Defective *Candida* killing in childhood malnutrition

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SUMMARY This study shows that malnourished children have impaired candidacidal activity of leucocytes, a finding which runs parallel to the higher rates of isolation of *Candida* sp. from throat secretions. In well nourished subjects the mean candidacidal ability was 44.5%, in the moderately undernourished group it was 17.6%, and in the severely malnourished group it was 13.7%. This impaired candidacidal capacity may be important in the establishment of large numbers of *Candida* sp. which are commonly found in the upper intestine and in the pathogenesis of diarrhoea in children with malnutrition.

Candida species are common in the upper intestinal contents of malnourished children (Mata *et al.*, 1972; Gracey *et al.*, 1974) and may contribute to the production of diarrhoea by their effects on intestinal absorption of sugar (Burke *et al.*, 1977) and water (Thelen *et al.*, 1978). The mechanism by which such large numbers of *Candida* become established in malnourished children is not clear. Previous studies have shown the ability of such children to secrete intestinal immunoglobulins to be unimpaired (Bell *et al.*, 1976), while depression of cell-mediated immunity *in vivo* and *in vitro* has been reported in malnutrition (Geefhuysen *et al.*, 1971; Chandra, 1972; Ferguson *et al.*, 1974). However Kulapongs *et al.* (1974) found lymphocyte responsiveness to phytohaemagglutinin and killing ability of polymorphs to be normal in a group of malnourished children with iron deficiency, while Macdougall *et al.* (1975) found cell-mediated immunity was impaired in such children. Bactericidal function of polymorphs was assessed using *Escherichia coli* as the test organism in the study of Kulapongs *et al.* (1974), but Macdougall *et al.* (1975) and other investigators, showing impaired killing ability of polymorphs (Seth and Chandra, 1972), used staphy-

lococci. It may be that bactericidal function is reduced only in relation to specific organisms.

Lehrer and Cline (1969) showed that leucocytic function was important in resistance to *Candida* infection. Using their technique, it was shown that the *Candida*-killing ability of polymorphs is reduced in newborn babies, a group known to be susceptible to mucosal candidal infections (Xanthou *et al.*, 1975). Because of our interest in gastrointestinal candidiasis in malnourished children we investigated the *Candida*-killing ability of polymorphs in such children.

Materials and methods

Patients. Blood and throat swabs were obtained from 67 children and adults. Patients were classified according to the recommendations of the Wellcome Working Party (Lancet, 1970) into a normal group with body weights >80% standard weight for age (SWFA), an underweight group 60-80% SWFA, and a marasmic group <60% SWFA. Samples of 4 ml heparinised blood were obtained by venepuncture from normal adults, well nourished children without known immunological deficiencies attending Princess Margaret Hospital in Perth, Indonesian children admitted to hospital in Jakarta, and from Australian aboriginal children in hospital in Derby, an isolated country town on the coast in the far north of Western Australia.

***Candida*-killing ability of leucocytes.** The method of Lehrer and Cline (1969) was used with the modifications of Xanthou *et al.* (1975) to minimise the volume of blood taken from the children. Killing

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ability was measured by counting the number of *Candida* stained by methylene blue which distinguishes killed organisms, after incubating comparable numbers of neutrophils and viable *Candida* in serum with Hanks's solution for 30 minutes at 37°C. All tests were done using patients' sera as well as with normal adult sera. At least 300 cells were counted for each sample and results are expressed as the number killed—that is, blue-stained *Candida*/100 phagocytosed organisms. Differences in percentage of *Candida* killed in groups classified according to nutritional status were assessed using the Kolmogorov-Smirnov test.

Throat swabs. Specimens were collected into Stuart's transport medium and refrigerated at 4°C if not processed immediately. Those from Derby and Jakarta were flown to Perth on ice and processed within 24 hours of collection. Throat swabs were cultured in Sabouraud's dextrose agar and incubated for 48 hours at 25°C.

Results

Candida-killing ability was not related to age, race, or diagnosis on admission to hospital. Consequently, results were considered only in relation to the patient's nutritional state.

In the well nourished group the mean candidacidal ability was 44.5% (SEM 8.8), for those with weights 60–80% SWFA the mean was 17.6% (SEM 4.4), and for children with weights <60% of SWFA the mean was 13.7% (SEM 3.4). Results are shown in the Figure. Comparison of each malnourished group with the well nourished subjects showed the difference to be highly significant ($P < 0.001$). However, there was no significant difference in candidacidal ability between the two groups of malnourished children. Addition of normal sera in place of the patients' own sera did not alter the *Candida*-killing ability of their leucocytes.

We previously showed (Gracey *et al.*, 1973) that oropharyngeal flora probably reflect environmental contamination, so that it is invalid to compare isolation rates of organisms from such different areas as Perth, Derby, and Jakarta. As there were no malnourished children from Perth, results of throat swabs taken in Perth were not included in the analysis. Children from Derby and Jakarta were grouped according to nutritional status and separate comparisons made for each area. In Jakarta, 92% of malnourished children had *Candida* in their throat swabs compared with 66% of normal children; in Derby *Candida* was grown from throat swabs of 80%

Figure *Candida*-killing capacity (expressed as percentage of *Candida* killed) according to nutritional status expressed as a percentage of standard weight for age compared with the 50th centile of the Harvard standards (Lancet, 1970).

of malnourished children but from only 14% of normal children.

Discussion

We show that malnourished children have reduced candidacidal activity of their leucocytes. This finding runs parallel to the higher isolation rate of *Candida* sp. from throat swabs of malnourished children. The defective *Candida*-killing ability of these leucocytes was not corrected by adding normal serum. Lehrer and Cline (1969) found that serum factors did not affect the candidacidal property of leucocytes and the addition of normal serum did not correct defective *Candida*-killing by leucocytes in the newborn (Xanthou *et al.*, 1975). It would seem that malnourished children also have impaired leucocyte function in relation to *Candida* killing. We found that the *Candida*-killing ability of leucocytes was reduced in patients with a high isolation rate of *Candida* sp. from their throat swabs. Organisms isolated from the upper small bowel are similar to the flora of the oral cavity (Dickman *et al.*, 1976) and our previous investigations suggest that *Candida* sp.

are likely to be found in the upper intestinal contents of these children (Gracey *et al.*, 1974). The relationship between gastrointestinal infection with *Candida* sp. and impaired *Candida*-killing ability of leucocytes is not clear. Although several investigators have established the importance of intestinal antibodies in controlling bacterial contamination of the upper intestinal contents (Walker and Isselbacher, 1977), their role in relation to intestinal candidiasis is not known. However, malnutrition does not impair secretion of immunoglobulins into the intestinal lumen as intestinal immunoglobulin levels have been shown to be raised in malnourished children with infections (Bell *et al.*, 1976). The importance of leucocytic function in maintaining the relative sterility of upper intestinal contents has not been widely investigated. However Takeuchi *et al.* (1965) and Kenworthy (1970) showed that phagocytosis was important during the establishment of experimental infections with *Shigella* and *E. coli*. It may be that neutrophils are also important in controlling candidal infections in the small intestine.

Our findings show that polymorphs from malnourished children have an impaired ability to kill *Candida* and extend the observation that the ability to kill *Staphylococcus pyogenes* is reduced in malnutrition (Seth and Chandra, 1972). This mechanism may contribute to the significantly increased incidence of candidal infections in malnourished children.

We thank everyone in Australia and Indonesia who took part in this study, and the doctors and nurses who helped to collect and transport the specimens.

R.T. was seconded from the Princess Margaret Hospital for Children, Perth, Western Australia.

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Received 12 September 1978