The Capacity of Noninflammatory (Steady-State) Dendritic Cells to Present Antigen in the Primary Response Is Preserved in Acutely Protein- or Energy-Deficient Weaning Mice

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ABSTRACT The objective of this investigation was to determine the influence of wasting protein and/or energy deficits on the capacity of dendritic cells to initiate primary responses. Weaning male and female C57BL/6J mice were permitted free access to a complete purified diet, free access to an isocaloric low protein purified diet (combined deficiencies of protein and energy) or restricted intake of the complete diet (energy deficiency) for up to 14 d; a 19-d-old zero-time control group was also included. Malnourished mice lost 1.5–2% of initial body weight daily. Antigen presentation by dendritic cells from spleen and lymph nodes was assessed in vitro by the primary one-way allogeneic mixed lymphocyte reaction using CBA/J mononuclear or CD4+ T cells as responders. This function was sustained despite advanced weight loss and, remarkably, was increased in cell suspensions from 14-d energy-deficient mice. Antigen presentation by dendritic cells in mononuclear suspensions was examined in vivo using the host-vs.-graft response in CBA/J recipients, and an ontogeny-related increase was sustained in both malnourished groups through 14 d of weight loss. Neither wasting protocol influenced the proportion of mononuclear cells (1–2%) exhibiting dendritic cell phenotype (CD11c+/F4/80−) in the cellular suspensions used to study antigen-presenting activity. Consequently, these functional studies are interpretable on a per dendritic cell basis. In the absence of infectious or inflammatory pressure, the dendritic cell retains antigen-presenting capacity despite acute (wasting) deficiencies of protein and/or energy. The results are relevant to presentation of both foreign (adjuvant role) and self (tolerizing role) antigens by the dendritic cell. J. Nutr. 132: 2748–2756, 2002.

KEY WORDS: antigen presentation • dendritic cell • energy deficiency • protein deficiency • mice
influence of malnutrition on cell numbers or on antigen-presenting capacity on a per cell basis. In addition, the previous work with rodents (13) involved study of stunting disease, and the investigation of marasmic children (12) focused on a form of wasting pathology that is usually superimposed on a preexisting stunting condition (15). Therefore, the first objective of the present investigation was to determine the influence of weight loss through weaning protein or energy deficit on dendritic cell–dependent antigen-presenting capacity in vitro, while accounting for any effect on cellular numbers.

The classical one-way allogeneic MLR involves mixed mononuclear stimulator and responder populations, and recent evidence shows that a synergistic interaction between dendritic cells of the stimulator and responder populations underlies much, even most, of the response (16,17). Strictly speaking, therefore, unambiguous information pertaining to the competence of dendritic cells affected by deficiencies of protein or energy is not provided by the previous reports (13,18). Therefore, the present investigation included a modified MLR assay in which purified CD4+ T cells served as the responder population. In addition, it was intended that this investigation would extend the study of dendritic cells in protein and energy deficiencies by providing an assessment of antigen-presenting capacity in vivo. Finally, this investigation was aimed at steady-state dendritic cells, i.e., cells unchallenged by overt infectious or other inflammatory stimuli that force their differentiation from antigen-capturing mode toward antigen-presenting maturity (11,18,19). The intent was to determine the effect of wasting protein and energy deficits on the readiness of dendritic cells in secondary lymphoid organs to initiate a primary acquired immune response.

MATERIALS AND METHODS

Animals, facilities, diets and feeding protocols. Male and female C57BL/6J and CBA/J mice were used from in-house breeding colonies derived from animals of the Jackson Laboratory (Bar Harbor, ME). Caging and housing conditions were exactly as described previously (2–8), and the investigation was approved by the Animal Care Committee of the University of Guelph in accordance with the Canadian Council on Animal Care. C57BL/6J mice were weaned at 18 d of age and acclimated for 1 d. As described elsewhere (20), the purified low protein diet contained 0.6% crude protein (as fed) and was prepared by mixing equal parts of the diet ad libitum, a group that consumed a low protein diet ad libitum, and the age-matched control, a zero-time control group was included in each treatment group. In addition to the two malnourished groups and the age-matched control group, 40 mice were studied for the 9-d and 14-d groups, respectively. Experiment 2 was similar except that purified CBA/J CD4+ T cells were used as responders, and C57BL/6J stimulator cells were taken from the two malnourished groups and the age-matched control (n = 4 per group) after 14 d, only. In addition, four zero-time control mice were included in the second experiment.

