Adoptively Transferred Dendritic Cells Restore Primary Cell-Mediated Inflammatory Competence to Acutely Malnourished Weanling Mice

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Immune depression associated with prepubescent malnutrition underlies a staggering burden of infection-related morbidity. This investigation centered on dendritic cells as potentially decisive in this phenomenon. C57BL/6J mice, initially 19 days old, had free access for 14 days to a complete diet or to a low-protein formulation that induced wasting deficits of protein and energy. Mice were sensitized by i.p. injection of sheep red blood cells on day 9, at which time one-half of the animals in each dietary group received a simultaneous injection of 10^6 syngeneic dendritic cells (JAWS II). All mice were challenged with the immunizing antigen in the right hind footpad on day 13, and the 24-hour delayed hypersensitivity response was assessed as percentage increase in footpad thickness. The low-protein diet reduced the inflammatory immune response, but JAWS cells, which exhibited immature phenotypic and functional characteristics, increased the response of both the malnourished group and the controls. By contrast, i.p. injection of 10^6 syngeneic T cells did not influence the inflammatory immune response of mice subjected to the low-protein protocol. Antigen-presenting cell numbers limited primary inflammatory cell-mediated competence in this model of wasting malnutrition, an outcome that challenges the prevailing multifactorial model of malnutrition-associated immune depression. Thus, a new dendritic cell-centered perspective emerges regarding the cellular mechanism underlying immune depression in acute pediatric protein and energy deficit. (*Am J Pathol* 2008, 172:378–385; DOI: 10.2353/ajpath.2008.070456)

age,¹ and this toll is undoubtedly dwarfed by the burden of infection-related morbidity also associated with prepubescent malnutrition. Immune depression is widely accepted as the link between malnutrition and susceptibility to infection,² and repairing inflammatory immune competence has been identified recently as one of three preferred, complementary approaches to reducing the burden of malnutrition-related infection.¹ Importantly, this applies to both acute (wasting) and chronic (stunting) forms of protein and energy deficit. In this connection, four distinct rodent models of acute malnutrition have been used in studies showing that depression in inflammatory immune competence, both innate and adaptive, can be prevented despite ongoing weight loss.^{2,3} In addition, one report documents restoration of a thymus (T)-dependent antibody response in the face of ongoing wasting disease.⁴ Proof of principle is available, therefore, that immune competence can be dissociated physiologically from weight loss in acute deficits of protein and energy. This fundamental principle permits optimism that improved understanding of malnutrition-associated immunological change will provide a basis for rational gains in the management of infection even in the most advanced stages of acute pediatric protein and energy deficit. Immune restoration, if achievable in the early stages of clinical management, may be the only viable strategy for the most severely wasted patients whose infections will overwhelm them before their weight loss can be addressed.³

A substantial catalog of descriptive information demonstrates that acute, prepubescent deficits of protein and energy reduce the capacity of humans and animals to generate inflammatory T-dependent adaptive immune responses to foreign agents.² However, malnutrition-associated immune depression remains poorly understood,

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Five million deaths result each year from infection among protein-energy malnourished children under 5 years of

and fundamental revision may be needed to the conceptual foundation that governs interpretation of the knowledge base.² Lymphoid atrophy is characteristic of acute deficits of nitrogen and energy and is legitimately regarded as an important contributor to the associated immune depression. Moreover, within the involuted T-lymphocyte system, an overabundance of the relatively quiescent naïve type of T cell is apparent phenotypically in acutely malnourished children⁵ and rodents,⁶ and this observation has been extended more recently by studies of polyclonal T-cell activation *in vitro*.⁷ The foregoing evidence of a naïve-shift within the T-cell compartment focuses attention on the dendritic cell because of its unique role as the antigen-presenting cell for the naïve T cell, ie, for initiating primary T-dependent immune responses.⁸

Dendritic cells constitute a small population of systemically distributed immunological sentinels.⁸ In the extra-lymphoid periphery, these cells are immature and particularly suited to antigen uptake. Microbial signals delivered through pattern recognition receptors stimulate immature dendritic cells to migrate to the T-dependent regions of secondary lymphoid organs and to differentiate to maturity, in which form the cells exhibit their unique capacity for presenting antigen to T cells.⁸ In recent years, the dendritic cell has emerged as a key player in the response to both foreign and self antigens.^{9,10} Notably, the mature inflammatory dendritic cell promotes classical adaptive responses to foreign antigens, whereas the immature cell promotes self-tolerance, which is the apparent physiological default function of this antigen-presenting cell.^{9,10} Importantly, even presentation of foreign antigens stimulates a suppressive (tolerance) response when the presenting dendritic cell is immature and, hence, tolerogenic.^{9,10}

