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Immunological Aspects of Infantile Protein-Calorie Malnutrition

K. SCHOPFER, S. D. DOUGLAS

Introduction

Increased susceptibility to infectious diseases is one of the major causes of the high morbidity and mortality of children with protein-calorie malnutrition (PCM). Many reports from different part of the world, where infantile malnutrition is prevalent, have demonstrated impaired immune reactions in these children.

Antibody production has been shown to be adversely affected by protein deficiency, and there is an extensive involution of the thymus dependent lymphatic system leading to impaired cell-mediated immunity. Furthermore, defective phagocyte function has been demonstrated in malnourished children. These are all factors favoring the risk of infections, and which may be partly responsible for the severe course of infections in these children.

During the past two years we have investigated in the Ivory Coast immune functions, mainly in vitro lymphocyte and phagocyte function, in children with kwashiorkor. – We will first summarize some basic findings of the interactions between the immune response and malnutrition and then present briefly some of our own findings concerning peripheral blood phagocyte function.

Humoral Immunity

Immunoglobulin levels are generally high in African children and malnutrition doesn't seem to affect immunoglobulin concentration in borderline or moderate cases. However, we have found that in dividing kwashiorkor children into two groups according to their serum albumin levels, the kwashiorkor patients with albumin levels below 2 g/100 ml serum show significantly decreased total gamma-globulin concentrations when compared to age-matched African control children. This observation could indicate that gamma-globulin levels are reduced only by very severe protein deprivation.

Reports dealing with antibody production after antigenic stimulation in PCM give conflicting results. It has been demonstrated that kwashiorkor children can mount an adequate specific antibody response after polio vaccination [1], but show a weak response after yellow fever vaccination [1, 2]. Restoration

of the antibody response after recovery from kwashiorkor could be correlated with the amount of protein introduced in the diet [3]. In a recent study in New Guinean schoolchildren protein supplementation has been shown to improve specific antibody production also in borderline malnutrition after antigenic stimulation with flagellin [4].

Serum complement levels were reduced (with the exception of C_4) in children with PCM in an investigation carried out in Thailand [5].

Cellular immunity and in vitro lymphocyte function

Characteristic of a failure of cell-mediated immunity is the low or absent tuberculin response after BCG vaccination. Kwashiorkor children always show a negative response also in cases with tuberculosis. Furthermore, the degree of the tuberculin sensitivity has been found to be less in children with growth failure than in healthy controls [6]. We have recently conducted a field study in the northern part of the Ivory Coast to test if protein supplementation in apparently healthy preschool children influences the tuberculin sensitivity after BCG vaccination. We were able to demonstrate that children receiving a diet rich in protein showed an increased tuberculin response compared to age-matched, non-supplemented control children.

These functional in vivo findings can be probably correlated with the extensive involution of the thymus dependent lymphatic system in severe PCM [7]. An outstanding feature of the histological examination of thymus preparations from kwashiorkor children is sparsity or absence of Hassall's corpuscles [8]. In vitro lymphocyte function is also impaired. Isolated lymphocytes of children with kwashiorkor show a highly decreased response to phytohaemagglutinin [7, 9] as well as a reduced number of rosette-forming thymus-dependent lymphocytes [10].

In vitro granulocyte function

Peripheral blood granulocyte (PMN) function is defective in severe protein deprivation, as e.g. in children with kwashiorkor [11, 12, 13]. Granulocytes belong to the first line of defense in host resistance against infections and their dysfunction is associated with bacterial infections. Their function can be divided into three different stages:

1. *chemotaxis*; this is the active, direct migration of cells towards a chemotactic stimulus and is a primary event in the early inflammatory response;
2. the *engulfment phase* which consists in the attachment of the particle to the cell membrane and subsequent ingestion;
3. the *postphagocytic events* include fusion of the lysosomes with the phagocytic vacuole and concomitant activation of several metabolic pathways. These processes are related to microbial killing.

According to this outline we proceeded to test peripheral blood phagocyte function in children with PCM.

