Methods

Subject:
- ★ Patients with FPGID (n = 60)
- ★ Patients of IgE-mediated cow’s milk allergy (n = 13)
- ★ Patients with Atopic Dermatitis who showed no clinical symptoms upon ingestion of cow’s milk (n = 16)
- ★ Cord blood samples from healthy infants (n = 10)

Mononuclear Cells

Incubation

α-lactalbumin 100 µg/ml each
β-lactoglobulin
α-casein
β-casein
κ-casein
Lipopolysaccharide (LPS)

Proliferation assay

Cytokine analysis

3H-thymidine uptake

Luminex assay

Lipopolysaccharide (LPS) concentrations in commercially available cow’s milk protein preparations

<table>
<thead>
<tr>
<th>Cow’s milk protein preparation</th>
<th>Before treatment (pg/mg)</th>
<th>After treatment (pg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Lactalbumin (Sigma, L-6010)</td>
<td>184,200</td>
<td>14</td>
</tr>
<tr>
<td>β-Lactoglobulin (Sigma, L-3905)</td>
<td>206,700</td>
<td>1,880</td>
</tr>
<tr>
<td>α-Casein (Sigma, C-6780)</td>
<td>540</td>
<td>23</td>
</tr>
<tr>
<td>β-Casein (Sigma, C-6905)</td>
<td>500</td>
<td>34</td>
</tr>
<tr>
<td>κ-Casein (Sigma, C-0406)</td>
<td>400</td>
<td>41</td>
</tr>
<tr>
<td>LPS-depleted β-Lactoglobulin (Bean Stalk Snow Co., Ltd.)</td>
<td>29</td>
<td>-</td>
</tr>
</tbody>
</table>

LPS concentration was measured by limulus amebocyte lysate (LAL) assay.

Impact of LPS on lymphocyte proliferation assay

Even as low as 100 pg/ml of LPS was sufficient to induce significant proliferative responses.

Mononuclear cells from younger children were more strongly affected by LPS than those from older children.

lympho-proliferative responses against LPS depleted cow’s milk components

The dot plots represent the maximum values obtained from each patient among 5 milk components.
Cytokine releases against LPS depleted cow’s milk components

**TNF-α**

**IL-6**

**IFN-γ**

**IL-17**

**IL-5**

**IL-13**

**Summary**

<table>
<thead>
<tr>
<th>Proliferation</th>
<th>++</th>
<th>++</th>
<th>+</th>
<th>±</th>
<th>+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine analysis</td>
<td>TNF-α</td>
<td>TNF-α</td>
<td>TNF-α</td>
<td>TNF-α</td>
<td>IL-6</td>
</tr>
<tr>
<td>IL-6</td>
<td>IL-8</td>
<td>IL-8</td>
<td>IL-8</td>
<td>IL-8</td>
<td></td>
</tr>
<tr>
<td>IFN-γ</td>
<td>IL-4</td>
<td>IL-5</td>
<td>IL-5</td>
<td>IL-5</td>
<td></td>
</tr>
<tr>
<td>IL-13</td>
<td>IL-13</td>
<td>IL-13</td>
<td>IL-13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Cluster 1**
- Vomiting
- Bloody stool

**Cluster 2**
- Vomiting
- Bloody stool

**Cluster 3**
- Bloody stool

**Cluster 4**
- Bloody stool

**Control**
- Type 1

**FPID**

**EGID**

**Proctocolitis**

**Enteropathy**

**Profound**