Immunobiology 216 (2011) 309–315

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.de/imbio

T lymphocytes from malnourished infants are short-lived and dysfunctional cells

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ARTICLE INFO

Article history:
Received 15 April 2010
Received in revised form 6 July 2010
Accepted 9 July 2010

Keywords:
Apoptosis
Malnutrition
Infants
Flow cytometry

ABSTRACT

To investigate T-cell functional molecules and inflammatory cytokines and to assess T-cell apoptosis in malnourished infants, 64 infants from undernourished women and 28 healthy control infants were recruited to the study. Malnourished infants showed a significant decrease in the levels of circulating IL-2 and IL-7 and increases in the levels of IL-1β, IL-6, IL-10 and TNF-α, as measured by flow cytometry. There was a significant reduction in the number of $CD3^+$ T cells and an increase in apoptotic T cells, which was associated with an up-regulation of CD95 and PD-1 expression on $CD3^+$ T cells in malnourished compared to control infants. Significant reductions were also observed in the phosphorylation of AKT and STAT5 and in the expression of CCR7 and CXCR4 receptors in malnourished children, and these reductions were associated with a significant reduction in T-cell migratory capacity to their ligands CCL21 and CXCL12, respectively, as measured using an *in vitro* chemotaxis assay. Taken together, these data suggest that lymphocytes from malnourished infants are short-lived and dysfunctional.

Introduction

Severe malnutrition occurs as a consequence of deficient food intake and/or low-protein diets. Maternal malnutrition prior to and during pregnancy, which is manifested by low body weight, short stature, inadequate energy intake during pregnancy and coexisting micronutrient deficiency, are considered major determinants of infant malnutrition in developing countries, where the total number of underweight and stunted children has not changed dramatically since 1980 (Heird 2004). A poor diet during pregnancy has been reported to have a long-term impact on the health of the child (Alam 2009). Malnourished children are more susceptible to infections than well-nourished children; consequently, malnourished children are considered immunodeficient (Nájera et al. 2004). Previous studies have indicated that malnutrition is the primary cause of secondary immunodeficiency (Chandra 1999; Islam et al. 2002), which is related to changes in cellular immunity (Chandra 1999; Das et al. 1977) and in the subsets of peripheral lymphocytes (Nájera et al. 2004).

Malnutrition in children is frequently associated with an increase in the incidence of bacterial, fungal and viral infections. Around the world, 12 million children under 5 years old die due to the malnutrition–infection cycle each year (Nájera et al. 2004). There are also some indications that apoptosis of immune cells in undernourished organisms might lead to an increase in susceptibility to diseases related to immune suppression (Chandra 1999; Hetts 1998; Hotchkiss et al. 1999; Voll et al. 1997).

Effective immune responses depend on an array of soluble mediators that are collectively called cytokines (Nájera et al. 2004). The binding of cytokines to their receptors results in the activation of the receptor-associated Janus family tyrosine kinases (JAKs). Activated JAKs phosphorylate latent cytoplasmic transcription factors called Signal Transducers and Activators of Transcription (STATs), which induce their dimerization, nuclear accumulation and DNA binding. STATs regulate genes that control cell proliferation, differentiation, development and survival of T lymphocytes and are required for the adaptive immune response. This response is characterized by the capacity to recognize and remember pathogen-specific antigens (Levy and Darnell 2002). Lymphocytes use a complex array of signal transduction molecules to regulate their proliferation, differentiation and effector functions (Cantrell 2003). Chemokines and chemokine receptors are important regulators of cell migration to specific anatomic areas, such as lymph nodes and sites of infection, and thus have an important effect on cell function (Mengshol et al. 2009). Lymphocyte apoptosis has been described in peripheral blood and lymphatic organs during infection and malnutrition (El-Hodhod et al. 2005; Zuniga et al.

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doi:10.1016/j.imbio.2010.07.007

2002). However, the molecular mechanisms of the perturbation of T-cell function in malnourished infants are not yet understood. The identification of apoptotic pathways in T cells leads to predictions about the pathophysiological consequences and practical implications of disrupting or augmenting these pathways (Van Parijs et al. 1998). Therefore, we studied T-cell functional molecules and inflammatory cytokines and assessed T-cell apoptosis in malnourished infants.

