thus seems that feeding intact cow's milk proteins to normal healthy infants during their first days of life indeed affects their later immune response to these proteins and that partially hydrolyzed proteins are able to reduce this priming effect.

REFERENCES


DISCUSSION

Dr. Collin-Williams: Were you not concerned that the babies on hydrolysate were possibly getting cow's milk protein via the mother's milk?
Dr. Schmitz: We cannot exclude that, but the effect would have been the same in the two groups.

Dr. Iikura: Could you comment on why you measured IgG antibody?

Dr. Schmitz: Because we wanted to see the normal physiological reaction of these babies to proteins of different molecular arrangement.

Dr. de Weck: Your group of breast-feeding women whose infants were supplemented with hydrolyzed formula was, I think, rather heterogeneous in terms of when they started supplementing. Is that correct.

Dr. Schmitz: Yes, it was rather heterogeneous.

Dr. de Weck: Have you tried to analyze whether there were any differences according to the time at which the supplement was started?

Dr. Schmitz: No, because the population is too small.

Dr. de Weck: Accepting that your data on specific IgG and volume represent a true phenomenon, an interesting possibility is that the mixture of hydrolyzed proteins may contain some immunogenic molecules and some tolerogenic molecules.

Dr. Schmitz: That is a very good proposition. Some of our results argue in favor of this.

Dr. Strobel: I have to stress that we were not looking for clinical symptoms; we were looking for a single variable in an unselected population. I think that this makes the study unique in comparison with all other studies, where many variables are studied and there are always problems in identifying what is cause and what is effect.

Dr. Husby: Would not the logical thing to do be to supplement with water? Most pediatricians would probably agree that babies don’t need nutritional supplements during the first few days.

Dr. Schmitz: You are probably right but as I said, we had two aims and one was to see whether modification of the protein would make a difference to the immunological response. Certainly we lacked a third or fourth arm of the study to look at the effect of pure water, but then it would have been a longer and more difficult study.

Dr. Marini: In some maternity hospitals, the cesarean section rate is as high as 25%. Some of these mothers cannot feed their babies in the first few days of life. It is not easy to keep their babies only on glucose and water for 2 or 3 days. That is the reason why we need less antigenic formulas in the first days of life.

Dr. Husby: We are talking about mature babies here. I don’t think they need any supplementation during the first few days.

Dr. Bock: I come from a part of the world where there is a high cesarean section rate. We do not have a big problem in enabling a cesarean section mother to feed her baby fairly promptly.

Dr. Brasseur: We have performed blood sampling in 300 mother–infant pairs at 5 days postpartum, and we found a very strong correlation between the level in mother and the level in the baby for α-lactalbumin antibody; we did not find such a strong correlation for β-lactoglobulin. Have you performed such an analysis?

Dr. Schmitz: No, only in cord blood.

Dr. Brasseur: I think that is quite a restriction on the interpretation of the data because for IgG antibody, there is such a strong correlation with maternal levels.

Dr. Schmitz: But you have seen from our data that for those antigens that were fed for the first time to the baby, for example casein, the IgG level rises after the time when the feeds were initiated.

Dr. Brasseur: My intention was purely to make sure of the interpretation of such antibodies after birth and I think your results are misleading.

Dr. Schmitz: They may be misleading at 5 days but not after that.