In Experiment 3, mononuclear cells were taken from C57BL/6J mice of the two malnourished groups and the age-matched control after either 9 or 14 d of their dietary protocols, and the cells were used to elicit a host-vs.-graft response in CBA/J recipients. At d 9, sample sizes were 8, 8 and 5 for the age-matched control, low protein and restricted intake groups, respectively, whereas n = 5 for each of the dietary groups maintained for 14 d. Zero-time control groups were also included such that n = 8 and 6 for the 9- and 14-d groups, respectively.

Procedures to obtain mononuclear cell suspensions from spleen and lymph nodes. Mice were anesthetized with diethyl ether (Fisher Scientific, Fair Lawn, NJ) and killed by cervical dislocation. To prepare stimulator cells for the MLR, donor cells for the host-vs.-graft response, or cell suspensions for CD11c enrichment (to facilitate surface marker analysis), the spleen as well as submandibular, mesenteric and inguinal nodes were removed aseptically, diced and incubated at 37°C in 5% CO2 for 60 min in 5 mL Hanks’ balanced salt solution (HBSS; Sigma Chemical, St. Louis, MO) supplemented with 1 g/L collagenase (type IV, Sigma Chemical). The tissue was then filtered through sterile stainless steel wire screen (100-mesh) and a single-cell suspension was produced by discontinuous gradient centrifugation as described previously (22). Viability was determined by eosin Y exclusion and always exceeded 95%. Mononuclear cell suspensions to provide responder cells for the MLR, whether the classical reaction or the reaction using purified CD4+ responder T cells, were prepared in the same manner except that they were not exposed to collagenase.

One-way primary allogeneic MLR using unenriched splenic and lymph nodal mononuclear cells as responders. Stimulator mononuclear cells from C57BL/6J mice were incubated in RPMI 1640 medium (Flow Laboratories, Mississauga, Canada) containing 10% heat-inactivated fetal calf serum (Sigma Chemical), 1 mM/L HEPES (ICN Biomedicals, Aurora, OH), 10 mM/L penicillin, 100 mg/L streptomycin and 70 mg/L mitomycin C (Sigma Chemical). This culture fluid, when formulated without mitomycin C, is henceforth referred to as “medium.” The cells, 50 × 10⁶ in a volume of 1 mL, were incubated for 30 min at 37°C in a humidified atmosphere containing 5% CO2. After three washes in HBSS, graded numbers (1 × 10⁵, 20 × 10⁵, 40 × 10⁵, and 10 × 10⁶) of the mitomycin C–treated stimulator cells were cocultured, in 200 μL of medium, with 10⁵ CBA/J responder mononuclear cells. Stimulator cell numbers were selected on the basis of a preliminary dose-response study (n = 7 at each stimulus dose tested) in which the response increased with stimulator cell numbers up to, but not beyond, a level of 35 × 10⁵ mononuclear cells. Thus, two stimulator cell numbers were selected to limit the magnitude of the MLR, and a third set of cultures was set in which stimulator cell numbers, 40 × 10⁵, were not limiting to the response. The cultures were maintained for 112 h at 37°C in 96-well V-bottom microplates (Corning, Corning, NY) in a humidified atmosphere containing 5%
CO2 and were pulsed, for the last 16 h, with 37 kBq/well of 1H-thymidine (methyl-1H, specific activity 250 GBq/mmol; ICN Biomedicals). MLR-specific proliferation by responder cells was expressed as the mean disintegrations per min (dpm) of quadruplicate wells after accounting for the uptake of 1H-thymidine by stimulator and responder cells incubated separately from one another (also in quadruplicate).