The stochastic interactions between T cells and inflammatory dendritic cells are rate limiting to T-cell priming *in vitro*.¹¹ Moreover, studies of healthy adult mice given dendritic cells pulsed with antigen show that T cells compete for access to dendritic cells and suggest that dendritic cell numbers, in mature form, limit naïve T-cell responses *in vivo*.¹² In this context, it is noteworthy that a combined acute deficiency of nitrogen and energy, in its advanced stages, is reported to produce disproportionate involution of this already-limiting cellular population in the spleen of the weanling mouse.¹³ Collectively, these findings raise the possibility that dendritic cell numbers limit adaptive immune competence, at least in the advanced stages of this form of acute malnutrition. This proposition departs radically from the prevailing multifactorial paradigm of malnutrition-associated immune depression in which no cellular event can be singled out as individually determinative.² Although widely accepted, the multifactorial model has little formal experimental support but rather has evolved from extensive data showing that essentially no component of immune defense escapes the impact of wasting deficits of nitrogen and energy.² The objective of this investigation, therefore, was to determine whether adoptively transferred syngeneic dendritic cells could restore a primary cell-mediated inflammatory immune response in an experimental system with established relevance to acute pediatric protein and energy deficit. The affirmative outcome reported

herein provides a new perspective on the cellular mechanism underlying malnutrition-associated depression in inflammatory cell-mediated immune competence.

Materials and Methods

Animals, Facilities, Diets, and Feeding Protocols

Male and female C57BL/6J mice were used from an in-house breeding colony. Caging and housing conditions were as described previously,^{6,13,14} and the investigation was approved by the Animal Care Committee of the University of Guelph in accordance with the Canadian Council on Animal Care.

The mice were weaned at 18 days of age and acclimated for 1 day with free access to a complete purified diet as described previously.¹⁵ After acclimation, the mice were assigned to one of two dietary groups: an age-matched control group that consumed the complete purified diet *ad libitum* and a group that consumed a low-protein diet *ad libitum*. The experimental period was 14 days, after which the mice were euthanized by CO₂. The low-protein diet¹⁵ contained 0.6% crude protein (as fed) and was prepared by replacement of most of the egg white (U.S. Biochemical, Cleveland, OH) of the complete diet with an equal weight of cornstarch (ICN Biomedicals, Inc., Aurora, OH). All mice had free access to clean tap water throughout the investigation, and coprophagy was permitted.

Design of Studies Imposing Acute Weanling Malnutrition

Two experiments were conducted. In the first experiment, mice were immunized to elicit a delayed hypersensitivity response to sheep red blood cells (SRBCs), and a 2 × 2 design was used such that animals within each dietary group were allocated either to receive dendritic cells by adoptive transfer or to receive only the carrier fluid in which the cells were suspended. Fifteen mice were included in each of the four groups of sensitized mice. Unsensitized negative control animals were included, according to an identical design (*n* = 4 per group), as an additional component of the first experiment. In the second experiment, 12 mice were fed the low-protein diet and immunized to elicit a delayed hypersensitivity response to SRBCs. Within this number, six mice were allocated to receive purified syngeneic T cells by adoptive transfer and six mice received only the carrier fluid. Four additional mice served as unimmunized negative controls. Comparable numbers of males and females were included within each group of mice.

Assessment of Delayed Hypersensitivity Response to Sheep Red Blood Cells

As described previously,¹⁴ an immunizing dose of SRBCs in 100 μ l sterile, endotoxin-free physiological saline (MTC Pharmaceuticals, Cambridge, Canada) was given i.p. on

day 9 of the feeding protocol. Subsequently, a challenge dose of the same antigen was given into the right hind footpad on day 13, whereas an equal volume of saline carrier was injected into the left hind footpad. Accumulation of inflammatory fluid was assessed 24 hours later as the difference in maximum footpad thickness expressed as a percentage of the thickness of the unchallenged footpad. Unsensitized animals received 100 μ l of saline i.p. on day 9, and their footpad response after SRBC challenge on day 13 was subtracted from the response of the sensitized mice to produce an estimate of lymphocyte-mediated inflammation.