Patients and methods

Patients

Sixty-four malnourished infants (29 female, 35 male) aged 15.2 ± 7.5 months (mean ± SD) from a poor suburb of Assiut, Egypt and 28 healthy control infants (14 female, 14 male) aged 14.6 ± 5.4 months were recruited to the study. All malnourished infants had a history of undernourished mothers during pregnancy. Thirty-five children presented with second-degree marasmus, 19 with third degree marasmus, and 10 with kwashiorkor. The clinical signs and symptoms of malnutrition and weight deficits were used to determine the type and severity of malnutrition according to the established values (Gomez et al. 1956). Forty-four patients were fed with artificial diets, 15 were breastfed and 5 were fed both. The children had been admitted to the hospital for gastroenteritis, and 10 also had respiratory infections. Informed written consent was collected from the parents of the patients and controls in accordance with the Assiut University ethical committee guidelines. The medical ethical committee of Assiut University approved this study. A medical history was taken from the parents of all patients, and controls were subjected to a thorough clinical examination. The following were also measured: serum levels of sodium (Na) and potassium (K) as determined by an AVL 9180 analyzer (Roch Diagnostic, Germany); serum calcium (Ca) level and liver and kidney functions as measured by a Beckman model CX-9 chemistry analyzer (CA, USA); and a complete blood count using a Celltac E automated hematology analyzer (Tokyo, Japan).

All patients and controls enrolled in the study were negative for antibodies to human immunodeficiency virus (HIV), hepatitis C and hepatitis B virus surface antigen (HBsAg).

Methods

Isolation of peripheral blood mononuclear cells (PBMCs) and survival assay

Blood was collected in sodium heparin vacutainer tubes for immunological assays. Plasma was isolated, frozen and stored for subsequent enzyme-linked immunosorbent assays (ELISAs). PBMCs were separated on a Ficoll–Hypaque density gradient (Wisent, St-Bruno, QC, Canada) and cryopreserved in 90% fetal bovine serum (FBS; Hyclone, Logan, UT) and 10% dimethyl sulfoxide (DMSO; Hybri-max, Sigma–Aldrich, France). All experiments were performed on frozen samples. Cells were cultured in R-10 (RPMI 1640 with L-glutamine containing 10% FBS and antibiotics) at a cell concentration of 10^6 cells/ml. The cells were cultured at 37 °C overnight and then were counted and analyzed by flow cytometry. The cell survival assay was performed using annexin-V–FITC/propidium iodide. Apoptotic cells showed double positivity for annexin/propidium iodide.

ELISA for plasma cytokine profiles

Sera were tested in duplicate by ELISA for interleukin-1 beta (IL-1β) and IL-2 from Mabtech (Nacka, Sweden), IL-6, IL-7 and IL-10 from Endogen (Woburn, Mass.) and Tumor Necrosis Alpha (TNF-α) from Medgenix (Stillwater, Minn.) according to the manufacturers' instructions. The optical densities (OD) were measured at 405 nm using a Vmax™ Kinetic microplate reader (Menlo Park, CA, USA). The detection limits were set according to the log–log correlative coefficient of the standard curve. The lower limits of detection were as follows: IL-1β, 2 pg/ml; IL-2, 2 pg/ml; IL-6, 1 pg/ml; IL-7, 1 pg/ml; IL-10, 3 pg/ml; and TNF-α, 3 pg/ml.

Flow cytometry antibodies

Cell surface antigens were analyzed by single parameter or multiparameter FACS analysis using the following directly conjugated monoclonal antibodies (mAbs): CD3-phycoerythrin (PE), CD127-PE, CD95-PE, PD-1-PE and CD3-FITC (all from BD Biosciences, Le Pont aux Claix, France). CCR7-PE and CXCR4-PE mAbs were purchased from R&D Systems (Abingdon, UK). Mouse isotype-matched FITC- and PE-conjugated control IgG1 and IgG2a were purchased from BD Biosciences. A FACSCaliber flow cytometer was used for data acquisition and the CellQuest® and FlowJo software (BD Biosciences) were used for data analysis. After gating based on the viable cells, 20,000 cells/sample were analyzed. The following intracellular antibodies were used: IL-2-PE, IFN-γ-FITC, pAKT-PE and pSTAT5-PE from BD Biosciences. All antibodies were titered on PBMCs (0.5×10^6) before use to determine the optimal dilutions.

Cell surface and intracellular phospho-specific flow cytometry

PBMCs were stimulated with IL-7 (for 10 min at 37 °C) or left unstimulated and then fixed for 10 min in pre-warmed cytofix buffer (BD Cytofix # 554655). Cells were permeabilized for 30 min on ice in PERM-III buffer (BD PERM-III buffer # 558050). Permeabilized PBMCs were washed twice and then re-suspended in staining buffer (phosphate-buffered saline plus 0.5% bovine serum albumin) and stained in a final volume of 100 μl for 30 min at room temperature. Directly conjugated antibodies from BD were against human CD3, phospho-STAT5 (Y694), phospho-AKT (S473) and control IgG. Cells were fixed, and the phospho-AKT and phospho-STAT5 cells in the $CD3^+$ population were analyzed.