Material and Methods

Only children with a kwashiorkor syndrome were examined. Blood was sampled upon admission of the children to the Pediatric Ward of the University Hospitals of Treichville, Abidjan, Ivory Coast. PMNs were isolated within two hours after the blood sampling. Control experiments were always done in parallel.

Chemotaxis was tested in diffusion chambers separated by a Millipore filter in two compartments. A chemotactic stimulus was filled into the lower compartment, the cells into the upper compartment. PMNs migrating completely through the filter towards the attractant fluid were counted after different incubation intervals.

The engulfment phase was assessed by incubating isolated PMNs or blood monocytes with either latex, antibody coated erythrocytes or *C. albicans*. Cells ingesting particles and particles ingested per cell were evaluated.

In vitro microbicidal assay were performed using *S. aureus*, *E. coli* or *C. albicans*. PMNs were incubated with the microorganisms, and after different incubation intervals the PMNs were lysed to release phagocytised bacteria or yeast, and viability was assessed by either colony count method [11] or by dye exclusion [14].

The activity of the intracellular, microbicidal myeloperoxidase-peroxide-iodide system [15] was examined by incubating phagocytising PMNs in the presence of labelled iodide [16]. The extent of iodide incorporation by the PMNs into the bacterial proteins can be evaluated by measuring the radioactivity of the precipitated proteins after different incubation intervals.

Results

All the children showed a classical kwashiorkor syndrome including mucocutaneous lesions, edema, hepatomegaly and weight loss despite extensive edema. Hypoproteinemia and hypoalbuminemia were always present. The chemotactic response of the cells from the kwashiorkor children was impaired in the early incubation intervals, namely after 30, 60 and 120 minutes. After 3 hours, however, the values were equal to controls (fig. 1).

Phagocytosis, that is the engulfment phase, was not impaired in phagocytes of malnourished children. The phagocytic indices didn't differ between control and test cells.

The in vitro microbicidal assay revealed a marked defect of the killing function of isolated granulocytes. There were significantly more viable *S. aureus* or *E. coli* in the cells of the kwashiorkor children than in those of the controls

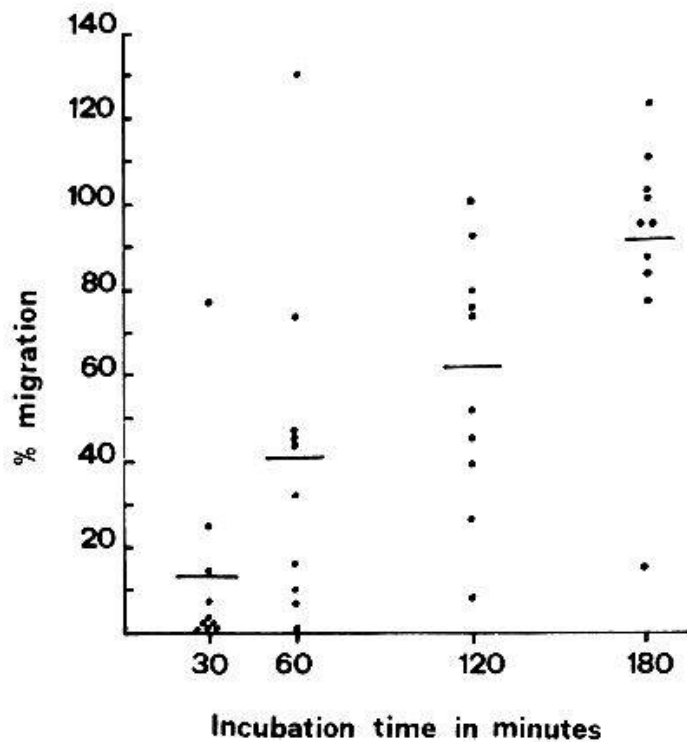


Fig. 1. The percentage migration of the PMNs of the sick children are related to the mean value of the controls, which is taken as 100%, at each incubation interval. Each point represents an individual kwashiorkor case; — indicates the mean value of the kwashiorkor patients.

		minutes		
Assay	Bacteria	30	60	120
E. coli				
1		1.39	4.24	5.83
2 ^x		1.56	0.48	0.26
3		0.93	9.69	5.91
4		3.97	2.56	2.04
5		0.90	1.38	1.91
S. aureus				
6		0.72	3.76	4.00
^x patient transfused				