Maintenance of Dendritic Cells in Vitro

An immortalized dendritic cell clone designated JAWS II was purchased from The American Type Culture Collection (CRL-11904) (Rockville, MD). This monocytic clone was derived from the bone marrow of a C57BL/6J mouse. The cells were maintained in Alpha Essential Medium (Sigma Chemical, St. Louis, MO) without antibiotics but supplemented with 0.05 g/L ribonucleosides, 0.03 g/L deoxyribonucleosides, 4 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, 2.2 g/L sodium bicarbonate, 200 ml/L heat-inactivated fetal bovine serum (Sigma Chemical), and 5 ng/ml murine granulocyte-macrophage colony-stimulating factor (Cedarlane Laboratories, Hornby, ON, Canada). Media and plasticware were free from endotoxin, and cells were cultured at 37°C in a humidified atmosphere containing 5% CO₂.

Adoptive Transfer of Dendritic Cells

JAWS II dendritic cells in exponential growth were washed twice in sterile, endotoxin-free physiological saline (MTC Pharmaceuticals). On day 9 of the experimental period, recipient mice received 10⁶ cells i.p. suspended in saline. Immunized mice received the cells together with the sensitizing dose of SRBCs.

Enrichment and Adoptive Transfer of Syngeneic T Cells

Single-cell suspensions of mononuclear cells were prepared as described previously⁶ from the mesenteric, inguinal, and submandibular lymph nodes of C57BL/6J mice at 4 to 6 months of age. The cells were suspended in RPMI 1640 (Sigma Chemical) supplemented with 100 ml/L heat-inactivated fetal bovine serum (Sigma Chemical), 1 mmol/L HEPES (ICN Biomedicals), 10⁵ U/L penicillin, and 100 mg/L streptomycin (complete medium). Enrichment of T cells from the suspensions was achieved by recovery of nonadherent cells after single passage over nylon wool as described previously.¹⁶ Each recipient animal received 10⁶ viable (eosin Y exclusion) cells by i.p. injection in 100 μ l of endotoxin-free physiological saline (MTC Pharmaceuticals) together with an immunizing dose of SRBCs on day 9 of the experimental protocol.

Stimulation of JAWS II Cells in Vitro

Exposure to endotoxin for 24–48 hours is a common strategy for stimulating maturation of dendritic cells *in vitro*.¹⁷ Hence, JAWS II cells in exponential growth were stimulated by inclusion of *Escherichia coli* lipopolysaccharide (Sigma Aldrich, Mississauga, ON) in the culture medium for 48 hours at a concentration of 10 μ g/ml.

Assessment of JAWS II Dendritic Cells and Nylon-Wool Nonadherent Mononuclear Cells by Flow Cytometry

Analyses were performed using a Becton Dickinson (Mississauga, ON, Canada) FACSCalibur flow cytometer equipped with BD CellQuest software (2001). Generic aspects of staining procedures in this laboratory were described previously.⁶ Each analysis was based on at least 10⁴ events after dead cells and residual erythrocytes were eliminated by gating on the basis of forward-angle light scatter.

JAWS II cells from cultures in exponential growth were subjected to single-color analysis to determine their surface expression of major histocompatibility complex class II molecules (anti-mouse I-A^{D,K,b,q,r,s,j}, 7-16.17, phycoerythrin-conjugated mouse IgG2a; Cedarlane Laboratories), CD80 (anti-mouse CD80, RMMP-1, phycoerythrin-conjugated rat IgG2a; Cedarlane Laboratories), and CD86 (anti-mouse CD86, RMMP-2, phycoerythrin-conjugated rat IgG2a; Cedarlane Laboratories). Each antibody was used at a concentration of 0.5 μ g/250 $\times$ 10³ viable cells. Corresponding negative controls were stained with the same concentration of phycoerythrin-conjugated mouse or rat IgG2a (Cedarlane Laboratories), as appropriate.

Nonadherent lymph node mononuclear cells from nylon wool columns were stained with phycoerythrin-conjugated anti-mouse CD3 (145-2C11, hamster IgG; eBiosciences, San Diego, CA) at a concentration of 0.1 μ g/250 $\times$ 10³ viable cells. Negative control samples were stained with biotin-conjugated hamster IgG (Cedarlane Laboratories) followed by phycoerythrin-conjugated streptavidin (Cedarlane Laboratories), each at a concentration of 0.1 μ g/250 $\times$ 10³ viable cells.