CFSE proliferation assays

Thawed PBMCs (20×10^6/ml) were stained with 0.63 μM carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, UK) for 8 min at room temperature (RT). The reaction was stopped with FBS and cells were washed three times in PBS and re-suspended at 2×10^6 cells/ml in warm R-10. CFSE-labeled cells were stimulated for 6 days with Staphylococcal Enterotoxin B (SEB) (final concentration of 10 ng/ml) at 37 °C and 5% CO_2 in polypropylene culture tubes set at a 5° angle. Recombinant human IL-2 (10 IU/ml; Roche) was added 3 days after stimulation. On day 6, cells were stained with CD3-PE and analyzed with flow cytometry.

Intracellular cytokine staining (ICS) assay

Thawed PBMCs (2×10^6/1 ml R-10) were stimulated with SEB (10 ng/ml) or left unstimulated for 1 h at 37 °C and 5% CO_2 in 5 ml snap-cap polypropylene tubes set at a 5° angle. After 1 h of stimulation, 10 μg/ml of Brefeldin A (Sigma–Aldrich, France) and 6 μg/ml of Monensin sodium salt (Sigma–Aldrich, France) were added, and the cells were incubated for a total of 12 h. The cells were then washed with FACS buffer and stained for 30 min at 4 °C with CD3-FITC. The cells were washed and permeabilized using BD Cytofix/Cytoperm solution (BD Bioscience, France), stained with anti-IL-2 and anti-IFN-γ for 30 min, washed twice in BD Perm/Wash buffer (BD Biosciences, France) and fixed in 200 μl of FACS fix buffer.

In vitro chemotaxis assay

A chemotaxis assay was performed as follows: 5×10^5 PBMCs in 100 μl pre-warmed RPMI 1640 containing 10 mM HEPES and 1% FBS were allowed to transmigrate through 5-μm pore size bare filter Transwell inserts (Costar, Cambridge, MA) for 3 h at 37 °C in response to 250 ng/ml of CCL21 and CXCL12 (R&D Systems). The

Table 1
Comparison of weight, CBC and macro-mineral measurements between malnourished children and controls.

Parameters	Patients (63)	Controls (27)	*p* value
Weight (kg)	5.1 ± 1.61	9.7 ± 1.07	<0.001
Hb (g/dL)	9.2 ± 1.71	11.6 ± 0.67	<0.001
WBCs (10^9/L)	10.5 ± 4.5	7.3 ± 1.4	0.001
Platelets (10^9/L)	236 ± 120	287 ± 85.3	0.046
Na level (mmol/L)	132.1 ± 5.9	138.1 ± 2.4	0.001
K level (mmol/L)	3.4 ± 0.77	4.0 ± 0.27	<0.001
Ca level (mg/dl)	7.1 ± 1.25	9.5 ± 0.58	<0.001

Values are presented as mean ± SD, and significance was determined with Student's *t* test.

input cells and migrating cells were stained with CD3-FITC mAb and analyzed within gated $CD3^{high}$ and $CD3^-$ T cells. After exclusion of cell debris by forward and side scatter gating, the migrating cells were counted by FACscan™ for 60 s. The results are shown as the percentage of specific migrating cells, from which background migration was subtracted to control for the medium.

Statistical analysis

Data are expressed as means ± SD unless otherwise indicated. Differences between groups were assessed using one-way analysis of variance (ANOVA), and *p* values <0.05 were considered significant.

Results

The present study revealed significant decreases in the weight, hemoglobin level, and Na, K and Ca levels and a significant increase in the white blood cell count in the malnourished patients compared to the controls (Table 1). Liver and kidney functions were within the normal ranges for the age and sex of the patients according to Nicholson and Pesce (2004).

None of the clinical and routine laboratory parameters of the studied patients showed any significant correlations with apoptotic and functional indices.

Alterations in the plasma levels of different cytokines

Cytokines are secreted by specific cells of the immune system, carry signals locally between cells and are critical for the development and function of both the innate and the adaptive immune responses. Malnourished infants showed significant decreases in the levels of circulating IL-2 and IL-7 and increased levels of the inflammatory cytokines IL-1β, IL-6, IL-10 and TNF-alpha (Fig. 1).

