Impaired Dendritic Cell Function Resulting from Chronic Undernutrition Disrupts the Antigen-Specific Immune Response in Mice

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Abstract

We examined whether antigen-specific immune responses are lower in mice with protein energy malnutrition (PEM mice) compared with nourished (control) mice. The mechanisms underlying reduced antigen-specific immune responses of PEM mice were evaluated through analysis of the functional capacities of antigen-presenting dendritic cells (DC). PEM mice were produced by subjecting male C57BL/6 mice for 52 wk to a daily food intake equivalent to 70% of the mean amount consumed by the control mice that consumed food ad libitum. PEM mice and control mice were immunized with hepatitis B vaccine containing hepatitis B surface antigen (HBsAg) at 52 wk and humoral and cellular immune responses to HBsAg were evaluated at 58 wk. Lymphoproliferative assays were performed to assess the functional capacities of lymphocytes and DC. After 52 wk of food restriction, PEM mice had a 49% lower body weight than controls, almost no subcutaneous fat, severe muscle wasting, and atrophied spleen. All control mice developed antibodies to HBsAg (anti-HBs) in the sera and HBsAg-specific lymphocytes in the spleen as a result of immunization with the hepatitis B vaccine. PEM mice, however, were almost unresponsive to immunization with the hepatitis B vaccine. In PEM mice, the numbers of spleen DC, the T lymphocyte stimulatory capacities of DC, and their production of IL-12p70 and IFN-γ were less than those of control mice (P<0.05). We suggest that chronic undernutrition disrupts antigen-specific immune responses and that this disruption can be attributed at least in part to reduced frequencies and impaired functions of DC.


Introduction

The immune system protects individuals from infectious agents in the environment (e.g., bacteria, viruses, fungi, and parasites) and other noxious insults. People with severely subnormal immune responses are more susceptible to infectious agents and exhibit increased infectious morbidity and mortality (1). Nutrition is an important contributor to immune competence; undernourished and malnourished animals are susceptible to infection (2) and patients with malnutrition require prolonged recovery periods after surgery and illness (3). In addition, responses to prophylactic vaccines depend on nutritional status. Undernourished or malnourished subjects and patients on hemodialysis suffering malnutrition exhibit low responses to hepatitis B vaccines (4–6) and the protective effect of influenza vaccine is also diminished in undernourished subjects (7,8).

In this context, investigators have shown that the functional capacities of different immunocytes decrease in subjects with nutritional deficiencies (9–13). However, some have reported that the proliferative capacities of T and B lymphocytes either remain unaltered or increase in subjects under dietary restriction (14–16). These discrepancies regarding the impact of nutrition on the function of T and B lymphocytes may be attributable to differences in experimental design and analytic procedures. In addition, some important aspects about the impact of nutrition on immune responses have not been well addressed. Most studies of nutrition and immune responses have mainly evaluated the effect of dietary restriction on lymphocyte proliferation in response to nonspecific stimulants (mitogenic stimulation). Although antigen-specific immune responses are critical for host protection, there is a paucity of information regarding antigen-specific immune responses in chronically undernourished subjects. To test properly the effect of nutrition on immune responses, experiments should incorporate both in vivo and in vitro approaches. Yet, most investigators use only one of these approaches in subjects with dietary restriction.

Although T and B lymphocytes represent effector cells of the immune system, the functional capacities of lymphocytes, especially the induction and functions of antigen-specific lymphocytes, are regulated by antigen-presenting dendritic cells (DC)2 (17). DC are present in almost all tissues and can recognize, capture, process, and present microbial agents and different types of antigens (18). We and others have recently demonstrated that

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2 Abbreviations used: anti-HBs, antibody to HBsAg; DC, dendritic cell; HBsAg, Hepatitis B surface antigen; PEM, protein energy malnutrition.

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Materials and Methods

Mice. Eight-week-old male C57BL/6 mice were purchased from Nihon Clea. The mice were housed individually in polycarbonate cages in a temperature-controlled room (23 ± 1°C) with a 12-h-light/-dark cycle at the Ehime University animal facility. Mice were fed a commercial laboratory chow (Oriental Corporation). Approximately 100 g of the nonpurified diet contained 23.6 g protein, 5.3 g fat, 6.1 g ash, 2.9 g fiber, and 54.4 g nitrogen-free extracts. All mice received humane care and the study protocol was approved by the Ethical Committee of the Graduate School of Medicine, Ehime University, Japan.