Assessment of Cytokine Secretion by JAWS II Cells in Vitro

Interleukin (IL)-10 and IL-12p70 concentrations were determined in culture fluids by sandwich enzyme-linked immunosorbent assay. Commercial kits were used (BD Biosciences, Mississauga, ON, Canada) according to the manufacturer's instructions. The linearity (R^2) of standard curves exceeded 0.99 in both assays, and estimates of reliability (intra-assay coefficient of variation) were 1.5% (IL-10) and 1.9% (IL-12p70). Detection limits for the assays were estimated as described previously.¹⁸

Table 1. Performance Indices of C57BL/6J Weanlings: Influence of Adoptively Transferred Dendritic Cells*

Index	Complete diet		LP diet		Pooled SEM	Statistical probability		
	No DC	DC	No DC	DC		Diet	DC	d × DC
IBW (g/mouse) [†]	8.3	8.3	8.5	8.6	0.013	0.36	0.87	0.76
FBW (g/mouse) [†]	17.6	18.3	6.5	6.4	0.310	0.001	0.08	0.08
FI (g/mouse/14 days) [†]	66.9	70.3	22.9	24.7	0.183	0.001	0.35	0.69
FI (g/g BW/d) [†]	0.34	0.34	0.20	0.20	1.063	0.001	0.93	0.90
Carcass DM (%) [‡]	30.9	32.6	32.0	29.6	1.258	0.62	0.86	0.31
Carcass lipid (%) [‡]	8.7	8.2	5.4	4.6	0.469	0.006	0.42	0.84

*Means. Probability values refer to main effects (two-way analysis of variance) of diet and adoptive transfer of dendritic cells and to the interaction term between main effects. DC, dendritic cells; DM, dry matter; FBW, final body weight; FI, food intake; IBW, initial body weight.

[†]From analysis of variance of natural log-transformed data. Mean values are antilogs of log means.

[‡]From ANOVA of inverse-transformed data. Mean values are reciprocals of inverse means.

[‡]Percent wet weight.

Carcass Analysis

Carcasses were stored at -20°C to await analysis. Dry matter and lipid contents were determined as described previously.^{6,13,14}

Statistical Analysis

The predetermined upper limit of probability for statistical significance throughout this investigation was $P \leq 0.05$, and analyses were performed using the SAS system for Windows (version 8.2). Throughout this investigation, data sets that failed to conform to a normal distribution according to each of the four tests applied by the SAS system were transformed to comply with this underlying assumption of parametric testing. Where transformation attempts failed, data from two-group studies were analyzed by means of χ^2 comparisons of Wilcoxon two-sample rank sums, whereas data from studies that included multiple groups were subjected to Wilcoxon two-sample testing if justified by the statistical probability outcome ($P < 0.05$) of a Kruskal-Wallis test (χ^2 approximation).

Results

Consumption of the Low-Protein Diet Produced a Wasting Deficit of Both Protein and Energy That Was Not Influenced by the Adoptive Transfer of Syngeneic Dendritic Cells

Growth indices for the first experiment are presented in Table 1. Initial body weights did not differ among groups, but diet exerted an influence on the other performance indices recorded. By contrast, adoptive transfer of dendritic cells did not influence growth indices, and the absence of statistically significant interaction terms showed that the influence of diet on the various measures of performance was independent of dendritic cell transfer. The food intakes and gains in fat and lean tissue exhibited by the age-matched control groups were comparable with previous results pertaining to C57BL/6J weanlings given free access to the same complete purified diet.^{6,13} By contrast, consumption of the low-protein

diet induced decrements of both fat and lean tissue with resultant daily weight loss of approximately 1.7% of initial body weight. Furthermore, the protein-deficient diet produced low food intakes relative to the complete diet, including low levels of intake on a body weight basis. Therefore, as discussed previously,¹³ the low-protein diet protocol elicited a wasting deficit of both protein and energy that was comparable with the pathology reported previously^{6,14} in studies demonstrating depressed thymus-dependent immune competence in the same experimental system. Growth indices pertaining to the unsensitized immunological negative controls were closely similar to those of the immunized animals and are not shown.

