Defective local leukocyte mobilization in children with kwashiorkor1, 2

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ABSTRACT The Reubuck window technique was used to determine the functional integrity of the inflammatory response in nine children with kwashiorkor. The total numbers of leukocytes mobilized into skin abrasions were similar between kwashiorkor and control patients. However, children with kwashiorkor showed significantly delayed and decreased macrophage migration and increased polymorphonuclear leukocyte migration. These defects of leukocyte mobilization were corrected with nutritional repair. Am. J. Clin. Nutr. 30: 367-370, 1977.

Children with untreated protein-calorie malnutrition (PCM) are unable to mount a normal delayed cutaneous hypersensitivity response to Candida albicans antigen (1, 2) or to develop a normal skin inflammatory response to the chemical irritant, dinitrofluorobenzene (1). In an attempt to elucidate the mechanism of these defects, we examined one aspect of the inflammatory response—the local cellular exudative reaction—in children with kwashiorkor. We observed a defect in the qualitative leukocyte response after skin abrasion and report our findings here.

Materials and methods

Patients

Nine children with kwashiorkor (3, 4) (1 to 5 years of age) were admitted to the metabolic ward of the Anemia and Malnutrition Research Center in Chiang Mai, Thailand, where they remained throughout a 70-day study period. All of the children had primary PCM and weighed between 3.0 and 12.0 kg. They were treated for fluid and electrolyte imbalance during their first 7 hospital days and received supplemental vitamins and minerals. Virtually all the patients had bacterial infections on admission and received appropriate antibacterial therapy until the infections cleared. By at least the eighth hospital day, each child was placed on a calorie-and-protein, milk-based diet that gradually reached a maximum of 175 cal (4 g of protein/kg per day). Nutritional repair was judged to be complete when the clinical stigmata of PCM had disappeared and when laboratory studies, such as serum albumin, produced normal results. Four to 8 weeks after admission, all nine patients showed clinical and biochemical signs of recovery. Each child was examined on admission for leukocyte mobilization response. Seven of these were retested after clinical recovery, and constituted the “PCM recovered” control group.

We also studied 10 clinically well-nourished Thai children with febrile illnesses, including pneumonia, empyema, meningitis, dysentery, and upper respiratory infections. The third control group consisted of 10 well-nourished Thai children (1 to 5 years of age).

Local leukocyte mobilization

Leukocyte mobilization was measured according to published methods (5)—i.e., by holding glass cover slips over 1-cm-square skin abrasions made with a scalpel. Care was taken to avoid bleeding at the window site. Cover slips were changed at 2, 4, 6, 8, 12, and 24 hr after abrasion and stained with Wright's stain. All of the easily identifiable leukocytes were differentiated. Because leukocytes did not migrate in large numbers for several hours after skin abrasion, only 40 to 100 cells were counted on most cover slips after 2 and 4 hr. After 6 or more hr, a greater number of leukocytes migrated, and as many as 500 leukocytes could easily be differentiated.

We determined each patient's cellularity by microscopically scanning each cover slip at low power. One person double-blindly viewed and estimated the density of leukocytes in each cover slip with a scoring system of 0 to 4+ (6).

We attempted to validate the total cell scoring system by comparing it to a more exact counting technique. A grid was placed on each cover slip, dividing it

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2 Supported by NIH Grant AM-11044, by the United States Army Medical Research and Development Command, and by a grant from the Rockefeller Foundation.
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into 25 equal squares. Each small square was then graded by the following system, according to the percent of its area filled with cells: 0 = no cells, 1+ = 1 to 25%, 2+ = 26 to 50%, 3+ = 50 to 75%, 4+ = 76 to 100%. The scores of the 25 grid squares were then totaled, and this total for each cover slip was compared with the score obtained without the grid. A significant correlation existed between the grid and no-grid methods of grading total cellularity ($P < 0.001$), a finding that suggests the validity of both methods. In this study we have arbitrarily chosen the no-grid method to represent total cellularity.

**Results**

Table 1 presents peripheral leukocyte differential counts of the kwashiorkor and febrile control patients for whom admission data were available. Comparison of the peripheral blood counts revealed no significant differences between the children with kwashiorkor and those with febrile illnesses.

The total number of leukocytes migrating onto the cover slips was similar among the three control groups and the malnourished children (Fig. 1). Although the mean cellularity score in the kwashiorkor children was higher than that of the control patients (particularly after 12 and 24 hr), this difference was not significant.

The qualitative cellular responses were similar among the three control groups, but the kwashiorkor patients differed in several ways. Firstly, their polymorphonuclear (PMN) response was more rapid (Fig. 2): compared to values obtained from febrile control children, a significantly higher percentage of PMN’s were counted on the cover slips of kwashiorkor patients at 2, 6, 8, 12, and 24 hr ($P < 0.02$). Secondly, the initial macrophage response in kwashiorkor patients was more sluggish, being delayed through the 6th hr after abrasion (Fig. 2). The percent macrophage migration in kwashiorkor patients was significantly less than that of febrile controls at 6 ($P < 0.001$), 8 ($P < 0.01$), and 24 ($P < 0.05$) hr.