One-way primary allogeneic MLR using purified CBA/J CD4⁺ T cells as responders. Single-cell suspensions containing 10⁶ CBA/J splenic and lymph nodal mononuclear cells were incubated at 37°C for 45 min in 1 g of nylon wool (type 200L; Robbins Scientific, Sunnyvale, CA) saturated with medium and held in a humidified atmosphere containing 5% CO₂. The resulting nonadherent population of cells was suspended in PBS (0.15 mol/L, pH 7.3) containing 1 g/L bovine serum albumin (BSA; Boehringer Mannheim, Mannheim, Germany). Anti-CD4 (L3T4)-coated Dynabeads (Dynal A.S., Oslo, Norway) were then added to the suspension to achieve a level of 1.5 × 10⁶ beads per 10⁶ mononuclear cells. The resulting suspension, containing 10⁶ cells/100 μL of 1 g/L BSA/PBS, was mixed at 4°C for 50 min on a rotator (speed setting of 8, Roto-torque model 7637, Cole-Parmer Instrument Company, Chicago, IL), and washed CD4⁺ T cells were recovered in medium using the appropriate Detachable reagent (Dynal A.S.), according to the manufacturer’s instructions, at a level of 1 × 10⁶ presumptive CD4⁺ T cells. Using these cells, the MLR was elicited and assessed in exactly the same manner as described for the MLR involving unenriched responder cells, except that 50 × 10⁶ purified responders were used in each culture and only two stimulator cell numbers were used, namely, 40 × 10⁶ and 20 × 10⁶. Cultures involving smaller numbers of stimulators (10 × 10⁶) were also set, but yielded an MLR response that was too small to interpret with confidence.

Enrichment of CD11c⁺ mononuclear cells. Mononuclear cell suspensions from the spleen and lymph nodes of C57BL/6J mice were subjected to Fc receptor blockade by incubation at 4°C for 20 min in heat-inactivated mouse serum (20 × 10⁶ cells in 500 μL of 5 g/L BSA/PBS containing 50 μL mouse serum). After the addition of 18 μL of magnetic beads conjugated with anti-CD11c [N418, hamster immunoglobulin (Ig)G; Miltenyi Biotec, Auburn, CA], the cells were incubated at 4°C for a further 30 min, washed in BSA/PBS (5 g/L), and subjected to two cycles of magnetic separation on 25M⁺ columns (Miltenyi Biotec) according to the manufacturer’s instructions.

Flow cytometric analysis of purified CD4⁺ T cells and of CD11c-enriched mononuclear cell populations. Single-cell suspensions, containing all mononuclear cells, were subjected to Fc receptor blockade by incubation for 10 min at 4°C in ~50 μL of 5 g/L BSA/PBS containing 5 μL heat-inactivated mouse serum. Saturation concentrations of each staining reagent were determined in preliminary studies, and cells were exposed to each reagent in 5 g/L BSA/PBS for 30–40 min at 4°C. Stained cells and isotype control suspensions that were not analyzed immediately were stored in darkness at 4°C in 5 g/L BSA/PBS containing 10 g/L paraformaldehyde for up to 7 d.

After Fc receptor blockade, each sample of purified CD4⁺ T cells (MLR responder cells, CBA/J strain) was subdivided to produce four suspensions. Two cell suspensions were used as isotype controls; one was incubated with biotin-conjugated rat IgG2b (Cedarlane Laboratories, Hornby, Canada; 1 μg/10⁶ cells) and the other with phycoerythrin (PE)-conjugated rat IgG2a (LODPN16; Cedarlane Laboratories; 1 μg/10⁶ cells). The remaining two cell suspensions were incubated with PE-conjugated anti-mouse CD4 (L3T4, rat IgG2b; Cedarlane Laboratories; 0.5 μg/10⁶ cells), or with PE-conjugated anti-mouse CD8 (7–16.17, mouse IgG2a; Cedarlane Laboratories; 0.5 μg/10⁶ cells), respectively. The isotype control cells exposed to rat IgG2b were subsequently incubated in PE-conjugated streptavidin (Cedarlane Laboratories; 0.5 μg/10⁶ cells).

CD11c-enriched C57BL/6J mononuclear cells were fixed in 10 g/L paraformaldehyde (Fisher Scientific) at 4°C overnight and washed in 5 g/L BSA/PBS before Fc receptor blockade. For the purpose of staining, the cells were exposed, first, either to hamster IgG (Cedarlane Laboratories; 1 μg/10⁶ cells) together with rat IgG2b (Cedarlane Laboratories; 1 μg/10⁶ cells) to produce the isotype controls, or to anti-CD11c (N418, hamster IgG; generous gift of Dr. Ralph Steinman, Rockefeller University, NY; 1 μL of culture supernatant per 10⁶ cells) together with F4/80 (HB-198, rat IgG2b). The clone producing the latter antibody was purchased from the American Type Culture Collection (Rockville Pike, MD), and 1 μL of culture supernatant was applied per 10⁶ cells. After a second Fc receptor blockade, cells of both isotype control and stained suspensions were exposed first to biotin-conjugated goat anti-hamster IgG (Cedarlane Laboratories; 1 μg/10⁶ cells) and then to tricolor-conjugated streptavidin (Cedarlane Laboratories; 2 μg/10⁶ cells). Finally, after a third Fc receptor blockade, cells were exposed to biotin-conjugated goat anti-rat IgG2b (BioCan; Mississauga, Canada; 2 μg/10⁶ cells) followed by PE-conjugated streptavidin (Cedarlane Laboratories; 0.5 μg/10⁶ cells).