Preparation of PEM mice. The food consumed by 1 adult C57BL/6 mouse per day was calculated by keeping 11 normal C57BL/6 mice separately (1 mouse per cage) and allowing them to consume food ad libitum for 12 wk. The mean daily intake was 268 g. Then, we produced 2 groups of mice. The control mice (n = 25) were housed in individual cages and consumed food ad libitum (2.7–3.0 g/d) for 52 wk. PEM mice (n = 25) were housed individually and received 1.7–1.9 g food/d for 52 wk. This was ~70% of that consumed by control mice. We measured body weights of all mice once each week. Blood samples were taken at regular intervals and the serum concentrations of total protein (A/G B-Test, Wako), total albumin (A/G B-Test, Wako), and total cholesterol (Cholesterol E Test Wako kit) were measured using commercial kits (Wako, Osaka, Japan). The numbers of leukocytes and lymphocytes were estimated at weekly intervals for the first 4 wk and then at monthly intervals for the next 52 wk. Some control (n = 5) and PEM mice (n = 5) were killed after 52 wk to determine the weights of different organs and body fat depots. Other mice (n = 20) were used for immunization with hepatitis B vaccine and for isolation of T lymphocytes, B lymphocytes, and DC.

Immunization with hepatitis B vaccine containing hepatitis B surface antigen. After 52 wk of treatment, control mice and PEM mice of the same age were immunized with a commercially available hepatitis B vaccine containing hepatitis B surface antigen (HBsAg) (Heptavax-II, subtype adw, Banyu Pharmaceutical). The levels of antibodies to HBsAg (anti-HBs) in the sera were measured 4 and 6 wk after immunization using a commercial kit (anti-HBs, Institute of Immunology) for 48 h. DC were recovered from the cultures and washed 5 times with PBS. The final wash solution was preserved to assess whether free HBsAg was present in HBsAg-pulsed DC.

Lymphoproliferative assays. We conducted a series of experiments to optimize the protocols for the lymphocyte proliferation assays, as described previously (26). T lymphocytes and B lymphocytes were cultured with concanavalin A (Sigma Chemical) and LPS (Sigma Chemical), respectively, for 72 h. Lymphocytes were isolated from control mice and PEM mice that had been immunized with hepatitis B vaccine. These lymphocytes were cultured in the absence or presence of HBsAg (1 mg/L) for 120 h to evaluate HBsAg-specific cellular immune responses.

We evaluated the stimulatory capacities of DC to induce proliferation of HBsAg-specific memory lymphocytes by culturing HBsAg-specific memory lymphocytes and DC from control mice and PEM mice without or with HBsAg (1 mg/L) for 120 h.

We assessed the functional capacities of HBsAg-pulsed DC by culturing HBsAg-specific memory lymphocytes and HBsAg-pulsed DC for 120 h.

All cultures were performed in 96-well U-bottom plates (Corning).

Estimation of IL-12p70 and IFN-γ. The levels of IL-12p70 and IFN-γ in culture supernatants were estimated using a commercial kit for the cytokometric bead array method, as described (24). The levels of cytokines in the culture supernatants were calibrated from the mean fluorescence intensities of the standard negative control, standard positive control, and samples by Cytometric Bead Array software (BD Biosciences) using a Macintosh computer (SAS Institute).

Statistical analysis. Repeated measures ANOVA was used to determine whether which groups differed. Other data were analyzed by unpaired t tests if the data were normally distributed and by the Mann-Whitney rank-sum test if they were skewed. Differences were considered significant if P < 0.05. Data are shown as means ± SEM.

Results

Characteristics of PEM mice. The body weights of control mice (23.73 ± 0.27 g, n = 25) and PEM mice (23.12 ± 0.31 g,
n = 25) did not differ before the study began. Body weights of control mice increased 24% to 31.6 ± 0.4 g. On the other hand, the body weight of PEM mice decreased by 25% to 17.6 ± 0.4 g (Fig. 1). Serum cholesterol concentrations were lower in PEM mice at wk 52 (12.4 ± 0.26 mmol/L, n = 10) compared with their baseline value (20.7 ± 1.09 mmol/L, n = 10) (P < 0.05). Visceral fat, subcutaneous fat, and spleen weights were significantly lower in PEM mice compared with those of control mice (Table 1). The relative spleen and fat weights (g/100 g body weight) also were lower in PEM mice compared with control mice (P < 0.05).