Expression of CD127 and its signaling pathway in T cells

T cells from malnourished children expressed low levels of CD127 (IL-7 receptor alpha) (MFI = 134 ± 3.99) compared to that of the control (MFI = 623 ± 6.01). After stimulation with IL7, T cells from malnourished children exhibited decreased AKT (MFI = 334 ± 7.77) and STAT5 (MFI = 411 ± 7.9) phosphorylation compared with the T cells of controls (MFI = 455 ± 7.8 and 545 ± 7.3, respectively) with a *p* value <0.02 (Fig. 2).

Mitogen-induced T-cell proliferation

The measurement of cell proliferation after mitogenic stimulation revealed that T cells from malnourished children exhibited a diminished proliferative capacity as measured by the CFSE dilution assay (Fig. 3). The percentage of CFSE negative cells (proliferating cells) was 27.4 ± 3.7% in subjects and 42 ± 4.3% in controls ($p < 0.01$).

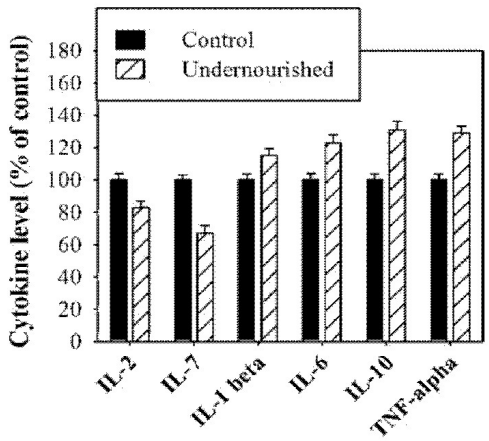

Fig. 1. Alterations in the plasma levels of different cytokines in malnourished children. Malnourished infants showed significant decreases in the levels of circulating IL-2 and IL-7 and increased levels of the inflammatory cytokines IL-1β, IL-6, IL-10 and TNF-alpha. Data are expressed as the mean ± SD of the percentages of the cytokine levels in undernourished (hatched bars) compared with the control (black bars); $p < 0.05$.

Cytokine production by T cells

Analysis of cytokine production following SEB stimulation revealed that T cells from malnourished children exhibited a decrease in cytokine production as demonstrated by ICS (Fig. 4). The percentage of T cells from malnourished subjects that produced IL-2 was 3.5 ± 0.8%, whereas the percentage of control T cells was 6 ± 0.9%. Similarly, the percentage of T cells that produced IFN-γ was 4.8 ± 1.1%, whereas that of control T cells was 8 ± 1.3% ($p < 0.02$).

Surface expression of CCR7 and CXCR4 and chemotactic activities of T cells

The expression of CCR7 and CXCR4, which are receptors that mediate homing of peripheral blood lymphocytes to secondary lymphoid organs (site of immune response initiation), was markedly reduced. The MFI of CCR7 expression was 67 ± 5.1 in malnourished T cells versus 97 ± 5.6 in control T cells. The MFI of CXCR4 expression was 610 ± 5.8 in malnourished T cells and 877 ± 5.6 in control T cells. The decreased expression of CCR7 and CXCR4 was correlated with a significant reduction in T-cell migratory capacity to their ligands CCL21 and CXCL12, respectively, as measured with an *in vitro* chemotaxis assay (Fig. 5). The percentages of CCL-21-mediated chemotactic cells were 19.9 ± 5.3% of malnourished T cells and 34 ± 4.6% of control T cells. The percentages of CXCL-12-mediated chemotactic cells were 28.9 ± 4.4% of malnourished T cells and 47.3 ± 5.9% of control T cells ($p < 0.02$).

Surface expression of CD95 and PD-1 and apoptosis of T cells

There was a significant reduction in the $CD3^+$ T-cell count (from 11.1 ± 3.2 in control to 6.88 ± 2.8 in patients, $p = 0.0023$) that was accompanied by an increase in apoptotic T cells in the malnourished children. The percentage of apoptotic T cells was 34.3 ± 4.3% in malnourished children and 8.6 ± 3.4% in controls. This increase in apoptotic cells was correlated with a marked up-regulation of CD95 and PD-1 expression (T-cell exhaustion marker) on $CD3^+$ T cells in malnourished children (MFI = 132 ± 5.6 and 112 ± 4.3, respectively) compared to the control infants (MFI = 89 ± 5.8 and 37 ± 3.3, respectively). The difference was statistically significant with a *p* value <0.01 (Fig. 6).