Cellular subsets were identified by means of an Epics XL-MCL flow cytometer (Coulter, Hileah, FL) equipped with version 1.5 (1993) software. Each analysis, including those of negative controls, was based on at least 10⁵ events after dead cells and residual erythrocytes were eliminated by gating on the basis of forward angle light scatter. Purified CD4⁺ T-cell suspensions were subjected to two independent single-color analyses to determine the percentage of CD4⁺ and major histocompatibility complex (MHC) II⁺ cells, whereas CD11c-enriched suspensions were analyzed to determine the percentages of CD11c⁺ cells. CD11c⁺ dendritic cells were identified using anti-CD11c (N418, hamster IgG2a; Cedarlane Laboratories; 0.5 μg/10⁶ cells) and anti-CD4 (L3T4)-conjugated rat IgG2a (LODNP16; Cedarlane Laboratories; 0.5 μg/10⁶ cells).

RESULTS

The malnutrition protocols produced wasting disease including lymphoid involution in secondary lymphoid organs. Growth indices and numbers of viable mononuclear cells recovered from several secondary lymphoid organs (spleen and
mesenteric, submandibular and inguinal lymph nodes) are presented in Table 1 for the experiments in which the primary outcome measures were the one-way primary allogeneic MLR (Experiments 1 and 2) and the host-vs.-graft response (Experiment 3). Mice used in Experiment 4 (numbers and percentage of CD11c^+/H11001^F4/80^+/H11002^low dendritic cells) exhibited comparable growth indices and mononuclear cell numbers (results not shown). The malnutrition protocols produced daily weight loss ranging between 1.5 and 2.1% of initial body weight, whereas mice that consumed the complete diet steadily gained both fat and lean tissue and doubled their body weight after 14 d. Loss of fat by the malnourished groups was particularly remarkable, and the decrement in fat tissue was more pronounced in the restricted intake group than in mice consuming the low protein diet. In terms of carcass energy decrement, therefore, the restricted intake protocol imposed more severe wasting than the low protein protocol. The loss of carcass fat produced by the restricted intake protocol was sufficient to result in a low carcass dry matter content, not reflective of edema (results not shown), within 9 d relative to age-matched controls. In contrast, this index was not consistently affected in the low protein groups even after 14 d. Finally, although the age-matched control groups exhibited increases in numbers of mononuclear cells recoverable from secondary lymphoid organs (relative to zero-time controls), both malnutrition protocols produced lymphoid involution discernible within 9 d. Overall, in each experiment, the malnutrition protocols produced wasting disease that was comparable to the pathologies reported in studies demonstrating depressed T-dependent immunocompetence in the same experimental systems (2–5,7,22). Although sex effects were apparent on some indices, e.g., weight gain and food intake by

