Note

Effects of Vitamin B₆ Deficiency on Cytokine Levels and Lymphocytes in Mice‡

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The effects of vitamin B₆ (B₆) deficiency on cytokine levels and proportions of lymphocyte subsets in BALB/c mice were investigated. The proportion of lymphocytes from the thymus and spleen of mice given no B₆, that were CD4⁺ CD8⁻ T cells, was larger than in mice given B₆, and the ratio of CD8⁻ to CD4⁺ T cells in the thymus of mice given no B₆ was lower. The concentrations of interleukin-5 and -10 in spleen cells stimulated in vitro with concanavalin A were significantly higher in the mice with B₆ deficiency, as was their plasma corticosterone concentrations. These results suggested that B₆ is necessary to maintain cytokine levels and lymphoid function in the thymus and spleen of mice.

Key words: vitamin B₆ deficiency; cytokine; lymphocyte

Vitamin B₆ (B₆) has long been recognized for its roles in growth, development, and biological functions as a cofactor in many enzyme reactions that regulate amino acid metabolism. Deficiency of this vitamin in animals including humans affects both humoral and cell-mediated immune responses.¹,² Meydani et al.⁹ report that B₆ deficiency impairs interleukin (IL) 2 production and lymphocyte proliferation in elderly. Cytokines including IL are important in the communication network that links inducer and effector immune and inflammatory cells.⁹ The balance of cellular and humoral immune responses to antigens seems to be controlled by communication between immunocompetent cells.

We have reported in a preceding paper¹⁰ the effects of B₆ deficiency on antibody production in BALB/c mice. The splenocytes of such mice not given B₆ have a higher concentration of IL-4 and a lower concentration of IL-2 when stimulated with concanavalin A (ConA) than such splenocytes of mice given B₆. To the best of our knowledge, the effects of B₆ deficiency on other cytokines and on lymphocytes in mice have not been reported. Appropriate amounts of B₆ improve the deficiency and restore cytokine regulation to normal. Cell recognition of cytokines produced by T cells underlies the immune response to antigens. We investigated cytokine levels and lymphoid function in the thymus and spleen of mice.

Female BALB/c mice 3 wk old and weighing about 8 g were obtained from Japan SLC (Hamamatsu, Japan). The 72 mice were randomly divided into six dietary groups. The groups were given isoenergetic six diets but with either 20% or 60% casein and with pyridoxine hydrochloride at 0.0, 0.7, 7.0 mg%, respectively. Mice were housed individually in cages in an air-conditioned room at 23 ± 1° C. Water was available ad libitum.

Thymic and splenic lymphocytes were collected aseptically by the method of Yada and Fujitawara from mice on six diets for 4 wk. T lymphocytes were treated with rat anti-mouse monoclonal antibodies (L3T4) to CD4 conjugated with fluorescein isothiocyanate or with rat anti-mouse monoclonal antibodies (LY-2) to CD8 conjugated with (R)-phycoerythrin (PharMingen, San Diego, CA, USA). The T-cell subsets were sorted and counted with a flow cytometer (FAC Sort, Becton Dickinson, Mountain View, CA, USA). By the method of Mosmann,¹¹ the splenocyte proliferation caused by ConA was assayed with 3-((4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). The stimulation index (SI) was defined as the mean absorbance of MTT formazan in splenocytes stimulated by ConA being divided by the mean absorbance of MTT formazan in untreated splenocytes.

Mouse IL-5 and IL-10 in culture supernatants were assayed with a kit for an enzyme-linked immunosorbent assay. (Endogen, Cambridge, MA, USA). In brief, spleen cells from one mouse were cultured in triplicate on ninety-six well flat-bottomed culture plate with culture medium. The splenocytes were then stimulated with ConA (190 µg/ml, 100 µg/well, Sigma Chemical Co., St. Louis, Mo, USA) for induction of IL-4, IL-5, and IL-10 during 72 h of incubation at 37°C in a 5% CO₂ incubator. After incubation, the mixtures in the wells were centrifuged for 10 min at 800 × g and the supernatants were assayed for cytokines, respectively.

