Polycrnonal B-Cell Response to Stimulation with *Escherichia coli* Lipopolysaccharide in Dietary Protein Restriction

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The polyclonal B-cell response to *Escherichia coli* lipopolysaccharide was studied in C57BL/6 mice maintained after weaning on either a moderate protein-restricted diet with 8% casein or a normal diet. After in vitro or in vivo stimulation with the endotoxin, autoreactive and anti-hapten antibody-producing cells were quantitated by direct plaque assay, using bromelain-treated mouse erythrocytes and trinitrophenylated sheep erythrocytes as targets. Larger numbers of plaque-forming cells were generated in cultures of spleen cells from dietary-restricted than from normal mice stimulated with various doses of lipopolysaccharide. The number of background plaque-forming cells was also higher in nonstimulated spleen cell cultures from restricted animals. After injection of lipopolysaccharide in vivo, the number of cells producing antibodies to bromelain-treated mouse erythrocytes per 10^7 spleen cells was significantly increased in dietary-deficient mice. The results are discussed in relation to the different sensitivities of lymphocyte populations to protein deficiency and to the possible presence of high levels of endogenous polyclonal B-cell activators in the restricted mice.

Polyclonal B-cell activators (PBA) such as bacterial lipopolysaccharide (LPS) induce B-cell proliferation as well as polyclonal antibody synthesis manifested by the production of antibodies against synthetic antigens (1) and autoantibodies (7, 12, 13, 15, 24, 30). Prolonged or intense PBA stimulation is now considered an important factor in the pathogenesis of autoimmune reactions (10, 16, 36).

Treatment of mouse erythrocytes with the proteolytic enzyme bromelain exposes the surface antigen Hb normally hidden in the cell surface (19). Plaque-forming cells (PFC) with specificity against bromelain-treated mouse erythrocytes (Br-MRBC) are normally present in small quantities in the spleen, lymph nodes, and peritoneal cell suspensions from mice of the autoimmune and nonautoimmune strains (6, 8, 20, 26), but their number increases significantly after stimulation with LPS (6, 7, 15).

In previous studies we have observed increased immunoglobulin M PFC response in vivo to alloantigens (21) and in vitro to sheep erythrocytes (SRBC), to trinitrophenyl (TNP)-Ficoll, and to TNP-LPS (29), as well as alterations in the regulation of the antibody response (2, 29), in mice with moderate dietary protein restriction starting after weaning. To further evaluate the influence of nutritional deficiency on B-cell function, we have studied the polyclonal B-lymphocyte response to *Escherichia coli* LPS in C57BL/6 strain mice that were fed diets with either low or normal protein content.

**MATERIALS AND METHODS**

**Experimental animals and diets.** Weanling male C57BL/6 mice obtained from the Jackson Laboratory (Bar Harbor, Maine) or bred at Instituto Venezolano de Investigaciones Científicas were rested for 1 week and then distributed at random into two groups. The experimental protein-deficient group (D) received a low-protein diet containing 8% casein, and the normally nourished group (N) received a diet with 26% casein (21). Both diets, obtained from ICN Nutritional Biochemicals (Chicago, Ill.), were isocaloric with complete vitamin and mineral supplements and were administered ad libitum. D mice appeared healthy, and their life span was not affected during a 24-month observation period. Restricted animals constantly failed to gain weight during the initial 4 weeks of diet and thereafter showed a progressive rise in their body weight. However, after the end of the first week of diet, the mean body weight of D animals was significantly reduced as compared with that of the N controls at all times (21). At 2, 4, and 12 weeks after the initiation of the diet the mean (± standard error) weight of D and N mice as assessed in at least 20 animals per group was: (i) week 2—D, 16.5 ± 0.5; N, 20.5 ± 0.4 (P < 0.001); (ii) week 4—D, 16.6 ± 0.6; N, 23.8 ± 0.6 (P < 0.001); (iii) week 12—D, 23.3 ± 0.5; N, 30.8 ± 0.6 (P < 0.001).

