Preliminary Communication

Occurrence of the Major Food Allergen, Ovomucoid, in Human Breast Milk as an Immune Complex

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The major food allergen, ovomucoid (molecular weight of 28 kDa) could be detected in 12 of 37 human breast milk samples by using three types of enzyme-linked immunosorbent assay. By gel-filtration, ovomucoid in breast milk was only eluted in the fractions corresponding to a molecular weight of about 450 kDa, suggesting its occurrence as an immune complex with IgA. In fact, almost the same elution profile as that for ovomucoid was obtained for its immune complex with IgA by gel-filtration.

Key words: human breast milk; ovomucoid; food allergy; monoclonal antibody; secretory IgA

The increase of food allergy in infants is one of the most severe problems for immediate solution in the developed countries. Although breast milk is considered to be the most appropriate food for infants nutritionally, immunologically, physiologically, and mentally, there are some reports that unfortunately suggest allergic sensitization via breast milk. Infants who had not taken any baby food have shown allergic symptoms.1 Ecwzema in 10 breast-fed babies disappeared within 11 days when their mothers abstained from egg and reappeared quickly when either the mothers or infants were given egg to eat.2 Food allergens such as β-lactoglobulin,3-5 ovalbumin,4,5 ovomucoid,4 and gliadin6 have been detected in breast milk from lactating women who consumed them before sampling and who followed a normal diet without any enforced dose of these antigens.7,8 However, the relationship between allergic symptoms and the existence of allergens in breast milk, the mechanism for their transport into breast milk, and their molecular features in breast milk are not well understood.

Hen’s egg is the most frequent cause of food allergy.9,10 Ovomucoid, a glycoprotein with a molecular weight of 28 kDa in egg white, is thought to be the major allergen11,12 despite the evidence presented in a controversial report.13 Its immunological properties have been revealed14-16 and the methods for producing low-ovomucoid egg white preparations have recently been proposed.12,17 We describe here a unique molecular feature of ovomucoid in human breast milk which is meaningful to understand the digestion, absorption, transfer and secretion processes of food proteins taken orally, and their immunological effects on infants.

Ovomucoid was chromatographically purified from fresh hen’s eggs. Antibera were obtained from rats that had been immunized with ovomucoid. Hybridomas producing monoclonal antibodies (mAbs) were established by the fusion of splenocytes of BALB/c mice immunized with ovomucoid and myeloma cells (NSI/1-Ag4-1) essentially as described previously.18 IgG fractions were purified in Protein A-Sepharose (Amersham Pharmacia Biotech, Buckinghamshire, England) columns from the rat antisera and the ascitic fluids from BALB/c mice injected with hybridoma clone 1H or 7D. They are designated as polyclonal IgG (pIgG) and mAbs 1H and 7D. As far as tested by an enzyme-linked immunosorbent assay (ELISA) with several proteins involving ovalbumin, ovotransferrin and lysozyme, these three antibodies did not react with proteins other than ovomucoid. Portions of pIgG and mAb 1H were biotinylated with EZ-Link sulfo-NHS-LC-Biotin (Pierce, IL, U.S.A.). Ovomucoid in breast milk was determined indirectly with alkaline phosphatase conjugated with streptavidin (Oncogene, NY, U.S.A.) and p-nitrophenylphosphate by three sandwich-types of ELISA.19 pIgG as a solid-phase antibody and its

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; mAb, monoclonal antibody; pIgG, polyclonal IgG
Ovomucoid in Human Breast Milk