Corticosterone was assayed with a kit for radioimmunoassay (Teikoku-zoki Co., Kawasaki, Japan) containing mouse antiserum against corticosterone-3-oxime conjugated with bovine serum albumin. A diethyl ether extract of plasma was put on a Sephadex LH-20 column and the fraction presumably containing corticosterone was assayed by radioimmunoassay.

Differences were evaluated for statistical significance by ANOVA and Duncan's multiple-range test. Differences with p < 0.05 were taken to be significant.⁹

The B₆ requirement depends on the amount of protein in the diet.⁹ The diet with 60% casein and 0.0 mg% B₆ would give the most severe B₆ deficiency in this experi-

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¹ Effect of Vitamin B₆ Deficiency on an Antibody Production in Mice. Part II. For Part I, see Ref. 5.
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Cytokine Levels and Lymphocytes in Vitamin B₆ Deficiency

Table I. Effects of Vitamin B₆ Deficiency on T-Cell Subsets from Spleen and Thymus in Mice on Six Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Spleen</th>
<th>Thymus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (%)</td>
<td>B₆ (mg%)</td>
<td>CD4⁺CD8⁻</td>
</tr>
<tr>
<td>20</td>
<td>0.0</td>
<td>18.6±0.2²</td>
</tr>
<tr>
<td>20</td>
<td>0.7</td>
<td>15.4±0.3²</td>
</tr>
<tr>
<td>20</td>
<td>7.0</td>
<td>13.6±0.0⁹</td>
</tr>
<tr>
<td>60</td>
<td>0.0</td>
<td>23.4±0.7²</td>
</tr>
<tr>
<td>60</td>
<td>0.7</td>
<td>13.7±0.5³</td>
</tr>
<tr>
<td>60</td>
<td>7.0</td>
<td>10.5±1.0³</td>
</tr>
</tbody>
</table>

Values are means±SD of 12 mice. Values in the same column without the same superscript are significantly different (p<0.05, ANOVA and Duncan’s multiple range test).

Table II. Effects of Vitamin B₆ Deficiency on Splenocyte Cytokine Levels, the Splenocyte Stimulation Index (SI), and Plasma Concentrations of Corticosterone in Mice on Six Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Interleukins</th>
<th>SI</th>
<th>Plasma Corticosterone (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (%)</td>
<td>IL-4 (µg/ml)</td>
<td>IL-5 (µg/ml)</td>
<td>IL-10 (µg/ml)</td>
</tr>
<tr>
<td>20</td>
<td>0.0</td>
<td>0.32±0.08²</td>
<td>0.52±0.05³</td>
</tr>
<tr>
<td>20</td>
<td>0.7</td>
<td>0.09±0.02²</td>
<td>0.19±0.06³</td>
</tr>
<tr>
<td>20</td>
<td>7.0</td>
<td>0.19±0.03³</td>
<td>0.19±0.10³</td>
</tr>
<tr>
<td>60</td>
<td>0.0</td>
<td>0.40±0.09³</td>
<td>0.65±0.08³</td>
</tr>
<tr>
<td>60</td>
<td>0.7</td>
<td>0.12±0.04³</td>
<td>0.30±0.04³</td>
</tr>
<tr>
<td>60</td>
<td>7.0</td>
<td>0.14±0.02³</td>
<td>0.23±0.05³</td>
</tr>
</tbody>
</table>

Values are means±SD for 12 mice. Values in the same column without the same superscript are significantly different (p<0.05, ANOVA and Duncan’s multiple range test).

The control diet contained 20% casein and 0.7 mg% B₆, as recommended by the American Institute of Nutrition-76. The effects of B₆ on the growth of mice on the six diets were the same as previously reported. The thymus and spleen weighed significantly less in mice with B₆ deficiency than in the control group.