**Spleen cell cultures.** Spleen cells pooled from at least 5 animals per group were suspended in medium RPMI 1640 supplemented with antibiotics, 5% fetal calf serum (all from GIBCO laboratories, Grand Is-
TABLE 1. Polyclonal PFC response in 72-h cultures of spleen cells from D and N mice stimulated with E. coli LPS as tested on Br-MRBC and TNP-SRBC

<table>
<thead>
<tr>
<th>LPS dose (µg)</th>
<th>Br-MRBC</th>
<th>TNP-SRBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D</td>
<td>N</td>
</tr>
<tr>
<td>0.1</td>
<td>186 ± 50</td>
<td>121 ± 50</td>
</tr>
<tr>
<td>1</td>
<td>367 ± 92</td>
<td>201 ± 52</td>
</tr>
<tr>
<td>10</td>
<td>387 ± 45</td>
<td>231 ± 32</td>
</tr>
<tr>
<td>None</td>
<td>34 ± 17</td>
<td>18 ± 11</td>
</tr>
</tbody>
</table>

* Pooled results of three experiments.
* b Mean ± SE number of PFC per culture obtained in the three experiments.
* c Statistical analysis was performed by means of the t test for paired data. NS, Not significant.

Spleen cells from mice kept on the experimental and the control diets for 3 to 6 weeks were stimulated with doses of LPS ranging from 0.1 to 10 µg and tested for PFC after 72 h of culture. PFC against Br-MRBC and TNP-SRBC appeared in larger numbers in D than in N spleen cell cultures, and the maximal PFC response of N spleen cells remained below the maximal response of D spleen cells. Larger numbers of background PFC with specificity to Br-MRBC were also generated in unstimulated cultures of D than N spleen cells (Table 1).

To see whether the increased polyclonal antibody formation observed in 72-h cultures of D spleen cells was related to differences in the kinetics of PFC generation, spleen cells from D and N mice were stimulated with 10 µg of LPS and assayed against Br-MRBC and TNP-SRBC at 48, 72, and 96 h of culture. The number of PFC detectable on either target was higher in cultures of D than N spleen cells at all times tested (Fig. 1).

Despite the increased polyclonal antibody response of D spleen cells, the magnitude and the kinetics of the proliferative response, as evaluated by DNA synthesis, were similar in D and N spleen cell cultures (not shown). This observation agrees with previous work demonstrating that the polyclonal antibody response to LPS is independent of DNA synthesis (20).

![FIG. 1. Kinetics of the generation of PFC against Br-MRBC and TNP-SRBC in cultures of spleen cells from D and N C57BL/6 mice stimulated with 10 µg of E. coli LPS. Results are expressed as the mean ± SE (bars) number of PFC detected in triplicate assays.](http://iai.asm.org/Downloaded from http://iai.asm.org/ on October 19, 2017 by guest)
TABLE 2. Effect of dietary protein restriction on the generation of PFC reacting against Br-MRBC in the spleens of C57BL/6 mice stimulated in vivo with E. coli LPS

<table>
<thead>
<tr>
<th>Weeks on diet</th>
<th>Dietary group (no. of mice)</th>
<th>LPS dose (μg)</th>
<th>Cells per spleen (×10⁴)</th>
<th>PFC to Br-MRBC (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PFC per 10⁷</td>
</tr>
<tr>
<td>2</td>
<td>D (18)</td>
<td>10</td>
<td>145 ± 12</td>
<td>317 ± 73</td>
</tr>
<tr>
<td></td>
<td>N (18)</td>
<td></td>
<td>254 ± 25</td>
<td>80 ± 16</td>
</tr>
<tr>
<td></td>
<td>D (17)</td>
<td>50</td>
<td>118 ± 10</td>
<td>472 ± 103</td>
</tr>
<tr>
<td></td>
<td>N (17)</td>
<td></td>
<td>198 ± 13</td>
<td>241 ± 48</td>
</tr>
<tr>
<td>4</td>
<td>D (10)</td>
<td>10</td>
<td>139 ± 22</td>
<td>173 ± 37</td>
</tr>
<tr>
<td></td>
<td>N (11)</td>
<td></td>
<td>193 ± 26</td>
<td>46 ± 7</td>
</tr>
<tr>
<td></td>
<td>D (10)</td>
<td>50</td>
<td>252 ± 26</td>
<td>485 ± 100</td>
</tr>
<tr>
<td></td>
<td>N (11)</td>
<td></td>
<td>280 ± 26</td>
<td>184 ± 20</td>
</tr>
<tr>
<td>≥12</td>
<td>D (15)</td>
<td>10</td>
<td>155 ± 11</td>
<td>184 ± 37</td>
</tr>
<tr>
<td></td>
<td>N (13)</td>
<td></td>
<td>175 ± 12</td>
<td>77 ± 19</td>
</tr>
<tr>
<td></td>
<td>D (10)</td>
<td>50</td>
<td>154 ± 18</td>
<td>592 ± 163</td>
</tr>
<tr>
<td></td>
<td>N (11)</td>
<td></td>
<td>198 ± 20</td>
<td>117 ± 35</td>
</tr>
</tbody>
</table>