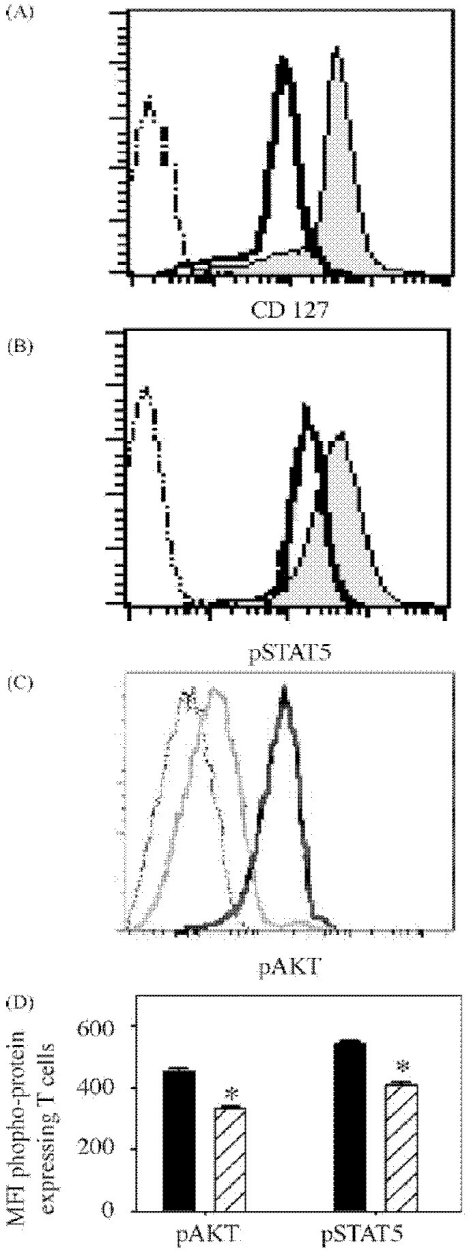

Fig. 2. Reduced surface expression of CD127 and its signaling pathway in T cells of malnourished children. (A) Surface expression of CD127 was analyzed by flow cytometry of T cells of control (gray-filled histogram) and malnourished children (open histogram, bold line) using anti-CD127 and IgG (open histogram, dotted line) isotype control antibodies. Histograms were gated based on viable $CD3^+$ T cells. T cells from malnourished children expressed low levels of CD127 compared to those of the control. (B) After stimulation with IL-7, T cells from malnourished children (open histogram, bold line) exhibited significant decreases in pSTAT5 compared with the T cells of controls (gray-filled histogram). (C) T cells from malnourished children (open histogram, gray line) exhibited significant decreases in pAKT following IL-7 stimulation compared with the T cells of controls (open histogram, bold line). Isotype control is represented by open histogram, doted line. (D) Phosphorylation of AKT and STAT5 upon binding of CD127/IL-7 in T cells from control (black bars) and malnourished (hatched bars) children is expressed as the mean ± SD of MFI; $p < 0.02$.

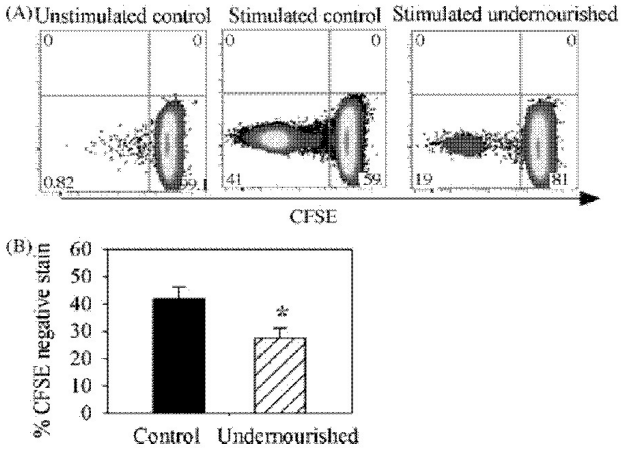

Fig. 3. Reduced mitogen-induced T-cell proliferation in malnourished children. (A) Plots are gated based on viable $CD3^+$ T cells. The numbers in the bottom left quadrant represent the percentage of CFSE-lo (proliferating cells) within the T-cell population, and the numbers in the bottom right quadrant represent the percentage of CFSE-high (non-proliferating cells) within the T-cell population. One representative experiment is shown. (B) The percentage of CFSE negative cells (% of proliferated T cells) is significantly reduced in malnourished children. Data are expressed as the mean ± SD and $p < 0.01$.