Table I shows the effects of B₆ deficiency on T-cell subsets from the spleen and thymus of mice on the six diets. CD4⁺CD8⁻ T cells, which are helper T cells, and CD8⁺CD4⁻ T cells, which are cytotoxic T lymphocytes, were counted, and expressed as a percentage of total lymphocytes. Both CD4⁺CD8⁻ and CD8⁺CD4⁻ T cells from the spleen of B₆-deficient mice because of their diet of 20% or 60% casein without B₆, accounted for a larger proportion of lymphocytes than in control mice fed 20% casein with 0.7 mg% B₆. The CD8⁺/CD4⁺ ratio for the spleen was not significantly different in the six groups. On the other hand, the CD8⁺/CD4⁺ ratio for cells from the thymus of B₆-deficient mice was 0.45 with 20% casein and 0.38 with 60% casein. For control mice, the ratio was 0.57. CD4⁺CD8⁻ T cells from the thymus of the mice fed 60% casein without B₆ accounted for a significantly larger proportion of lymphocytes than in most of the other groups, but CD8⁺CD4⁻ T cells of mice with B₆ deficiency were a smaller proportion both casein was 20% and 60%. These results suggested that the increased percentage of CD4⁺CD8⁻ T cells in the spleen and thymus were not caused by protein in the diet being abundant but by the severe B₆ deficiency.

Next, we examined other cytokines from T cells; these cytokines are related to immunoglobulin E production. IL-5 and IL-10 are secreted by T helper 2 (Th2) cells, as is IL-4, and they modulate the differentiation and proliferation of B cells. ConA is mitogenic toward T cells. The increases in IL-5 and IL-10 in splenocytes obtained from mice with B₆ deficiency and stimulated with ConA did not depend on the amount of protein in the diets (Table II). The same was found for IL-4, which is essential for immunoglobulin E production. Therefore, B₆ deficiency will cause CD4⁺CD8⁻ T cells shift from the T helper 1 (Th1) type to the Th2 type. Such cytokines as IL-4, IL-5, and IL-10 may help CD4⁺CD8⁻ T cells shift from Th1 type to Th2 type, or the leading Th2 cells in B₆ deficiency may also produce IL-4, IL-5, and IL-10, which participate in the humoral immune response.

The effects of B₆ deficiency on proliferation by splenocytes stimulated by ConA were investigated by spectrophotometry. ConA added to cell cultures decreased proliferation in B₆-deficient groups. The stimulation index was low in mice with B₆ deficiency (Table II). This decrease probably occurred because of a decrease in IL-2 in the B₆-deficient-splenocytes.

The endocrine system affects the secretion of cytokines such as IL-1, IL-6, tumor necrosis factor, and transforming growth factor β. The corticosterone concentration in plasma from mice not given B₆ was significantly higher than in the other groups. The corticosterone of mice given B₆ were not affected by the protein levels of the diets. Allgood and Cidlowski found that transcriptional activation by steroid hormone receptors is inversely modulated by changes in the
intracellular concentration of pyridoxal 5'-phosphate, the physiologically active form of B₆. They found reduced levels of hormone-mediated gene expression under conditions of elevated B₆ concentration and enhanced hormone responsiveness under conditions of B₆ deficiency. Thus, B₆ seems to participate in the regulation of steroid hormones. Katamura et al.¹² reported that prostaglandin E₂ inhibits the production of cytokines such as interferon-γ and IL-2 by acting directly on naive T cells. B₆ may facilitate the development of a Th2 type cytokine production profile by acting directly on naive T cells in mice.

In conclusion, B₆ seemed to improve the disturbed cytokine levels and proportions of lymphocyte subsets in B₆-deficient mice by regulation of the action of steroid hormone such as glucocorticoid. B₆ is essential for maintaining lymphocytes in the thymus and spleen.

Acknowledgments

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References