* Pooled results from two or three experiments, each one consisting of four to seven animals per dietary group.

* Statistical significance was calculated by Student's t test. NS, Not significant.

In agreement with the results of in vitro experiments, 72 h after stimulation with LPS in vivo, the number of anti-Br-MRBC PFC per 10⁷ spleen cells was higher in D than in N mice (Table 2). The total number of nucleated spleen cells was significantly diminished in mice with short-term (2 and 4 weeks) protein restriction and was also lower in mice with long-term (≥12 weeks) restriction than in the normally fed controls, although in this chronic group the differences were not significant. In spite of the reduction in the spleen cell content, the total number of PFC per spleen tended to be higher in D than in N mice due to the significant increase in the proportion of anti-Br-MRBC PFC in the restricted animals (Table 2). Background PFC against Br-MRBC per 10⁷ nucleated cells were also higher in the spleen of nonstimulated D mice (mean ± standard error PFC per 10⁷ cells in 17 mice per dietary group tested: D, 39 ± 6; N, 21 ± 3; P < 0.0255).

DISCUSSION

The results presented here show increased numbers of PFC against Br-MRBC and TNP-SRBC in spleen cell suspensions of protein-restricted C57BL/6 mice after in vitro or in vivo stimulation with E. coli endotoxin.

It has been shown that B cells responsive to LPS are more mature than those responsive to dextran sulfate (14). Furthermore, recent studies indicated that cells secreting autoantibodies against Br-MRBC are relatively mature B lymphocytes which bear the surface antigen Lyb3 (18), appear late in ontogeny, and are absent in CBA/N mice (18, 23) expressing a defect in the development of mature B lymphocytes (33). It is possible that preferential depletion of immature cells highly susceptible to protein deficiency (9, 22) determines a rise in the proportion of mature B lymphocytes, which respond to LPS with antibody synthesis, in the spleen of restricted-diet animals. On the other hand, since the synthesis of antibodies against self-antigens exposed on Br-MRBC appears to be controlled by suppressor T cells (7), a deficit of suppressor T-cell function could contribute to the increased numbers of autoreactive PFC in the spleens of D mice. This possibility is supported by previous work demonstrating failure in the suppressor regulation of the antibody response to SRBC in protein restriction (2, 29). A deficit in the number or function of adherent cells in the restricted mice (5), which participate in the suppressor control of the response to LPS (4), could also result in increased formation of PFC after LPS stimulation.

The increased numbers of background PFC observed in unstimulated cultures of D spleen cells and in the spleens of intact D mice could be determined by the presence of elevated levels of endogenous PBA which could have additive effects with LPS stimulation, resulting in increased polyclonal antibody synthesis. Endogenous PBA could also contribute to the high levels of serum immunoglobulins observed in some studies of nutritionally deprived children (3, 25, 35) and dietary-deficient mice (27). It is possible that bacterial or viral PBA appear in D animals as a consequence of their increased susceptibility to infections (34), or that abnormal distribution of T and B cells promotes cell interactions favoring the synthesis of endogenous PBA (36).

The high polyclonal antibody response in diet-restricted C57BL/6 mice, with increased autoan-
tibody formation, is in contrast with the retardation of autoimmune phenomena by nutrient deficiency in the autoimmune strains (11, 32), suggesting that the effects of dietary restriction on immune reactions could vary according to the genetic background and the developmental pattern of the lymphoid system.

ACKNOWLEDGMENT

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LITERATURE CITED