Preparation of a Milk Immunoglobulin Concentrate from Cow’s Milk

H. Hilpert

Research Department, Nestlé Products Technical Assistance Co. Ltd., CH-1814 La Tour de Peilz, Switzerland

There cannot be any serious doubt about the superiority of breast milk in the nutrition of the healthy term infant (1–3). However, it is frequently impossible for preterm infants and very-low-birth-weight infants to be nourished with their own mothers’ milk. They must therefore depend on banked human milk if they are to profit from the nutritional and immunological advantages of human milk.

The relatively high protein and energy requirements of the low-birth-weight preterm infant suggest that pooled human milk may have nutritional inadequacies (4,5). The concept of a human milk formula was proposed in order to meet these nutritional requirements (6–10).

To maximize the advantage of protein enrichment of human milk, care should be taken to insure that the human milk protein isolate has fully preserved immunobiological properties. From our work on the immunobiological modification of cow’s milk infant formulas, we have gathered some experience in the isolation of a “milk immunoglobulin concentrate” (MIC) from cow’s milk (11,12). This chapter describes the isolation procedure employed in the hope that it will be of use to those who work on adapting human milk.
THE RATIONALE FOR MILK IMMUNOGLOBULIN CONCENTRATE

Modern infant milk formulas attempt to provide nutritional equivalence to human milk, but even the most sophisticated products are devoid of any protective immune factors. We consider this to be a serious deficiency, especially when a formula is used to feed high-risk infants such as the very-low-birth-weight infant, who is prone to antibiotic-resistant nosocomial infections.

Immunoglobulins (Ig) are thought to play an important role among the protective immune factors in human milk. We have therefore investigated the possibility that these human milk immunoglobulins can be replaced by bovine milk immunoglobulins when babies are formula fed.

To reach this goal, a number of preliminary steps were essential:

1. Defining the specificity of bovine milk Ig.
2. Measuring the resistance of bovine milk Ig to proteolytic digestion.
3. Measuring the efficacy of bovine milk Ig in impeding the pathological mechanisms of common neonatal bacterial pathogens.
4. Demonstrating the clinical efficiency of bovine-Ig-enriched milk formulas.
5. Developing a technological treatment of the antibody-containing milk that insures bacteriological safety of the product while conserving its immunobiological activity. It is the last point that will be described in most detail in this present chapter.

Bovine milk immunoglobulins are part of the whey protein fraction. In order to avoid unnecessary losses of product and activity, we have not set out to isolate a pure immunoglobulin fraction but rather to separate the total whey proteins including the immunoglobulins, serum albumin, α-lactalbumin, β-lactoglobulin, and some minor proteins including bovine milk lactoferrin. The term MIC is justified when it is prepared from milk collected during the first 30 days of lactation, thus including colostral milk (see Fig. 1). This
Distribution of constituents in milk of first 30 days of lactation:

Liquid full milk

Dry matter full milk

Proteins

Whey proteins = "MIC"

FIG. 1. Relative increase of immunoglobulin concentration in cow's milk as a result of removal of milk constituents other than whey proteins.
milk contains on average 0.42% total Ig. Removal of water results in a dry matter containing 3.2% Ig. Elimination of lactose, fat, and minerals gives a protein fraction with 10.5% Ig. The separation of casein finally results in a whey protein fraction containing 35% Ig.

THE TECHNOLOGY OF MIC PREPARATION

Figure 2 shows the general flow sheet for MIC preparation.

Untreated raw milk—especially colostrum milk—sometimes contains blood and other cellular material. In order to remove erythrocytes, other cells, and coarse impurities, the cold milk (+8°C to +12°C) is centrifuged in a regular milk separator. Separation into skim milk and fat is achieved by running the milk through the same centrifuge after heating the milk to +40°C.

In order to accumulate the necessary quantities of milk for batch handling, the skim milk is kept frozen at -25°C. We have not observed any loss of antibody activity using this method of storage.

In order to inactivate any contaminating viruses, the thawed skimmed milk is heated to +56°C in a plate heat exchanger. The milk is held at this temperature for 30 min in a jacketed vat equipped with a stirrer before cooling to +37°C.

At +37°C, the milk casein is precipitated by acidification to pH 4.5 or coagulated by renneting. To obtain a good contraction of the curd, the coagulated milk is heated once more to +56°C for 10 min.

