

Impaired Cellular Immunity in Kwashiorkor with Improvement after Therapy

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Summary

Children with kwashiorkor showed a high incidence of deranged cellular immunity as evidenced by impairment of delayed cutaneous hypersensitivity reactions to candida and diphtheria toxoid antigens and of lymphocyte transformation after phytohaemagglutinin stimulation. This may contribute to their susceptibility to infection. A correlation was shown between the degree of impairment of tests of cellular immunity and the severity of the kwashiorkor. Once recovery was initiated the skin tests gave the expected positive results and the lymphocyte transformation index improved. Protein deprivation may result in impaired deoxyribonucleic acid (DNA) synthesis and in atrophy of both the thymus and the lymphoid tissue.

Introduction

Infection is prevalent in malnourished infants, and both bacterial and viral infections often follow a fulminating course in patients suffering from kwashiorkor (Smythe, 1958; Wittman *et al.*, 1967; Scrimshaw *et al.*, 1968). Published studies of immunoglobulin levels in infants with protein-calorie malnutrition do not suggest a defect in humoral immunity in this disease (Keet and Thom, 1969; Aref, 1970; Watson and Freese, 1970; El-Gholny *et al.*, 1970; McFarland *et al.*, 1970). Jackson (1925) and Vint (1936-7) reported that malnourished children show atrophy of the thymus and lymphoid tissue, so we tested cell-mediated immunity in malnutrition in a prospective study in children with kwashiorkor by the cutaneous delayed hypersensitivity reaction to antigens, and by the in-vitro transformation of lymphocytes by phytohaemagglutinin.

Patients

Eighteen African children aged 6 to 30 months admitted to Baragwanath Hospital, Johannesburg, were investigated. All had kwashiorkor as defined by Jelliffe (1966) and their weight was below the third Boston percentile for age. The severity of kwashiorkor on admission was assessed by the presence or absence of the following criteria (Kahn, 1959; Garrow and Pike, 1967): gross oedema, weeping dermatosis, severe wasting in the presence of oedema and dermatosis, hepatomegaly of 4 cm or more below the costal margin, jaundice, prothrombin index of 50% or less, disturbance of consciousness with or without hypoglycaemia, dehydration and/or electrolyte disturbance, the presence of infection, hypothermia to 95°F (35°C) or

less, and xerophthalmia. When four or more of these features were present the cases were regarded as "severe"; when at least one but fewer than four were present the cases were graded as "moderate," and "mild" cases had none of these poor prognostic criteria. Overt clinical evidence of infection was present in 13 of the 18 patients.

All patients were given a standard diet of ± 200 ml/kg/day of 66.6% Pelargon with added sucrose. This provided nearly 130 cal/kg/day and 4.5 g protein/kg/day. Feeding by nasogastric tube was often required initially. Potassium supplements and vitamins A, D, and C were given routinely, and intravenous fluids and antibiotics were administered when indicated. The surviving children were retested three weeks later when they showed considerable clinical improvement and weight gain.

Control Subjects.—The control group comprised nine well-nourished African children aged 6 to 18 months at the 50th Boston percentile for weight. All nine children had clinical evidence of infection—namely, gastroenteritis, otitis media, and pneumonia—similar to those diagnosed in the children with kwashiorkor.

Methods

Cutaneous Delayed Hypersensitivity Reactions.—Skin tests with diphtheria toxoid and candida antigen were carried out on admission to hospital on all patients and controls and, if negative, were repeated after three weeks. *Diphtheria toxoid:* 0.1 ml of a 1/10 dilution was injected intradermally with a tuberculin syringe into the flexor surface of the forearm. The reaction was read after 48 hours. *Candida antigen**: A somatic antigen was provided in a cream base. The material was applied to the flexor surface of the forearm and introduced into the skin with a Heaf gun set at 2 mm. The reactions were read at 48 and 72 hours.

In-vitro Lymphocyte Transformation.—Human peripheral blood leucocytes were cultured as described previously (Liknaitzky *et al.*, 1970). Ten millilitres of blood was collected aseptically in heparin (20 units per ml of blood), allowed to stand at room temperature for 45 minutes, and the supernatant leucocyte-rich plasma aspirated. The leucocyte suspension, which contained >70% lymphocytes, was then added to M150 medium and 10% bovine albumin to give a concentration of 1×10^6 lymphocytes per ml. The cultures were incubated at 37°C for three days after the addition of 0.1 ml of phytohaemagglutinin. After three days the cells were harvested and fixed in 75% alcohol and 25% glacial acetic acid. Two slides were prepared from each culture, stained with Wright's stain, and the cells counted. A total of 500-1,000 cells were counted and recorded as "transformed" or "small" lymphocytes. Unstimulated lymphocyte cultures were set up from each patient as controls. The number of transformed (stimulated) lymphocytes was expressed as a percentage of the total number of lymphocytes as follows:

$$\text{lymphocyte transformation index} = \frac{\text{transformed cells}}{\text{total no. cells counted}} \times 100$$

Serum immunoglobulins were estimated in 12 patients by the Hyland monospecific immunoplate method.†