Adoptive Transfer of Syngeneic Dendritic Cells Enhanced the Primary Delayed Hypersensitivity Response to Sheep Red Blood Cells in Both Well Nourished and Acutely Malnourished Weanling Mice

The primary delayed hypersensitivity responses of the four groups of sensitized mice (experiment 1) are shown in Figure 1. Comparison of the response elicited in the two groups not given exogenous dendritic cells confirmed previous reports¹⁴ that the low-protein protocol used herein almost eliminates this cell-mediated response in the weanling mouse. In addition, a statistically significant main effect of dendritic cell transfer was apparent ($P = 0.001$). Although the influence of dendritic cell transfer was diet dependent (P interaction = 0.004), receipt of JAWS II cells increased the delayed hypersensitivity response in both the malnourished mice ($P = 0.001$) and the age-matched control group ($P = 0.04$). No effect of diet or adoptive transfer of dendritic cells ($P = 0.58$ and 0.09 , respectively) was apparent on the response to challenge exhibited by unsensitized mice. Therefore, the cumulative mean response of the full cohort of 16 unsensitized mice, an increase of 3.7% in footpad thickness, was applied as a correction factor in calculating the responses shown in Figure 1 for sensitized mice in each of the four groups.

Figure 1. Delayed hypersensitivity response to SRBCs. C57BL/6J mice, initially 19 days old, were given free access for 14 days to a complete purified diet (groups designated "C") or to an isocaloric low-protein diet (LP groups). Mice were sensitized with SRBCs on day 9 of the experimental period and challenged by injection of the same antigen into the footpad on day 13. The immune response was assessed 24 hours after challenge by determining the increase in thickness of the challenged footpad. Groups C and LP each included 30 mice, and within each dietary group, 15 mice received 10^6 syngeneic dendritic cells (JAWS II) by i.p. injection together with the sensitizing dose of SRBCs. Data were subjected to two-way analysis of variance with the main effects being diet and receipt of dendritic cells. Bars represent antilogs of means from log-transformed data, and SDs are shown. Error mean square = 0.993; $P = 0.001$ (diet), 0.001 (receipt of dendritic cells), and 0.004 (interaction). Statistical probabilities are shown for the three predetermined comparisons permitted by least squares means analysis.

JAWS II Dendritic Cells: Surface Phenotype and Cytokine Secretion Profile in Vitro

The outcome of the adoptive transfer experiments with JAWS II cells gave rise to a need to assess the maturity of these cells as injected into the recipient animals. Representative flow cytometer histograms of JAWS II cells are shown in Figure 2 and illustrate the forward angle and side scatter characteristics of these cells together with

Figure 2. Representative flow cytometer histograms of JAWS II dendritic cells cultured under standard conditions. Histograms from stained suspensions are shown in gray outline, and isotype control histograms are shown in black. The percentage of viable cells judged positive is recorded in each surface marker histogram. **A:** Forward (FSC)- and side (SSC)-angle light scatter. Box R1 identifies viable single cells and vertical dotted line shows their mean forward scatter. Phycoerythrin-conjugated anti-mouse **B:** I-A^b, I-E^b, I-B^b, I-D^b, I-F^b, I-G^b, I-H^b, I-J^b (7-16.17, mouse IgG2a), **C:** CD80 (RMMP-1, rat IgG2a), and **D:** CD86 (RMMP-2, rat IgG2a).

Table 2. Phenotypic Characteristics of JAWS II Cells*

Phenotypic index	Culture conditions [†]			SEM
	Low GM-CSF	Standard	Endotoxin	
MHCII ⁺ (%)	35.9 ^A	64.7 ^B	61.3 ^B	4.63
CD80 ⁺ (%)	30.9 ^A	58.5 ^B	60.4 ^B	6.92
CD86 ⁺ (%)	57.6 ^A	80.7 ^B	77.8 ^B	4.25
Forward scatter [‡]	449 ^A	512 ^{AB}	615 ^B	0.0001

*Means. $n = 4$ (Low GM-CSF), 6 (Standard), and 5 (Endotoxin) except $n = 8$ (Standard, MHCII). Within a row, values not sharing a superscript letter differ according to Tukey's Studentized Range test ($P \leq 0.05$). GM-CSF, granulocyte-macrophage colony-stimulating factor; MHCII, major histocompatibility complex class II.

[†]Standard and endotoxin are as described in Materials and Methods; low GM-CSF is as described in Results.