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Initial</th>
<th>Final</th>
<th>Food intake</th>
<th>Dry matter</th>
<th>Lipid</th>
<th>Protein</th>
<th>Cells</th>
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<td>g</td>
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<td>10.3c</td>
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<td>79.7c</td>
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<td>31.7b</td>
<td>10.4c</td>
<td>16.3a</td>
<td>214.3d</td>
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<td>6.8a</td>
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<td>5.1b</td>
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<td>6.9a</td>
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<tr>
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<td>7.8a</td>
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1 Values are means. Within each column and experiment, and within each feeding period in expts. 1 and 3 (i.e., 9 or 14 d), values not sharing a superscript letter differ (P < 0.05) according to Duncan’s New Multiple Range test after one-way ANOVA.
2 B, zero-time control group (19 d old); C, age-matched control group given free access to complete diet; LP, group given free access to low-protein diet; R, group subjected to restricted daily intake of complete diet.
3 Cumulative intake for the period shown, i.e., 9 or 14 d (expt. 1) or 14 d (expt. 2).
4 Viable mononuclear cells recovered from spleen together with submandibular, mesenteric and inguinal lymph nodes.
5 Means are squares of means from square root-transformed data.
6 Means are reciprocals of means from inverse-transformed data.
7 Means are antilogs of means from log-transformed data.
Dendritic cell–dependent antigen-presenting activity was preserved in mononuclear cell suspensions from secondary lymphoid organs of weanling mice subjected to weight loss through protein and/or energy deficit. The results of the one-way primary allogeneic MLR using unenriched CBA/J responder mononuclear cells are shown in Figure 1 for the mice subjected to 14-d protocols. Two-way ANOVA revealed a diet-related main effect ($P = 0.0001$, inclusive of the zero-time control group). This outcome was independent of stimulator cell number as indicated by the nonsignificant interaction term ($P = 0.2660$). When this effect was examined using Duncan’s New Multiple Range test, the response elicited by cells from the restricted intake group exceeded that of the other three groups which, in turn, did not differ from one another. Further analysis by least-squares means comparisons within each stimulator cell number revealed that, in each case, mononuclear cells from restricted intake mice (but not from the low protein group) exerted a greater stimulus in the MLR than comparable cellular preparations from the age-matched control group. For mice subjected to 9-d feeding protocols (results not shown), no differences were apparent between the malnourished groups and the age-matched control, an outcome that was independent of stimulator cell number as indicated by the nonsignificant interaction term ($P = 0.7734$). In addition, no influence of ontogeny was apparent when either the 9- or 14-d age-matched control groups were compared with their zero-time control using Duncan’s New Multiple Range test. Finally, a main effect of stimulator cell numbers on the MLR was apparent in both the 9- and 14-d data sets ($P = 0.0013$ and 0.0001, respectively). The response increased with increasing cell numbers (Duncan’s New Multiple Range test), thereby confirming that at least the stimulator numbers below $40 \times 10^3$ mononuclear cells were limiting to the MLR.

Dendritic cells of the responder mononuclear cell population appear to present antigen in the one-way allogeneic MLR (16,17). Experiment 2, therefore, was a confirmatory investigation to eliminate this confounding factor. Flow cytometric analysis of the cell suspensions used as responders revealed a cellular composition of (mean ± SD) $95 \pm 1\%$ CD4$^+$ T cells and $0.2 \pm 0.1\%$ MHC II$^+$ cells, and the MLR outcomes are shown in Figure 2. By inspection, the proliferative responses were lower than those obtained when comparable numbers of stimulators were cultured with unenriched responders (Fig. 1), an outcome consistent with previous reports (16,17). Two-way ANOVA revealed a main effect of diet (including the zero-time control group, $P = 0.0002$) and the interaction term, which was nonsignificant ($P = 0.3255$), showed that the diet-related effect was independent of stimulator cell numbers at the two levels tested. Further analysis of the diet-related effect, by means of Duncan’s New Multiple Range test, showed that stimulators from the restricted intake group elicited a greater MLR than comparable mononuclear cell suspensions from the other three groups which, in turn, did not differ from one another in this respect. This outcome included a comparison of the age-matched and zero-time control groups, which did not reveal an influence of ontogeny on the capacity of mononuclear cells to elicit an MLR during this period of development, i.e., from 19 to 33 d of age. Further analysis by least-squares means comparison to age-matched controls confirmed the effect of the restricted intake protocol at both levels of MLR stimulation. Finally, a significant main effect of stimulator cell numbers ($P = 0.0168$) was also apparent, and further analysis of this outcome by Duncan’s New Multiple Range test showed that at least the lower number of stimulator cells ($20 \times 10^3$) was limiting to the MLR.

It was desirable to complement the results pertaining to stimulation of the MLR by determining the capacity of mononuclear cells from wasting animals to function in dendritic cell–dependent antigen presentation in vivo. The host-vs.-graft response is a manifestation, in vivo, of the phenomenon responsible for the MLR (25,27), and the results of this experiment are shown in Figure 3 for the mice subjected to the 14-d feeding protocols. One-way ANOVA followed by Duncan’s New Multiple Range test revealed no difference between