Discussion

Cytokines include a large group of glycoproteins that have the capacity to modulate the activity of individual cells during both physiological and pathological conditions (Nathan and Sporn 1991). These polypeptides are synthesized in response to microorganisms and other antigens, and they mediate and regulate immune and inflammatory reactions. Many studies have described alteration in the expression of different cytokines during malnutrition (Mengheri et al. 1992; Doherty et al. 1994; Ameho et al. 1997; Lofty et al. 1998; Malavé et al. 1998; Dülger et al. 2002; Amesty et al. 2003; Rodríguez et al. 2005; González-Martínez et al. 2008).

In experimental animals and humans, it has been demonstrated that the production of several cytokines, including IL-2 and IFN-γ, is diminished in malnourished patients (Mengheri et al. 1992; Ameho et al. 1997; Rodríguez et al. 2005; González-Martínez et al. 2008). Their results agree with ours, and this fact may be related to impairment of the immune response of malnourished patients.

TNF-α induces anorexia and cachexia in humans and animal models (Tisdale 2001); for this reason, the higher expression of TNF-α that we observed in malnourished children may be related to an aggravation of their nutritional status. The results of this study

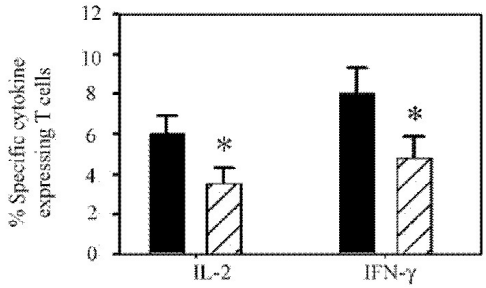

Fig. 4. T cells from malnourished children function poorly. For the analysis of cytokine production, which is a primary T-cell function, following SEB stimulation, data from 27 control (black bars) and 64 undernourished (hatched bars) children are expressed as the mean ± SD percentages of T cells that produce IL-2 and IFN-γ ($p < 0.02$).

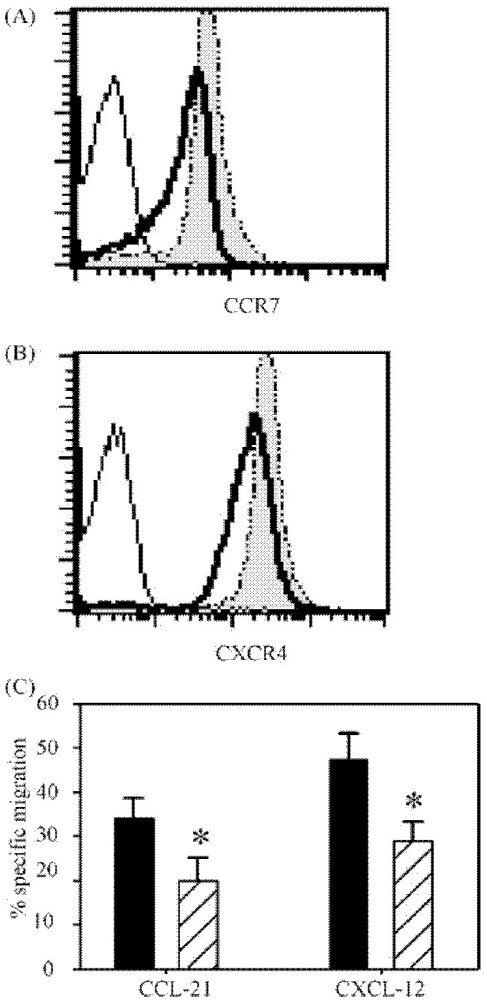

Fig. 5. Decreased surface expression of CCR7 and CXCR4 and chemotactic activities of T cells from malnourished children. (A) Surface expression of CCR7 was analyzed by flow cytometry on T cells of control (gray-filled histogram, dotted line) and malnourished children (open histogram, bold line) using anti-CCR7-PE and IgG (open histogram) isotype control antibodies. Histograms were gated based on viable $CD3^+$ T cells. One representative experiment is shown. (B) Surface expression of CXCR4 was analyzed by flow cytometry on T cells of control (gray-filled histogram, dotted line) and malnourished children (open histogram, bold line) using anti-CXCR4-FITC and IgG (open histogram) isotype control antibodies. Histograms were gated based on viable $CD3^+$ T cells. One representative experiment is shown. (C) PBMCs from 27 control (black bars) and 64 malnourished (hatched bars) children were analyzed for T-cell migratory capacity in response to 250 ng/ml CCL21 and 250 ng/ml CXCL12. Results are expressed as the mean ± SD of the percentage of specifically migrating cells obtained for each donor. $^*p < 0.02$.