A first separation of lactoserum from precipitated casein is done by simple decantation followed by two washings of the casein slurry with demineralized water. By centrifugation in a clarifier centrifuge, the casein is separated from the last washing. The original lactoserum and the casein washings, which are kept separately, are finally cleared of all fine particles by successive passage through the same centrifuge followed by filtration through a depth filter of the Seitz or Filtrox type. This final clarification is an essential step in order to avoid obstruction problems in the ultrafiltration procedure that follows.
FIG. 2. General flow sheet showing the applied techniques for the preparation of milk immunoglobulin concentrate from cow's milk.
Ultrafiltration (principle described in Fig. 3) is carried out in a membrane-type installation in order to remove the bulk of lactose and mineral salts. By the application of positive pressure on a constantly circulating solution, water and small-molecular-weight substances are forced to penetrate the membrane and are removed with the permeate. High-molecular-weight material cannot pass through the membrane and is preserved in the retentate. This separation according to molecular size depends, of course, on the type of membrane employed. We use a DDS (Danish Sugar Corporation) unit that is equipped with the membrane type GR 81 P, whose properties together with those of other possible membrane types are given in Table 1.

Ultrafiltration is divided into 3 steps:
1. preconcentration;
2. diafiltration; and
3. final concentration.

In the preconcentration step, the original lactoserum is concentrated three- to fourfold. In the diafiltration step, the volume of the
TABLE 1. Properties of DDS ultrafiltration membranes

<table>
<thead>
<tr>
<th>Membrane type</th>
<th>Water capacity (liters/m³/hr)</th>
<th>Lactose permeability (%)</th>
<th>Approx. cut-off value MW</th>
<th>Recommended operation range</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>300</td>
<td>100</td>
<td>65,000</td>
<td>pH 2–8, °C 0–50, MPa 0–0.5</td>
</tr>
<tr>
<td>600</td>
<td>150</td>
<td>98</td>
<td>20,000</td>
<td>pH 2–8, °C 0–50, MPa 0–1</td>
</tr>
<tr>
<td>800</td>
<td>80</td>
<td>95</td>
<td>6,000</td>
<td>pH 2–8, °C 0–50, MPa 0–2</td>
</tr>
<tr>
<td>GR61P</td>
<td>350</td>
<td>98</td>
<td>20,000</td>
<td>pH 0–14, °C 0–80, MPa 0–1.5</td>
</tr>
<tr>
<td>GR81P</td>
<td>100</td>
<td>95</td>
<td>6,000</td>
<td>pH 0–14, °C 0–80, MPa 0–1.5</td>
</tr>
<tr>
<td>FS60PP</td>
<td>300</td>
<td>100</td>
<td>30,000</td>
<td>pH 0–13, °C 0–80, MPa 0–1</td>
</tr>
<tr>
<td>GR60P</td>
<td>350</td>
<td>98</td>
<td>25,000</td>
<td>pH 1–13, °C 0–80, MPa 0–1</td>
</tr>
</tbody>
</table>

*Data measured after 1 hr of operation at 0.5 MPa (72.5 psi), +20°C. (From DDS membrane data sheet, DDS-Nakskov, Denmark.)

Preparation of Cow's Milk Ig

The retentate is kept constant by the successive addition of the two casein washings. The final concentration reduces the concentrate volume another twofold. This procedure is summarized in Fig. 4.

The resulting whey protein solution with 10% dry matter, 7 to 8% total protein, and 2 to 3% Ig is now submitted to sterile filtration. In order to avoid premature clogging of the sterile filter, careful prefiltration of the protein solution is extremely important. Seitz or Filtrox depth filter plates, type Supra EK, are used for prefiltration. Sterile filtration is accomplished by a combination of Seitz or Filtrox depth filter plates, type EKS, and a Millipore® membrane filter with 0.45-μm pore size.

In order to reduce the volume for the final step of drying by lyophilization, the sterile whey protein solution undergoes evaporation of water at low temperature and reduced pressure in a thin-film evaporator. The material is exposed to a maximum temperature of +40°C. Our evaporation installation is equipped to run under sterile conditions.

The concentrated whey protein solution with about 20% dry matter, 14 to 16% total protein, and 4 to 6% Ig is finally dried by freeze-drying under sterile conditions. We use a small production-sized freeze-dryer from Usifroid with a condenser capacity of 50 kg water. The composition of the resulting powder is shown in Table
Ultrafiltration of Lactoserum:

300 l Lactoserum
TP 1.3%
Ig 0.45%

UF -> 200 l Permeate

Pre-Concentration

200 l Casein Washing I

100 l Pre-Concentrate
UF -> 200 l Permeate

Dialfiltration

100 l Casein Washing II

100 l Pre-Concentrate
UF -> 100 l Permeate

Final Concentration

100 l Pre-Concentrate
UF -> 50 l Permeate

50 l Final Concentrate
TP 7.8% Ig 2.7%

UF-System : DDS Module 35-18
Membrane surface : 18 m²
Membrane type : GR 8 IP (cut-off MW - 6000)
Duration : 3 hours
Capacity : 10 l Permeate/m²/h

FIG. 4. Ultrafiltration of 300 liters of lactoserum with indication of retentate and permeate volumes in the course of preconcentration, diafiltration, and final concentration.
2. It can easily be mixed with milk powder and is perfectly soluble in water or liquid milk.