![FIGURE 1 One-way primary allogeneic mixed lymphocyte reaction (MLR) using unenriched responder mononuclear cells from the spleen and lymph nodes of CBA/J mice. Mononuclear cell stimulators were taken from the spleen and lymph nodes of male and female C57BL/6J mice subjected to experiment for 14 d, beginning at 19 d of age. Each culture included $10^6$ responder cells and 10, 20 or $40 \times 10^3$ stimulators; proliferation by responder cells was assessed using $\beta$H-thymidine. Malnourished mice were given free access to a purified low protein diet (group LP) or were fed a complete purified diet in restricted daily quantities (group R) to lose, in either case, $1.5-2\%$ of initial body weight daily. Age-matched controls (group C) were permitted free access to the purified complete diet, and 19-d-old zero-time controls (group B) were included; $n = 8$ in each dietary group, and each sample of malnourished mice was constituted by pooling 2–5 mice of the same sex. Data were subjected to two-way ANOVA, with main effects of diet (including the zero-time control group) and stimulator cell number. Square-root transformation was applied so that bars represent squares of transformed means; in each case, a $\pm$ is shown. Error mean square = 460.6, and $P = 0.0001$ (diet), 0.0001 (stimulator cell number) and 0.0064 (interaction). The letter “a” above a bar indicates difference from the corresponding age-matched control, i.e., within a stimulator cell number, according to least-squares means analysis ($P \leq 0.05$).]
which the number of dendritic cells was unknown. To permit interpretation of these results in terms of antigen-presenting capacity on a per dendritic cell basis, an analysis of the numbers of dendritic cells was required. Cells exhibiting a CD11c^+ F4/80^- low surface phenotype constituted (mean ± SD) 1.7 ± 1.3, 2.0 ± 1.8, 1.9 ± 0.8 and 0.8 ± 0.9% of the mononuclear cells in stimulator/donor suspensions from zero-time control 14-d age-matched control, low protein and restricted intake groups, respectively, and one-way ANOVA revealed no diet- or ontogeny-related differences (P = 0.3718, pooled SEM = 0.288). On this basis, it is apparent that the cellular suspensions used to elicit the MLR and the host-vs.-graft response contained comparable numbers of dendritic-phenotype cells regardless of their source. At the same time, it is important to understand this outcome in the context of the wasting-associated lymphoid involution that occurred, as expected (4–8,22), in both deficiency pathologies. Thus, the numbers of CD11c^+ F4/80^- low cells recovered from the spleen and lymph nodes were as follows (antilogs of log-transformed means ± SD, expressed as cells × 10^6): 1.5 ± 0.5 (zero-time control), 2.9 ± 2.6 (age-matched control), 0.3 ± 0.2 (low protein group) and 0.03 ± 0.02 (restricted intake group), and one-way ANOVA revealed that the malnourished groups yielded smaller numbers of dendritic-phenotype cells than either the zero-time control group or the age-matched control (P = 0.0001, pooled SEM = 0.316).

**DISCUSSION**

The dendritic cell presents antigen to T cells more potently than any other antigen-presenting cell and appears uniquely capable of eliciting the primary acquired immune response (11,28). To initiate a primary response, the dendritic cell must first perform antigen capture as an immature cell in the periphery and then migrate to the T cell–dependent regions of secondary lymphoid organs to interact with naïve T cells.
During migration from the periphery, the dendritic cell differentiates by losing its capacity for antigen uptake while acquiring the ability to present antigen to T cells, the hallmark of the mature dendritic cell (11,18). Infectious or inflammatory stimuli strongly promote migration and maturation of peripheral dendritic cells, but this sentinel function is maintained in the absence of overt inflammatory pathology (18,28). Under noninflammatory conditions, most dendritic cells in secondary lymphoid organs appear to be mature and to function in the maintenance of peripheral tolerance (11,18,19,29). However, these cells remain poised to develop an adjuvant capacity to elicit responses to foreign antigens if stimulated appropriately (11,28–30). It is important to note that both functions, i.e., tolerance-induction in relation to self-antigens and an adjuvant role vis-à-vis foreign antigens, depend on induction of T-cell proliferation and are modeled by the immunological indices assessed in this investigation (29). The present investigation of antigen presentation by noninflammatory (steady-state) dendritic cells, therefore, provides insight into the effect of acute protein and energy deficits on both the capacity of the dendritic cell of secondary lymphoid organs to promote tolerance to self-antigens and the readiness of this cell to initiate a primary T-dependent immune response to foreign antigens.

The metabolic dissimilarities between mice subjected to the low protein and restricted intake protocols, and their respective similarity to the human pathologies of kwashiorkor and marasmus have been discussed elsewhere (2,21,31,32). Briefly, weanling mice subjected to the low protein protocol lose weight as a consequence of dietary imbalance and develop fatty liver and edema, the classic signs of kwashiorkor, after 4 wk or more of wasting disease (31). Consequently, the low protein protocol, when imposed for the periods of time reported in this investigation, produces a condition that can be regarded as incipient kwashiorkor. Further to this point, calculations based on diet composition and food intake revealed that the low protein protocol produced a deficiency of both protein and energy when imposed for 14 d, but not at the earlier stages of weight loss examined (results not shown). In contrast, the restricted intake system produces energy deficit apart from dietary imbalance, fatty liver or edema, and the extreme mobilization of fat reserves by mice subjected to this protocol is consistent with expectations for a model of marasmus (15). In short, each experiment of this investigation produced pathologies similar to marasmus and incipient kwashiorkor as reported in previous studies of the same experimental systems (2–8,22,31,32).

