Acute and chronic immunological response to dietary antigen

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SUMMARY On separate mornings at 14 day intervals groups of six to eight healthy fasting male volunteers drank a 5.5 MJ test meal containing 20% milk, egg or soya protein, or a control protein free preparation. Using a Clq binding test, marked but transient rises in circulating immune complexes were detected 30–120 minutes after the milk and egg meals but not with soya or the control. No such changes were seen when complexes were measured by Raji cell immunoassay. Food antigen specific antibodies were present in the plasma of all subjects but showed no consistent pattern of variation in the postprandial period. In most volunteers a chronic increase in milk (three weeks) or soya (six weeks) consumption produced no changes in circulating immune complexes or antibodies to dietary protein. Although two of 16 milk and six of 52 soya volunteers had substantial rises of one or more classes of plasma food antigen specific antibodies they were matched by a similar number in which a decrease was recorded. These results indicate that the formation of circulating immune complexes may be a physiological response to a large load of dietary antigen but that in most adults a chronic increase of milk or soya consumption does not affect food antibody or immune complex concentrations.

At the turn of the century a series of ingenious human and animal1 2 experiments showed that unaltered food protein can reach the systemic circulation in small amounts. More recent studies have shown immunoreactive dietary antigen in both the mesenteric lymphatics3 and the portal venous system and suggest that up to 2% of food protein is absorbed in this way.4 5 Despite this continuous antigenic challenge most adults have only very low levels of circulating antibodies to common dietary proteins such as casein, ovalbumen, or gluten.6 7 Antibody levels may be increased, however, in coeliac disease or severe ulcerative colitis2 and some studies have suggested that antibodies to certain constituents of cow's milk are raised in patients with ischaemic heart disease.8 9

Because of the potential importance of systemic immune reactions to dietary antigens we studied the response to both animal and vegetable proteins on both an acute and chronic basis. Particular attention was paid to variations in the concentrations of plasma food antigen specific antibodies and circulating immune complexes.

Methods

SUBJECTS

Experiment 1 Acute feeding At 14 day intervals, after an overnight fast, groups of eight healthy male volunteers, aged 21–42 years, drank a 500 ml test meal containing soya, milk, or egg protein or an isoenenergetic control protein free preparation (Table 1). Food antibodies and circulating immune complexes were then estimated in venous blood samples taken at 0, 30, 60, 90, 120, and 180 minutes. Antigen specific antibodies were measured by an enzyme linked immunosorbent assay (ELISA). The assays were performed on polystyrene microtitre plates which had been coated with antigen (dried skimmed milk, hen ovalbumen, or a freeze dried extract of promine D) by overnight incubation at 4°C with 210 µl per well of a 10 µg/ml solution of test protein in pH 9.6 carbonate buffer. Between all stages of the assay plates were thoroughly washed with 0.9% saline containing, like all subsequent
buffering solutions, 0.05% Tween 20. Twenty microlitres of diluted test serum was added for three hours at room temperature. A 1:500 dilution of commercial IgA, IgG, or IgM rabbit antihuman antibody conjugated with horseradish peroxidase was then incubated for two hours. The subsequent assay mixture contained 1 ml of 1% aqueous hydrogen peroxide (substrate) and 0.85 ml of 1% dianisidine hydrochloride (chromogen) per 100 ml of pH 6.0 phosphate buffer. The reaction was read at one hour in a Dynatech plate reading spectrophotometer at 450 nm. The results were expressed as a product of the optical density reading and the serum dilution, corrected to a standard enzyme activity. The coefficient of variation of replicate analyses was always less than 10%.

Circulating immune complexes were measured by both Clq binding\(^\text{10}\) and Raji immunoassay,\(^\text{11}\) with minor modifications.\(^\text{12,13}\) The Clq binding test measures the ability of immune complexes to bind to radiolabelled purified Clq. Bound complexes are precipitated with 3% polyethylene glycol and the radioactivity of the centrifuged precipitate is expressed as the per cent of the activity of the same amount of labelled Clq precipitated with 20% trichloroacetic acid. The upper limit of normal was taken as 5% and the coefficients of variation of replicate analyses ranged between 6.5 and 23.3%.

In the Raji immunoassay cultured lymphoblastoid cells, rich in complement receptors but free of native surface Ig, are incubated with the test serum and a source of complement. Immune complexes which have activated complement bind to the cell surface and can be assayed with labelled antihuman immunoglobulin antibodies. Alkaline aggregated immunoglobulin was used as a standard and the results expressed as the amount of AHG which would produce the same result as the test serum. The upper limit of normal was taken as 41 µg AHG, the mean plus two standard deviations (15±13) of a group of 65 normal subjects. The coefficients of variation of replicate analyses was less than 20%.