In both kwashiorkor and control children, the number of mature, migrating lymphocytes was always less than 3% of the total leukocytes.

**TABLE 1**

Peripheral blood leukocyte counts

<table>
<thead>
<tr>
<th>Patients</th>
<th>No.</th>
<th>White blood cell/mm$^3$</th>
<th>Leukocyte differential*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>PMN</td>
</tr>
<tr>
<td>With kwashiorkor</td>
<td>7</td>
<td>13,900 ± 500</td>
<td>61 ± 19</td>
</tr>
<tr>
<td>Febrile control</td>
<td>9</td>
<td>17,200 ± 700</td>
<td>53 ± 16</td>
</tr>
</tbody>
</table>

* Average percent ± standard deviation.

**FIG. 1.** Total cellularity in Reubke skin windows in children with kwashiorkor and in healthy, well-nourished febrile, and PCM-recovered controls. Mean ± SEM measured 2 to 24 hr after the initial skin abrasion.
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FIG. 2. Percentage of macrophage and PMN leukocyte response in Re buck skin windows in children with kwashiorkor and in healthy, well-nourished febrile, and PCM-recovered controls. Mean ± SEM from 2 to 24 hr after the initial skin abrasion.

Discussion

The results of these experiments indicate that the early exudative cellular response into skin abrasion sites in kwashiorkor children is not quantitatively different from the response of healthy, well-nourished febrile, or PCM-recovered children. However, kwashiorkor children did show qualitative defects in leukocyte mobilization compared with the three control groups. These defects consisted of a significant delay and decrease of macrophage mobilization and a reciprocal increase of PMN migration. These studies, in part, confirm the observations of Freyre et al. (7), who also found decreased mononuclear migration among children with kwashiorkor. In addition, we have shown that the cutaneous inflammatory response after clinical recovery from kwashiorkor resembles in all aspects the inflammatory cycle of well-nourished children (Fig. 2).

Children with kwashiorkor, like newborn infants, have an early delayed macrophage migration into dermal abrasions (8). Both groups show decreased total numbers of macrophages, i.e., macrophages never account for more than 40% of total leukocytes 24 hr after the abrasion. In the well-nourished children, the majority (≥ 60%) of cells migrating at 24 hr are macrophages. These observations in humans gain support from animal experiments in which protein-depleted rats also had a deficient macrophage inflammatory response (9) but a normal neutrophil response (10). The first 2 to 5 hr of the inflammatory response are the crucial period that determines the success or failure of microbial invasion (11). The neutrophilic granulocyte (PMN) is the cell of major importance during this early phase of the defensive exudative reaction; its prompt arrival in adequate numbers at the site of injury constitutes the first line of host defense against bacterial invasion. In kwashiorkor PMN cells were present in normal, or even greater than normal, numbers early in the inflammatory cycle (Fig. 2). Deficiencies in the PMN phase of the local inflammatory response have been reported in diabetic acidosis (6), leukemia (12), and terminal shock, and also after alcohol (13) and steroid (14) administration. However, this report is the first to describe, to our knowledge, increases in PMN mobilization in vivo. Macrophages also participate in later phases of host defense against microbial invasion (15); therefore, one could speculate that the diminished macrophage response in our malnourished patients contributed to the establishment of their bacterial infections. The abnormalities in leukocyte migration cannot be ascribed to abnormalities in numbers or types of circulating leukocytes, both of which resembled those of well-nourished febrile children (Table 1).

The exact mechanism in kwashiorkor that mediated the differential migration of an increased number of PMN's and a decreased number of macrophages onto glass cover slips is unknown. Dale and Wolff (15) demonstrated in neutropenic patients that an adequate neutrophil accumulation at an inflammatory site was not necessary to initiate subsequent mononuclear cell migration. Complement defects reported in untreated PMC (16, 17) may participate in this effect, since the activated complement system gen-
erates leukocytic factors (18). These complement factors are leukotactic, however, for both PMN and macrophages, even though different chemotactic factors, specific for monocytes or PMN's, may exist (19). One can postulate that decreased monocyte chemotactic factor production or increased chemotactic factor inactivator (19) may exist in kwashiorkor. This possibility clearly requires additional study.

The results of this study do not support the contention that a totally diminished leukocyte exudative reaction following skin injury mediates the defect in inflammation previously noted after the skin application of a chemical irritant, such as dinitrofluorobenzene (1). Other mediators of inflammation—e.g., synthesis and release of pharmacologically active mediators—may be impaired in PCM, and may thus account for the defect in the reaction to a nonspecific irritant.

Macrophage exudation represents the major cellular-type response in the later phases of the delayed cutaneous hypersensitivity reaction. An impaired immunological recall response to skin test antigens has been reported in PCM (1, 2, 16, 20); hence, a defect in the ability to mobilize macrophages may play a role in this defective reaction.

The authors thank Mr. Tawat Tositarat, Mr. Pramote Teowsiri, Mrs. Somari Ruckphaophunt, and Miss Chanmet Suwanarach for their outstanding contribution toward the successful completion of this study. We also thank Susanna Fein for editorial assistance.

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