**Leukocytes and lymphocytes in the peripheral blood of PEM mice.** One wk after the study began, the numbers of leukocytes and lymphocytes in the peripheral blood had decreased in PEM mice compared with their baseline values (P < 0.05) (Fig. 2) and this continued throughout the study. However, numbers of leukocytes and lymphocytes did not change in control mice (data not shown).

**Humoral immune responses.** Anti-HBs were detected in the sera of all control mice (n = 10), but in only 2 of 10 PEM mice, 4 wk after immunization with the hepatitis B vaccine. The levels were lower in PEM mice (8.3 ± 5.5 IU/L, n = 10) compared with those in control mice (138.6 ± 51.3 IU/L, n = 10) (P < 0.05). This was also true 6 wk after immunization when the concentration in PEM mice was 9.6 ± 4.8 IU/L and in control mice was 203.9 ± 61.7 IU/L (P < 0.05).

**HBsAg-specific cellular immune responses.** Lymphocytes from PEM mice and control mice, immunized with hepatitis B vaccine containing HBsAg, were cultured with HBsAg to assess HBsAg-specific cellular immune responses. The proliferation of HBsAg-specific lymphocytes from PEM mice (119.4 ± 12.4) was less than in control mice (395 ± 68 Bq) (P < 0.05).

**Proliferative capacities of lymphocytes to polyclonal mitogens.** A total of 2 × 10^5 each of T and B lymphocytes from PEM mice and control mice were cultured with concanavalin A (1 g/L) and LPS (10 g/L), respectively, for 72 h. The levels of blastogenesis of cultures containing T lymphocytes from control mice (624.7 ± 99.7 Bq) and PEM mice (64.5 ± 72.8 Bq) did not differ nor did levels in culture containing B lymphocytes, where the values were 139.4 ± 12.6 Bq and 140.4 ± 24.7 Bq, respectively.

**Functional capacities of spleen DC.** Because antigen-specific humoral and cellular immune responses were impaired in PEM mice, we investigated the underlying mechanism by analyzing the frequencies and functional capacities of spleen DC. The numbers of spleen DC were significantly lower in PEM mice compared with control mice (Table 2). Spleen DC from both PEM and control mice were challenged to stimulate HBsAg-specific memory lymphocytes in the presence of HBsAg. Spleen DC from PEM mice had reduced capacities to stimulate HBsAg-specific memory lymphocytes compared with spleen DC from control mice (P < 0.05) (Fig. 3).

One method for assessing the antigen-processing and-presenting capacities of DC is to evaluate the functional capacities of antigen-pulsed DC. DC from PEM mice and control mice were cultured with HBsAg and HBsAg-pulsed DC were produced. HBsAg-pulsed DC from PEM mice (59.2 ± 7.0 Bq) had less capacity to stimulate HBsAg-specific memory lymphocytes in vitro than HBsAg-pulsed DC from control mice (129.2 ± 10.8 Bq; P < 0.05).

**Levels of IL-12p70 and IFN-γ in cultures containing DC.** The levels of IL-12p70 (154 ± 24 ng/L and 1324 ± 245 ng/L, respectively) and IFN-γ (53 ± 12 ng/L and 267 ± 16 ng/L, respectively) were lower in cultures containing DC from PEM mice compared with those in culture supernatants containing DC from control mice (P < 0.05).

**Discussion**

Chronic nutritional deficiencies are important causes of impaired immunocompetence and can be causal factors in increased

![Figure 1](https://academic.oup.com/jn/article-abstract/137/3/671/4664694/673)

**Figure 1** Body weights of control and PEM mice throughout the study. Values are means ± SEM, n = 25. Control mice and PEM mice differed beginning at wk 1.

![Figure 2](https://academic.oup.com/jn/article-abstract/137/3/671/4664694/673)

**Figure 2** Numbers of leukocytes (A) and lymphocytes (B) in the peripheral blood of PEM mice (n = 10). Values are means ± SEM, n = 10. *Different from d 0, P < 0.05.