**Experiment 2: Chronic** Thirty-nine male and 27 female volunteers, range 20–68 years, ate at least four meals per week based on textured vegetable protein, for 44 days. The products used included mince meat extenders, prepared dinners (goulash, curry, stroganov, etc), and a soya protein based milk. Sixteen different subjects (10 men and six women, range 21–42 years) increased their daily milk consumption for 21 days. They drank at least 700 ml milk (46 g protein) and 300 g commercial fruit flavoured yoghurt per day, although some drank 500 ml of a concentrate containing 192 g of dried skimmed milk instead.

In the soya experiment all volunteers completed a 24 hour dietary recall questionnaire before, and 35 of the 54 a detailed three day weighed food survey towards the end of the six weeks. In the milk experiment the 16 subjects and seven additional controls wrote up a three day dietary record before beginning the project. Mean daily nutrient intakes were then determined using computerised food tables.\(^\text{14}\) The programme used provided details of total energy intake, proportions of fat, carbohydrate and protein, and allowed individual food stuffs such as milk and soya protein to be calculated separately.

Fasting blood samples were taken at the start and finish of each experiment. Immune complexes were measured by the methods used in Experiment 1. Antibodies to milk protein were measured by ELISA in polystyrene microtitre plates but 64×10 mm diameter test tubes were used for the soya antibody assays. Total cholesterol and triglycerides were estimated in a Technicon autoanalyser and HDL cholesterol values by heparin-manganese chloride precipitation.

**Results**

**Experiment 1: Acute Feeding**

Antibodies to egg, milk, and soya were detected in all serum samples. There was considerable variation in concentrations between subjects (10–100 fold) but values in individual volunteers changed little from experiment to experiment. No consistent pattern of change was observed in the serial, postprandial blood samples. In most subjects values altered by less than ±25%, although the percentage change was sometimes greater when the fasting values were low. The greatest range and percentage variation was with ovalbumen antibodies.

Results of immune complex assays, as determined by Clq binding, are illustrated in the Figure. After either milk (subjects 4, 5, and 6) or egg (subjects 2,
3, 4, 6, and 7) there was a substantial postprandial rise of immune complexes. The time after which the highest values were recorded varied between 30–120 minutes and usually only one very high value was obtained in each subject. In the control (protein free) experiment only one volunteer showed a postprandial response whereas none of the six subjects reacted to the soya meal. In subject 5 there was an inexplicable rise in immune complexes in the fasting sample taken before the egg experiment.

In striking contrast to the C1q results there was no evidence of increased levels of immune complexes when Raji immunoassay was used (Table 2). Only occasional values exceeded the arbitrary upper limit of normal (41 μg AHG), the highest value being 59 μg AHG.

There were no significant changes in total serum cholesterol concentrations. Postprandial increases in triglycerides were most marked after soya protein and least evident after casein.

**Experiment 2: Chronic Feeding**

**Dietary data** In the soya experiment there was a substantial rise in total energy intake over the six week experimental period (11·0±0·6 SEM vs 10·1±0·7 MJ/day). Protein, fat, and carbohydrate were all increased but there was no significant change in the distribution of energy between the different nutrients. Soya consumption was negligible (0·5%) at the start of the experiment but six weeks later accounted for 22·7±2·3% of total protein.

At the start of the milk experiment volunteers and controls were eating between 12 and 48 g of milk protein per day (mean 22·4 g). With a minimum intake of 60 g milk protein during the experiment volunteers increased their consumption approximately three fold. Some subjects complained of

**Table 2** Acute experiments. Circulating immune complexes by Raji cell immune assay after a 5·5 MJ test meal

<table>
<thead>
<tr>
<th>Test meal</th>
<th>Time after test meal (min)</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>13±2</td>
<td>15±3</td>
<td>14±3</td>
<td>12±2</td>
<td>15±3</td>
<td>15±3</td>
</tr>
<tr>
<td>(no protein)</td>
<td></td>
<td>19±7</td>
<td>12±3</td>
<td>13±4</td>
<td>18±6</td>
<td>14±2</td>
<td>13±5</td>
</tr>
<tr>
<td>Milk</td>
<td></td>
<td>13±5</td>
<td>13±3</td>
<td>10±2</td>
<td>12±3</td>
<td>10±2</td>
<td>16±3</td>
</tr>
<tr>
<td>Egg</td>
<td></td>
<td>17±4</td>
<td>29±18</td>
<td></td>
<td>27±13</td>
<td>29±16</td>
<td>20±11</td>
</tr>
</tbody>
</table>

Results expressed as means ± SEM in μg AHG equivalents per ml serum.
flatulence in the soya study and most taking milk
reported a loss of appetite.