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TABLE II—Tests of Cellular Immunity and Immunoglobulin Levels in 18 Children with Kwashiorkor on Admission to Hospital, and after 3 Weeks' Treatment

Case No.	Age in Months	Clinical Severity	Clinical Infections*	Skin Reactions				Lymphocyte Transformation Index (%)		Immunoglobulin Levels (mg/100 ml)		
				Candida		Diphtheria		Initial	Repeat	IgG	IgA	IgM
				Initial	Repeat	Initial	Repeat					
1	22	Mild	No	-	+	-	+	30	-	1,200	350	36
2	12	Mild	No	-	N.T.	-	+	64	-	750	190	58
3	15	Moderate	Yes	+	N.T.	-	-	60	-	-	-	-
4	12	Moderate	Yes	+	N.T.	±	+	78	-	1,350	240	74
5	6	Moderate	Yes	-	+	-	+	68	-	1,250	145	68
6	12	Moderate	No	-	+	-	-	45	-	-	-	-
7	17	Moderate	No	-	+	-	+	7	30	1,600	130	135
8	19	Moderate	Yes	-	+	-	+	N.T.	-	800	110	80
9	12	Severe	No	-	+	-	+	52	-	750	97	86
10	30	Severe	Yes	-	Died	±	Died	33	-	820	180	90
11	15	Severe	Yes	-	Died	-	Died	51	-	100	260	100
12	12	Severe	Yes	-	N.T.	±	N.T.	45	-	940	270	90
13	14	Severe	Yes	-	+	+	N.T.	40	-	-	-	-
14	15	Severe	Yes	-	+	-	N.T.	33	-	820	230	62
15	18	Severe	Yes	-	+	-	+	14	30	940	370	48
16	12	Severe	Yes	-	+	-	+	5	N.T.	-	-	-
17	12	Severe	Yes	-	Died	-	Died	52	-	-	-	-
18	7	Severe	Yes	-	Died	-	Died	N.T.	-	-	-	-

*Overt clinical infections include otitis media, gastroenteritis, herpes simplex, pneumonia, and skin sepsis. N.T. = Not tested.

Results

Positive candida and diphtheria toxoid skin tests were significantly less frequent in children with kwashiorkor than in control children ($\chi^2 = 10.14$; $P < 0.005$ for candida, and $\chi^2 = 8.70$; $P < 0.005$ for diphtheria toxoid) (see Tables I and II). After three weeks of hospital treatment the skin reaction to candida became positive in all 10 patients with kwashiorkor retested (Table II), a statistically significant increase ($\chi^2 = 14.75$; $P < 0.005$), and positive diphtheria skin tests were obtained in 10 of the 12 patients retested ($\chi^2 = 15.56$; $P < 0.005$).

TABLE I—Tests of Cellular Immunity in 9 Well-nourished Control Infants with Intercurrent Infections

Subject No.	Age in Months	Clinical Infections	Skin Reactions		L.T.I.
			Candida	Diphtheria	
1	8	Yes	-	+	N.T.
2	10	Yes	+	+	65%
3	7	Yes	+	+	52%
4	8	Yes	+	+	N.T.
5	18	Yes	+	-	58%
6	9	Yes	+	+	60%
7	9	Yes	+	+	55%
8	6	Yes	+	-	N.T.
9	9	Yes	+	-	58%

L.T.I.—Lymphocyte transformation index. N.T. = Not tested.

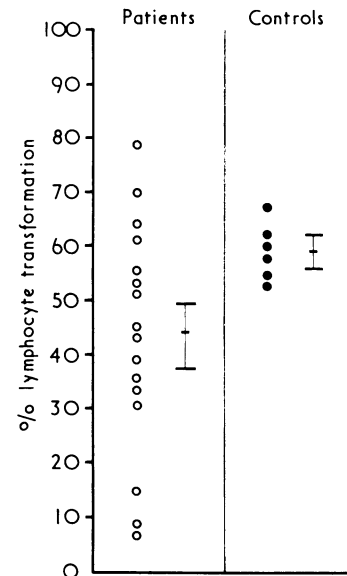
The lymphocyte transformation index was determined in six of the nine control children and in 16 children suffering from kwashiorkor, and the results are shown in the Chart. The mean lymphocyte transformation index was $58.0 \pm 1.63\%$ in the control children and $42.5 \pm 5.33\%$ in the kwashiorkor patients. The difference between the two means is statistically significant ($t = 3.33$; $P < 0.005$). Two patients with low initial values were retested after three weeks, when their nutritional state had improved, and in both of them the index had risen to 30%.

The four patients with lymphocyte transformation index values greater than 60% showed the least disturbance of the candida skin test reaction; three of these had a positive candida response initially. In the six children with an index of 40-59% the skin test responses were variable, and the six patients with an index of less than 40% showed the maximum suppression of cutaneous delayed hypersensitivity.