Interpretation of this investigation in terms of antigen-presenting capacity on a per dendritic cell basis depends on a meaningful estimate of mature dendritic cell numbers in the cellular suspensions used to elicit the MLR and the host-vs.-graft response. A lineage-specific marker useful during the late stages of differentiation has not been identified for the murine dendritic cell. However, in mice, all subsets of dendritic cells from the spleen and lymph nodes express CD11c (23,24,33). Among mononuclear cells, CD11c is also expressed by some mononuclear phagocytes (34), but these cells are F4/80-high (34) unlike the F4/80-low phenotype of dendritic cells from secondary lymphoid organs (24,33,34). In the absence of inflammatory stimuli, most dendritic cells of lymphoid organs seem able to promote T-cell tolerance (11,18,19,29) and require only brief stimulation to develop an ability to serve as adjuvants in the presentation of foreign antigens (11,30). Thus, the CD11c*F4/80-low phenotype identifies mature dendritic cells poised at, or near, a capacity for stimulating appropriate T-cell responses in murine secondary lymphoid organs. In turn, because the proportion of such cells in splenic and nodal mononuclear cell populations was unaffected by acute deficits of protein and/or energy in this investigation, it may be concluded that these experimental weight loss pathologies exerted no influence on the numbers of competent dendritic cells in the suspensions used to elicit the MLR and the host-vs.-graft response.

The results of this investigation show that the capacity of the dendritic cell to present antigen in a primary response, or to respond to stimuli that promote this capability, is preserved into advanced stages of metabolically diverse forms of acute weanling protein and/or energy deficit. This conclusion is independent of gender, and is based on evidence of dendritic cell function both in vitro and in vivo. On first inspection, these results appear at variance with previous reports of depression in the primary one-way allogeneic MLR using blood-derived stimulators from marasmic infants (12) and spleen-derived stimulators from protein-deficient mice (13). Both previous reports, like the present investigation, pertained to noninflammatory (steady-state) dendritic cells. However, the rodent study involved 10 wk of stunting malnutrition (13), and marasmus (12) is characteristically superimposed on pre-existing stunting disease (15). Therefore, this investigation appears to provide a first view of dendritic cell–dependent antigen presentation in wasting protein and/or energy deficits uncomplicated by chronic disease. Dendritic cells were not enumerated in the two previous investigations. Perhaps chronic (stunting) forms of protein and energy deficiency affect the numbers of dendritic cells disproportionately to the effect of acute (wasting) pathologies.

The one-way primary allogeneic MLR remains the standard in vitro assay of antigen presentation by the dendritic cell (14), which induces >90% of responder cell proliferation in this culture system (35). Antigen presentation in this system is relevant to the capacity of the dendritic cell to elicit a response to an abundant and strongly immunogenic foreign peptide antigen (36) and presumably, therefore, is also relevant to the ability of the dendritic cell to initiate tolerance to self-antigens. Thus, the MLR is germane to the actions of the dendritic cell in response to infections, vaccination procedures and large-scale challenge by self-antigens in catabolic pathologies, although this assay may fail to identify subtle effects (e.g., of acute protein and energy deficiencies in the context of this investigation) on the competence of the dendritic cell in presentation of weak and/or low level antigens. The use of limiting numbers of stimulators in these studies extends the relevance of the results to the late stages of a primary response when T cells must compete for position on a limiting number of dendritic cells (37). In addition, the host-vs.-graft response, an in vivo manifestation of the MLR (25,27), extends the investigation to the presentation of antigen by dendritic cells to T cells in vivo.