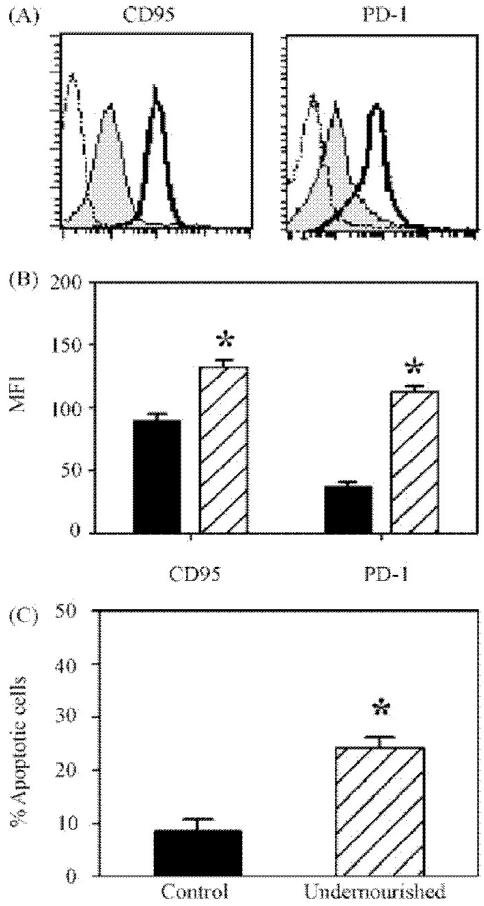

Fig. 6. T cells from malnourished children are characterized by up-regulation of the surface expression of CD95 and PD-1 and a marked increase in apoptosis. (A) Surface expression levels of CD95 and PD-1 were analyzed by flow cytometry on PBMCs of control (gray-filled histogram) and malnourished children (open histogram, bold line) using anti-CD95-PE, anti-PD-1-PE and IgG (open histogram, dotted line) isotype control antibodies. Histograms were gated based on viable $CD3^+$ T cells. One representative experiment is shown. (B) MFI for T cells that express CD95 and PD-1. Data from 27 control (black bars) and 64 malnourished (hatched bars) children are expressed as the mean ± SD, $p < 0.01$. (C) PBMCs from 27 control (black bar) and 64 malnourished (hatched bar) children were assessed for apoptosis using annexin-V/propidium iodide and flow cytometry. Data are expressed as mean ± SD of the percentage of $CD3^+$ T cells that were stained simultaneously with annexin-V and propidium iodide.

and that of González-Martínez et al. showed a significant increase in IL-10 expression in malnourished children, which may explain the deficiencies of IL-2 and IFN-γ (de Waal Malefyt et al. 1993).

Previous reports have shown inconsistent data concerning whether IL-6 production is affected in malnourished children. In agreement with our data, Malavé et al., Amesty et al. and Dülger et al. showed a higher concentration of IL-6 in the peripheral blood of malnourished children. Vethencourt et al. (1994) found that malnourished and well-nourished children have the same levels of IL-6 production. In contrast, Doherty et al. and González-Martínez et al. (2008) reported lower levels of this cytokine and its gene product in malnourished children. It is important to note that the differences in the results concerning IL-6 production could be due to the type of control group because some studies used children with infection as a control group. This suggests that the increase in IL-6 may be related to the presence of infection.

The present results showed significant decreases in the levels of circulating IL-7 in malnourished infants, and these reductions were associated with a low level of CD127 (IL-7 receptor alpha) and a significant increase in the levels of apoptotic T cells that correlated with a marked up-regulation of CD95 and PD-1 expression. The significant reduction in the level of IL-7 in malnourished T cells may enhance apoptosis in this population. IL-7 signals are vital for T-cell development, and IL-7−/− or IL-7Rα−/− mice exhibit severe T-cell deficiencies (von Freeden-Jeffry et al. 1995). The binding of IL7 to its high-affinity receptor CD127 results in the up-regulation of anti-apoptotic molecules, such as Bcl-2, and enhancement of TCR-mediated signaling. After T-cell activation by antigen or IL-7 binding, IL-7Rα is lost from the cell surface (Park et al. 2004)

and is expressed by de novo protein synthesis (Sasson et al. 2006), which is deficient in malnourished infants. This may explain the low level of CD127 (IL-7 receptor alpha) observed in malnourished children in this study. However, despite the stimulation of excess pro-inflammatory cytokines IL-1β, IL-6, IL-10 and TNF-alpha, no T lymphocyte response was found, which may be due to T-cell dysfunction or elimination by apoptosis.