It was possible to correlate the clinical severity of kwashiorkor with the results of the tests of cellular immunity. The three children with positive candida reactions and lymphocyte transformation index of 60% or greater showed mild or moderate kwashiorkor. In all children with severe disease the candida reaction was negative and the lymphocyte transformation index less than 55%. Two infants (Cases 1 and 7, Table II), however, showed poor correlation between the clinical findings and both

the skin tests and the lymphocyte transformation index. The skin tests initially were negative and the lymphocyte transformation index was $< 40\%$, yet clinical criteria suggested only mild or moderate clinical kwashiorkor. Of the 12 patients who showed definite diminished cutaneous delayed hypersensitivity initially, four died.

The serum immunoglobulin levels in the 12 patients tested are shown in Table II. IgG levels ranged from 100 to 1,600



Lymphocyte transformation index after PHA stimulation in 16 patients with kwashiorkor and six well-nourished control infants of comparable age, with intercurrent infection.

mg/100 ml, IgA from 97 to 370 mg/100 ml, and IgM from 36 to 135 mg/100 ml. There was no correlation with clinical infection or the degree of severity of the kwashiorkor. Of the two patients who died, one had a low IgG, with raised IgA and a normal IgM, whereas the second had normal immunoglobulin levels for his age.

Discussion

Harland (1965), Lloyd (1968), and Hutt (1969) showed that the delayed response to the tuberculin skin test is diminished in malnourished children. We have obtained similar results using diphtheria toxoid and candida. These antigens are appropriate because of the high probability of previous contact by immunization and candida infections (Fireman *et al.*, 1969).

This choice was validated by the finding that the candida skin tests were positive in eight of the nine controls and converted to positive in most of the surviving children with kwashiorkor after refeeding. This recovery of function with feeding suggests that the defect is directly related to the nutritional status. Our finding of impaired lymphocyte transformation to phytohaemagglutinin, not to our knowledge previously reported in malnutrition, is consistent with these findings.

The correlation between lymphocyte transformation, delayed cutaneous hypersensitivity, and clinical grading of severity of kwashiorkor, and the improvement after refeeding, support the premise that lymphoid cell function is partly dependent on protein availability.

This defect of delayed hypersensitivity in infants with kwashiorkor may contribute to their high incidence and great severity of infection, though other factors such as non-specific immunity, exposure to infections, and socioeconomic conditions may be important. The finding of a normal lymphocyte transformation index and positive reactions to the skin antigens in a group of well-nourished but infected children indicates that in kwashiorkor the impaired lymphocyte function is related to protein deprivation rather than to the presence of infection.

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PRELIMINARY COMMUNICATIONS

Lymphocyte Response Depressive Factor in Multiple Sclerosis

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Summary

Normal serum contains a lymphocyte response depressive factor and this is more active against the lymphocytes of the blood from which the serum was obtained than against lymphocytes from a different normal blood. The suppressive factor is thus "tailor-made" to its own lymphocytes though cross-reactivity with other lymphocytes does occur. The significance of this is discussed. The suppressive factor has a higher titre in serum from patients with multiple sclerosis or other destructive neurological disease than in normal serum. This may be an instance of a general phenomenon in which lymphocyte sensitization is ordinarily accompanied by production of a suppressor factor able to damp down response so that this is controlled by an "accelerator-brake" mechanism. The possibilities of imbalance in the pathogenesis of disease and therapeutic manipulation of the level of suppressor substance are briefly discussed.

Introduction

Whatever the aetiology of multiple sclerosis (M.S.) may ultimately turn out to be—infective or autoimmune—it is difficult to escape the conclusion that immunological processes (and delayed hypersensitivity in particular) play a part in the genesis of recurrent episodes of the disease. Since delayed hypersensitivity depends on lymphocyte sensitization and the autoaggression of these cells is believed to produce the disease, any means of damping-down the reactivity of such lymphocytes is of potential therapeutic importance. In the case of M.S. the putative antigen is a basic protein extractable from human brain (Caspary and Field, 1965, 1971a) and capable of producing experimental allergic encephalomyelitis in animals.

In multiple sclerosis (Hughes, Caspary, and Field, 1968; Knowles, Hughes, Caspary, and Field, 1968; van den Noort and Stjernholm, 1971) as well as in a whole variety of other conditions such as tuberculosis (Heilman and McFarland, 1966), hepatitis (Paronetto and Popper, 1970), secondary syphilis (Levene, Turk, Wright, and Grimble, 1969), ataxia telangiectasia (McFarlin and Oppenheim, 1969), and chronic candidiasis (Canales, Middlemas, Louro, and South, 1969) among others, serum has been shown to contain a factor which is able to damp down lymphocytic response to antigen. The present work studies the titre of this lymphocyte depressive factor in normal serum and in serum from patients with M.S. and other (destructive) neurological diseases. The isolation and characterization of the lymphocyte depressive factor might be of considerable therapeutic importance in the treatment of diseases due to "autoaggression" by these cells.

Research in human lymphocyte sensitization has hitherto been much hampered by the absence of a sensitive, reproducible, and quantitative method of estimation (Bloom, 1971), but recently we have described one such which we believe has these attributes—the macrophage electrophoretic slowing test (Field and Caspary, 1971b; Caspary and Field, 1971b)—and this has been used in the present work. Its principle is described below.

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