[‡]Forward angle light scatter (arbitrary units). From ANOVA of inverse-transformed data. Mean values are reciprocals of inverse means.

their expression of major histocompatibility complex class II, CD80 and CD86. These surface antigens were readily discernible on only a proportion of the cells. Culture in a low concentration of murine granulocyte-macrophage colony-stimulating factor (0.5 ng/ml) for 4 weeks reduced the proportion of cells discerned to express class II major histocompatibility molecules as well as CD80 and CD86, whereas exposure to endotoxin for 48 hours did not influence expression of these molecules (Table 2). It is noteworthy, also, that the fluorescence intensity of the selected surface markers was low (not shown) and that this characteristic was not influenced by the culture conditions tested. Forward angle light scatter was examined as an index proportional to cellular surface area and volume,¹⁹ both of which increase as dendritic cells mature.²⁰ This index increased in JAWS II cells only in response to the combined influences of granulocyte-macrophage colony-stimulating factor and endotoxin (Table 2). During 5 days of culture, unstimulated JAWS II cells were unable to generate concentrations of either IL-10 or IL-12p70 that exceeded the detection limits of the respective assays (Figure 3). However, exposure to endotoxin during the final 48 hours of culture resulted in the appearance of formally detectable levels of both cytokines (Figure 3). As injected into recipient mice, therefore, the JAWS II cells exhibited phenotypic and functional characteristics expected of immature dendritic cells.

Adoptive Transfer of Syngeneic CD3⁺ Mononuclear Cells Did Not Influence the Primary Delayed Hypersensitivity Response to Sheep Red Blood Cells in Acutely Malnourished Weanling Mice

A T cell-centric focus prevails vis-à-vis the cellular mechanism underlying malnutrition-associated immune depression.²¹ Therefore, it was of interest to determine whether adoptive transfer of syngeneic T cells could influence inflammatory immune competence in the man-

Figure 3. Concentrations of IL-10 and IL-12p70 generated by JAWS II dendritic cells cultured for 5 days in Alpha Essential Medium containing 10% fetal bovine serum and 5 ng/ml murine granulocyte-macrophage colony-stimulating factor with (+) or without (–) 10 μ g/ml *E. coli* lipopolysaccharide (LPS) for the final 48 hours of culture. Bars represent means and SDs are shown ($n = 5$ and 4 per group for IL-10 and IL-12p70, respectively). Statistical probabilities are outcomes of two-tailed Student's *t*-test. Horizontal dashed lines depict detection limits of each assay estimated as described previously.¹⁹

ner achieved using dendritic cells. Two preparations of lymph node mononuclear cells, enriched for CD3⁺ surface phenotype, proved sufficient for this small supplementary experiment. Purity of 97.4 and 98.0%, respectively, was achieved and a flow cytometer histogram illustrating the extent and quality of enrichment is shown in Figure 4. The key outcomes of the experiment are summarized in Table 3. The low-protein diet protocol produced a wasting condition and a weak delayed hypersensitivity response, each of which was comparable with the results of the preceding experiment of this investigation and to previous results from use of the same dietary protocol.¹⁴ Under these conditions, provision of syngeneic CD3⁺ mononuclear cells failed to influence the capacity of the malnourished animals to develop a cell-mediated response to SRBCs.

Discussion

In this investigation, adoptive transfer of syngeneic dendritic cells exhibiting characteristics of immaturity increased primary cell-mediated competence vis-à-vis a

Figure 4. Representative flow cytometer histogram illustrating the purity of the syngeneic CD3⁺ cells adoptively transferred into weanling C57BL/6j recipients subjected to acute protein and energy deficit and immunized to elicit an anti-SRBC delayed hypersensitivity response. Nylon wool enrichment was quantified using single-cell suspensions stained with phycoerythrin-conjugated anti-mouse CD3 (145-2C11, hamster IgG; gray outline). Isotype controls (black) were created with phycoerythrin-conjugated streptavidin after exposure of cells to biotin-conjugated hamster IgG.