Fig. 2. Studies of bactericidal capacity of neutrophils from kwashiorkor patients as compared with controls. The ratios of viable intracellular bacteria in phagocytes from kwashiorkor and control children are shown after various incubation periods. A greater number of *E. coli* or *S. aureus* are viable in the cells from kwashiorkor children than from the control (ratio greater than 1 after 60 and 120 minutes). (From Clin. Exp. Immunol. 17, 121 [1974].)

after 60 minutes [11] (fig. 2). The fungicidal capacity was also defective: the controls killed $32 \pm 11\%$ (mean \pm SD, n 8) of the ingested *C. albicans* after 60 minutes whereas the PMNs of the kwashiorkor children killed only $18 \pm 7\%$ (mean \pm SD, n9: $p < 0,01$).

The myeloperoxidase mediated killing system showed a decreased in vitro activity. Less radioactivity was measured in the precipitated proteins of the incubation mixture containing the PMNs from the sick children at 15, 30 and 60 minutes than in control experiments; hence, there was a reduced extent of iodide incorporation into bacterial proteins during the postphagocytic events by the test cells. The difference is significant ($p < 0,01$) for each incubation period.

Discussion

Our findings indicate impaired in vitro granulocyte function in children with kwashiorkor. The early chemotactic response is delayed, there is a bactericidal and fungicidal defect and the quantitative leucocyte iodination assay showed in vitro an impairment of the myeloperoxidase-peroxide-iodide mediated killing system. This is thus far the only biochemical parameter which is directly related to the observed in vitro killing defect in phagocytes of children with severe kwashiorkor. The same method has been found to be closely related to killing defects in other granulocyte dysfunction syndromes as e.g. in chronic granulomatous disease [17] or in leucocyte glucose-6-phosphate dehydrogenase deficiency [18].

The possible clinical significance of our findings remains to be elucidated. Nevertheless, there are some indications that these in vitro observations can be related to clinical conditions. Superficial infections occur frequently in kwashiorkor children and often undergo necrosis. In contrast purulent infections are rarely seen in these patients in spite of frequent isolation of pyogenic bacteria. Hence, the result of impaired chemotactic response could be complementary to the clinical observation. Also bacterial and fungal infections are frequently encountered in children with kwashiorkor and our finding of defective in vitro granulocyte function could support this. However, more studies are needed to assess the relations of the in vitro phagocyte dysfunction to the in vivo conditions. – *Candida* infections occur mainly in patients with impaired cell-mediated immunity, as e.g. in primary immunodeficiencies or in patients undergoing immunosuppressive therapy. Cell mediated immunity is known to be depressed in kwashiorkor children [7, 9, 10], the decreased in vitro candidacidal activity may further contribute to the increased susceptibility of these children towards *candida* infections. Decreased in vitro candidacidal activity of PMNs and disseminated candidiasis have been reported in a case with hereditary myeloperoxidase deficiency [19]; however, myeloperoxidase activity has been found to be normal in the granulocytes of the kwashiorkor children examined.

Summary

Children with protein calorie malnutrition have impaired immune functions. Immunoglobulin levels are low in children with severe protein deprivation, but are not affected in moderate or borderline protein malnutrition. Specific antibody response is either normal or reduced depending on the antigen used. Involution of the thymus dependent lymphatic system and severe impairment of the cell-mediated immune reactions are prominent features in children with kwashiorkor. Furthermore, *in vitro* granulocyte dysfunction in those children has been demonstrated as far as chemotaxis, microbiocidal activity and quantitative leucocyte iodination is concerned. There is a delayed chemotactic response in the early incubation intervals; there is decreased killing of *S. aureus*, *E. coli* and *C. albicans* after 60 minutes of incubation, and there is less iodination by phagocytising granulocytes of kwashiorkor children than in control experiments, indicating an impairment of the myeloperoxidase-peroxide-iodide mediated killing system. The possible clinical implications of these *in vitro* findings are briefly discussed.