**DISCUSSION**

None of the applied techniques is new, but their combination, as described here, is of relevance to the preparation of a human milk protein isolate. A problem that will arise concerns the volume of milk to be treated. We work with volumes varying between 200 and 500 liters of milk from immunized cows, but smaller quantities of milk will be available in the case of human milk. This does not affect the fundamental approach described here but will affect the type of equipment used. Therefore, Table 3 not only shows the description of the installation as it is used in our work but also proposes alternatives that might be useful for work with smaller milk volumes. These indications do not claim to represent the best solution. That could only be established after careful investigations by milk engineering specialists.
TABLE 3. Preparation of MIC from cow's milk: technical equipment and possible alternatives for smaller milk volumes

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Equipment used for 200–500 kg full cow's milk</th>
<th>Equipment proposed for 20–50 kg full human milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifugation for milk clarification and skimming</td>
<td>Diabolo milk separator, type AE-574</td>
<td>Alfa Perfect milk separator type AE-31</td>
</tr>
<tr>
<td>Thermization, pasteurization</td>
<td>APV Plate heat-exchanger-type paraflow</td>
<td>Small-dimensional plate heat exchanger with a corresponding lower throughput rate</td>
</tr>
<tr>
<td>Centrifugation for casein removal and final clarification</td>
<td>Alfa-Laval closed-bowl clarifier, type K-2939 H</td>
<td>Westfalia closed-bowl centrifuge, type LG 205-8</td>
</tr>
<tr>
<td>Ultrafiltration</td>
<td>DDS Module 35-18</td>
<td>DDS Lab Unit 36–2.25</td>
</tr>
<tr>
<td>Filtration for clarification and sterilization</td>
<td>Seitz filter holder, type A-207 with 9 20 x 20-cm plates</td>
<td>Seitz filter holder, type A-207 with 2 20 x 20-cm plates</td>
</tr>
</tbody>
</table>
**TABLE 3. (Continued)**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Equipment used for 200–500 kg full cow’s milk</th>
<th>Equipment proposed for 20–50 kg full human milk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Evaporation at low temperature</strong></td>
<td>Filtration surface, 0.36 m²</td>
<td>Filtration surface, 0.08 m²</td>
</tr>
<tr>
<td></td>
<td>Filtrox filter holder, type Econom with 7 40 × 40-cm plates</td>
<td>Millipore disk filter holder YY 30293 16 with Millitube cartridge CEHA</td>
</tr>
<tr>
<td></td>
<td>Filtration surface, 1.12 m²</td>
<td>Filtration surface, 0.05 m²</td>
</tr>
<tr>
<td></td>
<td>Millipore cartridge housing YY 14 131 00 with Millitube cartridge CEHA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Filtration surface, 0.12 m²</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LUWA mechanically agitated thin film evaporator, type NL-150/1100/14</td>
<td>LUWA mechanically agitated thin-film evaporator, type L-82/500/14</td>
</tr>
<tr>
<td></td>
<td>Heating surface, 0.5 m²</td>
<td>Heating surface, 0.125 m²</td>
</tr>
<tr>
<td></td>
<td>Capacity, 50 kg water/hr</td>
<td>Capacity, 15 kg water/hr</td>
</tr>
<tr>
<td><strong>Freeze-drying</strong></td>
<td>Usifroid, model SM.I.R.</td>
<td>Usifroid, model SM.I.50.F.</td>
</tr>
<tr>
<td></td>
<td>Ice capacity of condenser, 45 kg water</td>
<td>Ice capacity of condenser, 9 kg water</td>
</tr>
</tbody>
</table>

**CONCLUSION**

The procedures used in the isolation of a milk immunoglobulin concentrate from cow’s milk could serve as a basis for the isolation of a protein isolate from human milk. Paradoxically, the introduction and realization of new procedures and isolation techniques are
often linked to problems of "scaling up," but here we are faced with the necessity of "scaling down."

It is hoped that the procedures described in this chapter may assist in the work of supplementing pooled banked human milk with a standardized, immunologically active human milk protein isolate for the optimal nutrition of preterm and very-low-birth-weight infants.

REFERENCES