**Immunological assays**

The results of the antibody assays are summarised in
Table 3. Using a paired t test no changes were
observed in the mean levels of any class of antibody
to either soya or milk protein. In six individuals in
the soya experiment and two in the milk study a
substantial increase in one or more classes of
antibody was recorded. Conversely, a reduction was
noted in an almost exactly equal number. The
relative proportions of the various classes of serum
antibodies to both milk and soya protein was similar
to the distribution in normal serum. There were no
appreciable changes in immune complex concen-
trations in either experiment.

**Cholesterol and triglycerides**

A significant increase in both total and HDL-
cholesterol and triglycerides occurred during the
soya experiment. There was a positive correlation
between the initial and final values of plasma total
cholesterol (r=+0.56, p<0.05). In the milk study there
was a slight but significant fall in both total and
HDL cholesterol in the test group but no changes
were observed in controls. In both groups there was
a significant correlation between initial and final
cholesterol values (test r=0.9, p<0.01; control
r=0.95, p<0.001). Triglyceride values showed no
important changes in the milk feeding experiment.

**Discussion**

The object of these experiments was to study
variations in food antigen specific antibodies and
circulating immune complexes after acute and
chronic alterations in dietary protein. Although
food antibodies were detected in all subjects these
were not influenced by a three to six week milk or
soya rich diet. A substantial but transient rise in
immune complexes was observed in most volunteers
after a meal containing 66 g of milk or egg
protein. No such changes were found with soya or
with a single exception the control protein free
preparation.

There is no satisfactory explanation as to why
healthy adults have such low concentrations of
circulating food antibodies despite a continuous low
grade exposure to dietary antigens from the gastro-
intestinal tract. An efficient mechanism must exist to
maintain this form of tolerance or hyporesponsiv-
ness, as shown by our failure to alter antibody
centrations in the chronic feeding experiments.
There is good clinical and experimental evidence
that the liver plays a central role in the phagocytosis
of both antigenic material and immune complexes
arriving via the portal venous system. Increased
concentrations of bacterial and dietary antibodies
can be detected in patients with advanced liver
disease and hyporesponsiveness to orally adminis-
tered antigens is diminished in experimental animals
with hepatic cirrhosis. Cell transfer studies in this
animal model suggest that T as opposed to B
lymphocytes mediate the hyporesponsiveness to
gastrointestinal antigens, and indeed other groups
have implicated suppressor T cells in Peyer’s patches
of the small intestinal mucosa. In contrast André
and his colleagues found that tolerance produced
by gastrointestinal challenge could be transferred by
a cell free preparation rich in complexes of antigen
and IgA.

Animal experiments suggest that complexes
containing more than two molecules of antigen and
antibody are rapidly taken up by the liver and
spleen. It may well be that after a milk or egg rich
meal complexes of a variety of molecular sizes form
but only the smaller evade phagocytosis for any
period of time. Experiments in our laboratory have
shown that Clq binding and Raji immunoassay both
detect immune complexes greater than c 1×10^6
Daltons molecular weight, but that only Clq
measures complexes smaller than this. In this
context it is important that in the acute feeding
experiments described above only Clq binding was
positive, suggesting that postprandial circulating
complexes are of relatively small molecular size.

Three previous studies have investigated the
appearance of immune complexes in the circulation
after milk drinking. In two of these relatively small
amounts of milk were used (100 and 250 ml: 6-6 and
16-5 g protein) and complexes were detected only in
patients with IgA deficiency. There was no
measurable response in subjects with systemic lupus
erythematosus or normal controls. In contrast
Paganelli et al used much larger amounts of milk

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**Table 3 Experiment 2: food antibody concentrations in
chronic feeding studies**

<table>
<thead>
<tr>
<th></th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soya</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>63±6</td>
<td>17±1</td>
<td>26±3</td>
</tr>
<tr>
<td>Day 42</td>
<td>60±6</td>
<td>17±1</td>
<td>23±3</td>
</tr>
<tr>
<td>Milk volunteers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>2-85±0.43</td>
<td>0.79±0.16</td>
<td>0.48±0.05</td>
</tr>
<tr>
<td>Day 21</td>
<td>3-35±0.57</td>
<td>0.73±0.15</td>
<td>0.49±0.07</td>
</tr>
<tr>
<td>Milk controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>2.55±0.69</td>
<td>0.84±0.30</td>
<td>0.39±0.07</td>
</tr>
<tr>
<td>Day 21</td>
<td>2.56±0.71</td>
<td>0.84±0.39</td>
<td>0.37±0.07</td>
</tr>
</tbody>
</table>

* All values expressed as means ± SEM in arbitrary units.
  Milk and soya antibodies not quantitatively comparable.
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(1200 ml; 80 g protein) and found complexes by two separate techniques, including an antigen specific assay.

These results, and the findings of our own study indicate that healthy adults must consume relatively large amounts of protein before immune complexes can be measured in the circulation and that Clq binding is more likely to detect these complexes than Raji immunoassay.

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References

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