Table 1. Determination of Ovomucoid in Human Breast Milk

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Assay I (ng/ml)</th>
<th>Assay II (ng/ml)</th>
<th>Assay III (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.33</td>
<td>0.84</td>
<td>1.06</td>
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<tr>
<td>10</td>
<td>0.50</td>
<td>4.00</td>
<td>0.96</td>
</tr>
<tr>
<td>12</td>
<td>0.68</td>
<td>4.00</td>
<td>3.57</td>
</tr>
<tr>
<td>13</td>
<td>0.33</td>
<td>2.00</td>
<td>0.94</td>
</tr>
<tr>
<td>14</td>
<td>0.39</td>
<td>28.0</td>
<td>1.53</td>
</tr>
<tr>
<td>20</td>
<td>0.50</td>
<td>3.52</td>
<td>5.85</td>
</tr>
<tr>
<td>21</td>
<td>1.53</td>
<td>3.00</td>
<td>5.85</td>
</tr>
<tr>
<td>23</td>
<td>0.44</td>
<td>2.00</td>
<td>0.99</td>
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<td>29</td>
<td>37.0</td>
<td>28.0</td>
<td>2.04</td>
</tr>
<tr>
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</tr>
<tr>
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<td>2.00</td>
<td>0.32</td>
</tr>
<tr>
<td>37</td>
<td>1.74</td>
<td>0.99</td>
<td>1.53</td>
</tr>
</tbody>
</table>

Breast milk samples were collected in sterile containers and stored at -20°C. Before the assay, they were thawed, adjusted to 0.02% NaN3, and centrifuged at 10,000 x g for 15 min at 4°C to remove particulate materials and fat. Ovomucoid was determined with alkaline phosphatase conjugated streptavidin by three specific types of sandwich ELISA. Polyclonal IgG as a solid-phase antibody and its biotinylated derivative as a second antibody were used for Assay I, polyclonal IgG and biotinylated monoclonal antibody 1H for Assay II, and monoclonal antibody 7D and biotinylated polyclonal IgG for Assay III.

Fig. 1. Gel-filtration of Breast Milk

After pretreating with 0.45 µm Millex-HA (Millipore, MA, U.S.A.), 0.2 ml of a sample was applied to a column of Cosmosil 5Diol-300 (7.5 mm × 300 mm, Nacalai Tesque, Kyoto, Japan) and eluted with 10 mM Na-Pi buffer at pH 7.4 containing 150 mM NaCl and 0.02% NaN3 at room temperature. The flow rate was 0.4 ml/min, and fractions were 0.2-ml each. The column was calibrated with gel filtration calibration kits (Amersham Pharmacia Biotech). Blue dextran 2000, a marker of the void volume, was eluted at fraction No. 29. Ovomucoid (open circle) was determined by Assay III, and its IgA-immune complex (closed circle) was determined by monoclonal antibody 7D and alkaline phosphatase-conjugated goat anti-human IgA. The arrow indicates the position where free ovomucoid was eluted.

Table 1 is mainly attributable to the competition of binding to ovomucoid between the mother’s IgAs and the antibodies used for the assays. Fukushima et al. have reported that ovomucoid is present in human breast milk as an immune complex with its specific IgA in breast milk. The difference among the assays shown in Table 1 is mainly attributable to the competition of binding to ovomucoid between the mother’s IgA and the antibodies used for the assays. Fukushima et al. have reported that ovomucoid is present in human breast milk as an immune complex with its specific IgA in breast milk. The difference among the assays shown in Table 1 is mainly attributable to the competition of binding to ovomucoid between the mother’s IgAs and the antibodies used for the assays.
et al. have determined ovalbumin and its specific IgA separately in human breast milk. On the other hand, Kilshaw et al. have demonstrated that while ovalbumin was present partially as an immune complex in the blood stream, it was in a free form in breast milk after the ingestion of one raw egg. This is the first report to show the occurrence of a food allergen as an immune complex with its specific IgA in human breast milk. Further analyses are necessary to reveal whether the phenomenon we have clarified here is specific to ovomucoid and how it is related to the condition or constitution of the mother.

Orally administered ovomucoid can appear in breast milk only after passing through many barriers such as digestion, absorption, the immune system, and transcytosis. What is the mechanism to accomplish this and how does the immune complex in breast milk affect babies? It is possible that the absorption of allergens in infants with immature digestion and absorption systems is suppressed by making allergens to the immune complexes in breast milk. The relationship between the presence of allergens in a mother’s milk and the allergic symptoms of their baby should be addressed further in consideration of not only the level but also the molecular features of the allergen.

Acknowledgment

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References