foreign antigen in acute weanling protein and energy deficit. Importantly, the model of malnutrition used herein is relevant to the human pathology of incipient kwashiorkor,²² and its depressive influence on cell-mediated adaptive immune responses is documented.^{14,23} The cell-mediated anti-SRBC response used in this investigation was selected primarily because of its immunological similarity to the classical tuberculin reaction,²⁴ a common tool for assessing the attenuated inflammatory cell-mediated competence of acutely malnourished humans.² Delayed hypersensitivity manifests as an inflammatory response to challenge with sensitizing antigen and, hence, includes elements of innate defense. However, unsensitized negative controls revealed that the response assessed reflects an influence on primary adaptive competence. The results of this investigation demonstrate the need to move beyond the T cell-centric research focus that has prevailed with regard to adaptive immune competence in acute malnutrition^{2,21} and challenge the multifactorial paradigm that defines current understanding of malnutrition-associated immune depression.² In short, a new perspective is proposed on the cellular mechanism underlying depression in primary cell-mediated immune competence in acute deficits of protein and energy, and new optimism emerges with regard to the clinical management of the most severely debilitated patients.

In relation to the main objective of this investigation, a remarkable model emerges in which dendritic cell numbers independently and decisively limit primary cell-mediated inflammatory competence in acute deficits of protein and energy. Previous investigations of the same experimental system^{13,25} lend support to this proposition. In the first place, the splenic dendritic cell compartment is profoundly involuted in this model of incipient kwashiorkor.¹³ Moreover, splenic dendritic cells from weanling mice subjected to this experimental protocol exhibit mature inflammatory capacities when permitted to function either *in vitro* or following adoptive transfer to healthy animals.²⁵ It must be noted that these findings, although confined to splenic dendritic cells, are directly relevant to the anti-SRBC response of the present investigation, which arises within the spleen.²⁶ Consequently, a simple interpretation of the present investigation is that dendritic cells develop and function adequately in acute pediatric deficits of protein and energy but that the size of the dendritic cell compartment limits primary cell-mediated immune functions in this type of pathology. As a secondary outcome, the results suggest that dendritic cell numbers also impose a normal physiological limit on the primary cell-mediated inflammatory response of the healthy mouse. This finding (pertaining to an inflammatory immune response endpoint) is consistent with, and predicted by, evidence that T cells must compete for access to antigen-bearing dendritic cells *in vivo* in the adult mouse.¹² On the basis of both its antigen-presenting proficiency and its anatomical location, the dendritic cell is widely recognized as the unique antigen-presenting cell for the primary thymus-dependent immune response.^{8,9} The present investigation, therefore, provides new insight into the determining role of the dendritic cell

Table 3. Performance Indices and Anti-SRBC Delayed Hypersensitivity Response of C57BL/6J Weanlings Subjected to Acute Protein and Energy Deficit: Influence of Adoptively Transferred T Cells*

Index	Vehicle recipients	T-cell recipients [†]	P value
IBW (g/mouse) [‡]	8.5 (8.1 to 9.6)	8.7 (8.5 to 8.9)	0.15
FBW (g/mouse)	6.6 ± 0.36	6.8 ± 0.24	0.34
FI (g/mouse/14 days)	22.2 ± 0.95	22.6 ± 1.46	0.55
FI (g/g BW/d)	0.21 ± 0.012	0.21 ± 0.017	0.88
Anti-SRBC response (%) [¶]	5.4 ± 5.6	5.8 ± 2.5	0.90

*Means. SDs are shown for data sets analyzed by Student's two-tailed *t*-test. Range is shown in parentheses where Wilcoxon's two-sample test was applied. FBW, final body weight; FI, food intake; IBW, initial body weight.

[†]10⁶ syngeneic T cells by intraperitoneal injection in 100 μ l of physiological saline.

[‡]Wilcoxon two-sample test of rank sums that were 48.0 (T-cell recipients) and 30.0 (vehicle recipients).

^{||}Square root-transformed data. Means are squares of square root means.

[¶]Expressed as increase in footpad thickness 24 hours after challenge with SRBCs.

in adaptive immune competence both in health and in a model of a prevalent form of acute pediatric malnutrition.