**Table 1** Nutritional status of control and PEM mice at wk 52^1

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control</th>
<th>PEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length, cm</td>
<td>18.1 ± 0.17</td>
<td>15.88 ± 0.37*</td>
</tr>
<tr>
<td>Kidney, mg</td>
<td>222 ± 10</td>
<td>115 ± 4*</td>
</tr>
<tr>
<td>% body</td>
<td>0.70 ± 0.03</td>
<td>0.67 ± 0.05</td>
</tr>
<tr>
<td>Perigonadal fat, mg</td>
<td>201 ± 53</td>
<td>63 ± 1.2*</td>
</tr>
<tr>
<td>% body</td>
<td>0.66 ± 0.17</td>
<td>0.04 ± 0.007*</td>
</tr>
<tr>
<td>Perirenal fat, mg</td>
<td>383 ± 62</td>
<td>95 ± 0.37*</td>
</tr>
<tr>
<td>% body</td>
<td>1.18 ± 0.20</td>
<td>0.05 ± 0.01*</td>
</tr>
<tr>
<td>Mesenteric fat, mg</td>
<td>802 ± 88</td>
<td>143 ± 30*</td>
</tr>
<tr>
<td>% body</td>
<td>2.6 ± 0.29</td>
<td>0.81 ± 0.17*</td>
</tr>
<tr>
<td>Subcutaneous fat, mg</td>
<td>358 ± 38</td>
<td>Almost nil</td>
</tr>
<tr>
<td>% body</td>
<td>1.17 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>Spleen, mg</td>
<td>150 ± 7</td>
<td>26 ± 2*</td>
</tr>
<tr>
<td>% body</td>
<td>0.49 ± 0.02</td>
<td>0.15 ± 0.009*</td>
</tr>
</tbody>
</table>

^1 Values are mean ± SEM, n = 5. *Different from control mice, P < 0.05.
susceptibility to infectious diseases in both relatively healthy people and hospitalized patients (1,3). Undernourished persons also respond poorly to prophylactic vaccines (4–8). However, it is difficult to assess the direct impact of undernutrition on immune responses, because most patients with undernutrition also suffer from other pathological lesions that may affect host immunity in addition to nutritional factors. Undernourished patients on hemodialysis respond poorly to HB vaccine, but they obviously also suffer from concurrent kidney disease (4,5). Increased morbidity and mortality have been reported in undernourished older persons due to infection with influenza virus (8); however, the advanced age of these patients may also play an important role in the diminished immune responses.

Thus, the mechanism involved in the effects of nutrition on the immune system may be better addressed in experimental systems in which the influence of diet and nutrients can be more precisely controlled. Along these lines, investigators have prepared murine models of dietary restriction and have found an association between dietary restriction and reduced body weight gain, thymus atrophy, and reduced numbers of lymphocytes (9–13). Diminished proliferation of lymphocytes in diet-restricted subjects has been reported, although there are also reports in the literature of no effect in such subjects (14–16).

To study the effects of chronic undernutrition on immune responses, we prepared PEM mice by restricting male C57BL/6 mice to a daily food intake equivalent to 70% of the amount consumed by control mice for 52 wk (Figs. 1 and 2; Table 1). In comparison to control mice, HBsAg-specific immune responses were significantly decreased in PEM mice. Anti-HBs were not detected in the sera in 8 of 10 PEM mice and very low levels of anti-HBs were found in only 2 of 10 PEM mice due to immunization with hepatitis B vaccine containing HBsAg. This outcome contrasted with very high levels of anti-HBs in the sera of all control mice. The magnitude of HBsAg-specific cellular immune responses was significantly lower in PEM mice compared with control mice.

In this study, lymphocytes from control mice and PEM mice showed similar levels of proliferation in response to stimulation with polyclonal mitogens. However, there is lack of consensus about this in the literature. In fact, many questions regarding the effects of dietary restriction on cell proliferation have not been properly addressed. In particular, details of the response of cell proliferation to dietary restriction, including the time course, dose-response relation, and endocrine correlations, have not been established (13). Moreover, the effect of using different control groups in the dietary restriction field also has not been systematically explored. However, the results of this study are supported by those of Conzen et al. (16), who found that T cell proliferation was not altered in chronically protein-deprived mice. Nevertheless, although the proliferation of lymphocytes (on a per-cell basis) was not reduced in PEM mice in this study, the total numbers of lymphocytes were only 25% of those of control mice; thus, a role for lymphocytes in the impaired HBsAg-specific immune responses in these mice cannot be completely dismissed.