Recent evidence implicates the dendritic cells in MLR responder populations as antigen-presenting cells in the classical one-way allogeneic system, perhaps through uptake of MHC antigens from dendritic cells of the stimulator populations (16,17). In the present investigation, therefore, the MLR involving purified CD4+ T cell responders that included only trace numbers of potential syngeneic antigen-presenting cells provides a basis for interpreting the results specifically in terms of the capabilities of stimulator dendritic cells in vitro. The much larger proliferative response elicited by the classical mixture of responder and stimulator cells appears to result from a synergistic exchange of antigen, particularly antigen shed by the allogeneic dendritic cells and presented by their syngeneic counterparts (17). Consequently, the ability to release antigen
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for subsequent presentation by other dendritic cells is a characteristic thought to contribute to the potency of the dendritic cell in eliciting primary responses (17). This capability appears undiminished even in the advanced stages of wasting protein and/or energy deficit.

An ontogeny-related increase was apparent in the capacity of the dendritic cell to stimulate T cells in vivo, and this was sustained despite advanced wasting disease. No such ontogenetic change was apparent when antigen-presenting activity was assessed in the MLR, but wasting food intake restriction resulted in a surprising increase in the ability of dendritic cells to initiate this in vitro response. Thus, although the role of dendritic cells may be similar in the MLR and the host-vsgraft response (25,27), these assays provide different views of antigen presentation. It is important, therefore, that the outcome of each assay in the present investigation points to an ability to sustain this immune function despite profound wasting disease. The MLR provides more direct insight into the function of antigen-presentation, per se, than the host-vs-graft response, which is complicated by factors such as cellular migration. In turn, this renders particularly interesting the finding that dendritic cells from marasmic mice, but not from mice fed the low protein diet, exhibit an increased ability to initiate the MLR. In this connection, endotoxemia is reported in the most severe manifestations of wasting malnutrition regardless of metabolic type (38), and lipopolysaccharide powerfully stimulates maturation of dendritic cells (39). Perhaps responsiveness to endotoxin is retained by dendritic cells in the face of acute energy deficit, but becomes attenuated if dietary imbalance is superimposed on wasting energy depression as in the most advanced protein deficiency pathology produced here.

Ultimately, it is of interest to know whether an influence on the dendritic cell contributes to wasting-associated immune depression (1). In this investigation, dendritic cells were studied outside their physiologic, or pathophysiologic, microenvironment. Therefore, the results show that wasting protein and/or energy deficiencies bring about no fundamental change in the dendritic cell that reduces its inherent ability to initiate a primary response. However, the possibility remains of an indirect influence through the cellular microenvironment. For example, the high glucocorticoid levels that characterize wasting protein and energy deficiencies bring about no fundamental change in dendritic cell function pursued in this investigation. Studies of dendritic cells within the lymphoid environment produced by wasting protein and energy deficits are required to determine whether antigen presentation is a factor in the depressed primary immune competence of the experimental systems used in this investigation (2–5,7,22) and of wasting protein and energy deficiencies, more generally (1).

Lymphoid involution was inevitable (1) and substantial in the malnourished mice of this investigation and, not unexpectedly, included a decline in numbers of dendritic-phenotype cells. The question arises, therefore, whether functionally important numbers of dendritic cells remain in the wasted subject. In this connection, studies of the protein deficiency model used in this investigation reveal that supplements of triiodothyronine can prevent development of primary T-dependent immune depression despite exacerbation of the truly profound lymphoid involution that characterizes this experimental system (3,4). On this basis it appears that although cellular losses undoubtedly contribute to wasting-associated immune depression, the endocrine microenvironment determines the limits of immune competence in acute deficits of protein and energy (1).

In summary, this investigation demonstrates that the readiness of the noninflammatory dendritic cell to present antigen, or to acquire this capability, is sustained in the face of acute protein and/or energy deficiencies. This outcome was apparent in experimental systems known to deprive primary T-dependent immune competence, and suggests that wasting-associated immune depression occurs apart from an effect on antigen-presentation. Such a conclusion can not be finalized, however, without studies of dendritic cells functioning within the lymphoid microenvironment produced by protein and energy deficits. In a different vein, the noninflammatory dendritic cell is emerging as, first and foremost, a tolerizing cell (11,18,19,29) so that the significance of sustaining dendritic cell competence in acute protein and energy deficits may relate primarily to the maintenance of self-tolerance in these catabolic, but noninflammatory (1), pathologies. Thus, the results of this investigation connect current understanding of the noninflammatory dendritic cell with an earlier proposition (42) that wasting-associated immunological change confers benefit by minimizing the risk of autoimmune disease. Studies are warranted that directly address the activity of the dendritic cell in maintaining peripheral tolerance in wasting protein and energy deficiencies.

LITERATURE CITED