To date, the T cell has occupied center stage in the research effort aimed at understanding the depression in adaptive immune responses characteristic of acute malnutrition.^{2,21} Numerous factors pertaining to, and impinging on, the T-cell compartment have been cited as decisive contributors to malnutrition-associated immunopathology, eg, profound reduction in T-cell numbers,² imbalances among critical cellular subsets,² directly depressive endocrinological influences, notably of glucocorticoids,²⁷ and dearth of necessary endocrinological stimuli, notably of leptin.²⁸ However, in the present investigation, adoptive transfer of viable, syngeneic CD3⁺ (ie, T) cells failed to influence a cell-mediated immune response that was enhanced by an equivalent large number of syngeneic dendritic cells. The likely contribution of changes within the T-cell system must be acknowledged vis-à-vis malnutrition-associated immune depression, but the outcome of the present investigation suggests that the influence of acute malnutrition on primary cell-mediated immunity centers fundamentally within the antigen processing and presentation compartment rather than within the T-cell compartment. This proposition constitutes a fundamental revision to our perception of the cellular mechanism underlying cell-mediated immune depression in the advanced stages of acute pediatric protein and energy deficit.

The use of the JAWS II cell line permitted unambiguous attribution of the results to the dendritic cell and, thereby, eliminated an inevitable interpretation problem that has proven severe in closely related applications of enriched dendritic cell preparations.²¹ The present investigation confirms the phenotypic immaturity of JAWS II cells reported by others²⁹ who examined not only expression of class II major histocompatibility antigens, CD80 and CD86, but also expression of CD40, CD14, and CD11c under the culture conditions applied herein. The responsiveness of the cells to granulocyte-macrophage colony-stimulating factor and their insensitivity to endotoxin in terms of maturity-related phenotypic characteristics²⁹ were also confirmed. Surface marker phenotype, however, is an insensitive and inconclusive index of dendritic cell maturity relative to the index of cytokine production.¹⁷ In particular, an equivocal capacity to produce IL-10 and inability to secrete IL-12 each distinguish functionally

immature dendritic cells from their mature inflammatory counterparts.¹⁷ Therefore, the present investigation extends characterization of the unstimulated JAWS II cell by demonstrating its immaturity in terms of cytokine production and its responsiveness to endotoxin in relation to this functional characteristic, as one would expect of an immature dendritic cell.¹⁷ The JAWS II cells used for adoptive transfer in this investigation, therefore, exhibited characteristics of immaturity both phenotypically and functionally but could respond to a maturational stimulus *in vitro* by developing the ability to produce IL-12, a critical inflammatory cytokine. Furthermore, the support afforded by the JAWS II cells to a cell-mediated inflammatory response in this investigation reveals the capacity of these cells to differentiate to functional maturity *in vivo*.

It has been proposed recently that acute weanling malnutrition, including the experimental system used herein, limits T cell-dependent inflammatory responses by imposing maturational arrest on the dendritic cell compartment.³⁰ This proposition is based on the high blood levels of transforming growth factor- β ,³⁰ IL-10,³⁰ and glucocorticoid hormones^{2,27} reported in acute prepubescent deficits of energy and/or protein coupled with evidence that these molecules are potent physiological regulators of dendritic cell maturation.¹⁰ In the present investigation, however, the JAWS II cells evidently achieved antigen-presenting maturity after adoptive transfer regardless of the nutritional status of the recipients. Dose-response comparisons between immature and stimulated dendritic cells in the experimental system applied to this investigation should prove useful in determining the extent to which maturational blockade influences inflammatory adaptive immune competence in acute malnutrition.

The emergence of antigen processing and presentation as the focal point of primary immune competence in a model of incipient kwashiorkor appears irreconcilable with the prevailing multifactorial paradigm of malnutrition-associated immune depression.² In fact, attention must center on the dendritic cell in its role as the physiological primer for the naïve T cell.^{8,9} By contrast, the multifactorial paradigm dictates that no single immunological element is decisively limiting to the inflammatory immune competence of the malnourished. The present investigation, therefore, clearly presents a challenge of fundamental significance to our understanding of the cellular un-

derpinning of immune depression in acute protein and energy deficit. In addition, the multifactorial model can only lead to complexity in the immunological management of the acutely malnourished by requiring simultaneous clinical attention to a daunting diversity of immune defense components. By contrast, the findings of the present investigation offer the possibility of clinical simplicity or, at least, a basis for establishing clinical priorities. Before this investigation, the principle that the link between immune competence and weight loss is physiologically and clinically severable was based entirely on hormonal interventions, eg, using triiodothyronine, leptin, or anti-glucocorticoid strategies.^{2,3} The findings reported herein, therefore, add substantively to the base of data underlying this principle. It now appears reasonable to contemplate sophisticated but uncomplicated strategies to improve the management of infection in wasted pediatric patients, including those whose advanced debilitation precludes early stabilization of body weight.

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