In this context, the role of DC was evident for impaired HBsAg-specific immune responses in PEM mice. The numbers of spleen DC were significantly lower in PEM mice compared with control mice (Table 2). In addition, DC from PEM mice were less potent in processing HBsAg in vitro (as evidenced by the impaired stimulation of HBsAg-specific memory lymphocytes by HBsAg-pulsed DC of PEM mice). DC of PEM mice also had significantly diminished abilities to activate HBsAg-specific lymphocytes (Fig. 3). In addition, DC from PEM mice produced lower levels of IL-12p70 and IFN-γ compared with those produced by control mice. Because DC recognize and capture antigens in situ, process them in their endosomal compartments, and induce antigen-specific immune responses in situ (17–19), decreased antigen processing and presentation capacities of DC of PEM mice can explain why lower levels of anti-HBs and HBsAg-specific immune responses were induced in PEM mice. We and others have reported a relation between impaired DC function and pathogenesis of various diseases such as cancers, chronic viral infections, and autoimmune diseases (27,28). In addition, DC-based therapy has successfully been applied in various pathological conditions in mice and humans (18,19). In the context of nutritional immunity, Shi et al. (11) have documented defective antigen presentation in energy-restricted, gastrointestinal nematode-infected mice.

In this study, we intentionally assessed the efficacy of hepatitis B vaccine in chronically undernourished mice. Infection with hepatitis B virus is related to progressive liver diseases, such as liver cirrhosis and liver cancer. About 400 million people are chronically infected with this virus, and >90% of them live in the developing nations of the world (29). The WHO recommended adding the hepatitis B vaccination to all immunization programs for preventing further spread of the virus (30). We showed that the efficacy of the hepatitis B vaccine depends on the host nutritional status and the efficacy of the present regimen of hepatitis B vaccine should be evaluated in subjects afflicted with chronic nutritional deficiencies. However, there are some limitations in translating the findings of this study from the laboratory to clinical practice. In this study, PEM mice received reduced amounts of food for 52 wk, about one-half of the life span of mice. The efficacy of the hepatitis B vaccine should be evaluated in chronically undernourished subjects to determine whether there is a need to revisit the present regimen of global hepatitis B vaccination.

Moreover, the influence of feeding behavior on HBsAg-specific immune responses was not addressed in this study. PEM mice ate food comparatively more quickly than control mice, because they were given 30% less food each day. Future studies should evaluate the effect of feeding behavior on immune responses.

**TABLE 2** Numbers of spleen cells and spleen DC from control and PEM mice at wk 52

<table>
<thead>
<tr>
<th>Spleen cells</th>
<th>Spleen DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control mice</td>
<td>7.73 ± 1.03 × 10^7</td>
</tr>
<tr>
<td>PEM mice</td>
<td>1.77 ± 0.15 × 10^7</td>
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1 Values are mean ± SEM, n = 3. *Different from control mice, P < 0.05.

![Figure 3](https://example.com/image.png)

In this study, lymphocytes from control mice and PEM mice showed similar levels of proliferation in response to stimulation with polyclonal mitogens. Values are means ± SEM, n = 3. *Different from control mice, P < 0.05.
Understanding the biochemical and molecular targets of immune responses in PEM may lead to new prophylactic and therapeutic strategies to counter the impaired responses to vaccine and other adverse effects of malnutrition in hospitalized patients. The optimal approach would be to improve the nutritional status of the hosts, but this solution may not be possible in many cases because of complicating underlying diseases. The true benefit of these studies may be in identifying immune-associated biomarkers, which can be used in dietary supplementation and pharmacologic studies to prevent or treat various pathological conditions. Studies have shown that dietary supplements also regulate different parameters of immunity (31,32); however, little is known about the impact of these supplements on DC, the regulator of antigen-specific immune responses. Further study is required to address this question and to analyze the functional capacities of DC in people with undernutrition and malnutrition.

**Literature Cited**