Zusammenfassung

Die an einer kalorischen und proteischen Mangelernährung leidenden Kinder weisen eine Störung der immunologischen Funktionen auf. Bei den schweren Formen findet man niedrige Immunoglobulinwerte im Blut; bei den leichten oder minimen Formen sind diese hingegen unverändert. Die Bildung spezifischer Antikörper kann normal oder vermindert sein, je nach gebrauchtem Antigen. Die Zurückbildung des thymusabhängigen lymphatischen Systems und die schwere Störung der zellgebundenen immunologischen Reaktionen sind typische Merkmale bei Kindern, die an Kwashiorkor leiden. In solchen Fällen wurden weiterhin granulozytäre Funktionsstörungen «*in vitro*» gezeigt, insofern Veränderungen der Chemotaxis, der bakteriziden Wirkung und der Iodination vorhanden sind. Bei Kindern, die an Kwashiorkor leiden, stellt man, in Gegensatz zu Kontrollexperimenten, eine verspätete chemotaktische Antwort während der frühen Inkubationszeiten fest, in denen eine verminderte Zerstörung von *S. aureus*, *E. coli* und *C. albicans* nach einer Inkubationszeit von sechzig Minuten besteht; es zeigt sich ebenfalls eine schwächere Iodination durch phagozytierende Granulozyten. Dies weist auf eine Störung der auf der Myeloperoxydase und der Iodperoxydase beruhenden bakteriziden Verteidigungssysteme. Es wird noch die mögliche klinische Bedeutung solcher «*in vitro*» erhaltenen Resultate kurz diskutiert.

Résumé

Les enfants souffrant de malnutrition protéino-calorique présentent une altération des fonctions immunitaires. Dans les cas de malnutrition grave, les taux d'immunoglobulines sont bas alors qu'ils sont normaux dans les cas de malnutrition modérée ou marginale. La réponse des anticorps spécifiques est soit normale, soit diminuée selon l'antigène en cause. L'involution du système lymphatique et une altération grave de l'immunité cellulaire sont caractéristiques des enfants souffrant de kwashiorkor. Chez ces mêmes enfants, on a démontré «in vitro» une altération des granulocytes, en particulier de la chimiotaxie, de la bactéricidie et de l'iodination quantitative des leucocytes. La réponse chimiotactique est retardée au stade précoce de l'incubation; la destruction des *S. aureus*, *E. coli* et *C. albicans* est diminuée après 60 minutes d'incubation. De plus, l'iodination des phagocytes actifs est plus basse chez des enfants souffrant de kwashiorkor que dans un groupe témoin, indiquant un déficit du système myéloperoxydase-iodure peroxide participant à la bactéricidie. Les implications cliniques possibles de ces observations «in vitro» sont discutées brièvement.

Riassunto

I bambini con carenze nutritive caloriche e proteiche hanno delle funzioni immunologiche compromesse. In quelli con carenze severe, i valori sanguigni delle immunoglobuline sono bassi, mentre essi non sono alterati se tale carenza è moderata o minima. La formazione di anticorpi specifici è normale o ridotta, a seconda degli antigeni usati. L'involuzione del sistema linfatico dipendente dal timo ed il severo deterioramento delle reazioni immunologiche a livello cellulare sono le caratteristiche principali riscontrate nei bambini affetti da kwashiorkor. In tali casi inoltre sono state dimostrate «in vitro» delle disfunzioni granulocitarie in presenza di alterazioni della chemotassi, dell'attività battericidica e della iodinazione leucocitaria. Si constata una risposta ritardata della chemotassi durante gli intervalli precoci d'incubazione con una distruzione diminuita di *S. aureus*, *E. coli* e *C. albicans*, dopo 60 minuti di incubazione; esiste inoltre una iodinazione più bassa da parte di granulociti fagocitanti nei bambini affetti da kwashiorkor che negli esperimenti di controllo; ciò indica una compromissione del sistema difensivo battericidico basato sull'attività della mieloperossidasi e della perossidasi iodica. Si discutono brevemente le possibili implicazioni cliniche di tali risultati ottenuti «in vitro».

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