The analysis of cytokine production, which is a primary T-cell function, following SEB stimulation indicated that the T cells of malnourished children exhibited a significant decrease in cytokine production, which could be related to T-cell dysfunction. This was associated with a significant decrease in the percentage of $CD3^+$ cells in malnourished children compared with the normal controls. This finding agrees with the results of previous studies (Chandra 1999; Nájera et al. 2001), which indicated that some mineral deficiencies, such as Zn deficiency, are associated with profound impairment of cell-mediated immunity, such as the lymphocyte stimulation response, decreased $CD4^+$:$CD8^+$ cells, and decreased chemotaxis of phagocytes in children. El-Hodhod et al. (2005) reported that the abnormality in peripheral blood lymphocytes during malnutrition was not related to abnormal cell counts or an imbalance in the subset ratio as previously reported but can be explained by enhanced apoptosis of these cells. This agrees with the results of the present study, which showed a highly significant increase in apoptotic T lymphocytes in malnourished children compared to controls. Consistent with that observation, Fas (CD95) and PD-1 expression (exhaustion marker) were markedly up-regulated on $CD3^+$ T cells from malnourished infants. The Fas gene plays an essential role in activation-induced cell death in mature T lymphocytes; it has been reported to induce apoptosis in activated T cells when they are repeatedly stimulated by antigen and functions to maintain T-cell tolerance by deleting autoreactive cells (Nagata and Golstein 1995). The inhibitory receptor programmed death 1 (PD-1, also known as PDCD1) is a negative regulator of activated T cells (Day et al. 2006). Upon antigen re-stimulation, effector T cells up-regulate the expression of PD-1. Therefore, sustained stimulation of naive and/or effector T cells leads to accumulation of PD-1 T cells (Singer and Abbas 1994). The functional role of Fas and PD-1 in the immune system has been examined in a variety of experimental models (Singer and Abbas 1994; Su 2007). The results of these studies support the idea that the Fas/FasL and PD-1 pathways are critically involved in the elimination of mature but not self-reactive or infected lymphocytes. However, their function in apoptosis of lymphocyte populations in malnourished children has not been explored. This work has not excluded the possibility that $CD127^+$ T cells are differentially susceptible to Fas-mediated apoptosis, which was suggested by the observation that IL-7 increases susceptibility to Fas-mediated apoptosis (Fluur et al. 2007). Cell proliferation after mitogenic stimulation is an important parameter used in the diagnosis of immunodeficiencies in clinical laboratory research and in various fields of lymphocyte research. Recent methods have attempted to replace the standard tritiated [^{3}H]thymidine assay with flow cytometry methods that utilize different fluorochromes, such as CFSE (Lašt'ovička et al. 2009). Using flow cytometry, we found that T cells from malnourished children exhibited a diminished proliferative capacity.

Our results have shown that T lymphocytes from malnourished infants had depressed levels of the inflammatory chemokine receptors CXCR4 and CCR7. This decreased expression was correlated with a significant reduction in T-cell migratory capacity to their ligands CXCL12 and CCL21, respectively, as measured with an *in vitro* chemotaxis assay. Recent studies have shown that CCR7 and CXCR4 are involved in the recruitment of blood-borne leukocytes to sites of inflammation (Blades et al. 2002). As receptors, CXCR4 and CCR7 can be considered activation markers (Lašt'ovička et al. 2009). The chemokines CCL21 and CXCL12 belong to the family of homeostatic molecules that regulate immune and nonimmune cell homing and survival (Broxmeyer et al. 2003) and the control of lymphoid organogenesis and homeostasis (Mebius 2003).

In conclusion, the abnormality of peripheral blood lymphocytes during malnutrition can be explained by enhanced apoptosis and dysfunction of these cells. This might explain the increased incidence of infections and the induction of diverse clinical problems among these children, which may continue into adulthood; in British regional heart studies, people who were born in relatively poor areas and subsequently moved to more affluent parts of Britain experienced higher incidences of ischemic heart injury (Barker 1989), which could be an indirect effect of maternal malnutrition. In future studies, it will be interesting to compare the effects of these apoptotic and dysfunctional findings with the prognosis and outcome of the children.

Competing interests

None.

Acknowledgments

The authors are grateful to Professor DJ Parker of the MRC Epidemiology Resource Centre, Southampton General Hospital, Southampton, UK, for revising the manuscript. This paper was funded by the Research Chair "Fetal Programming of Adult Disease" of King Saud University, Riyadh, Saudi Arabia.

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