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A controlled trial of treatment of acquired immunodeficiency in severe measles with thymic humoral factor

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SUMMARY

A randomized controlled trial of treatment with thymic humoral factor (THF) in 20 children with severe complicated acute measles infection, resulted in objective benefit as evidenced by improvement in the ESR and a fall in C-reactive protein, fewer complications and a reduced incidence of secondary herpes infection. An increased ratio of helper to suppressor T cells (OKT4/OKT8 ratio) and a greater lymphocyte transformation response to phytohaemagglutin was seen in those children receiving THF. We conclude that THF treatment helps to prevent the development of complications particularly secondary viral infections possibly by enhancing cell-mediated immune responses.

Keywords acquired immunodeficiency thymic hormones measles

INTRODUCTION

Measles, one of the common childhood infectious diseases, is accompanied by high morbidity and mortality particularly in malnourished children. In North America, death from respiratory or neurological complications occurs in 1 in 3,000 reported cases (Measles prevention, 1982) whereas in Africa measles in a malnourished population may result in a mortality rate of up to 50%, particularly in the very young child (Morley, 1962; Dosseter, Whittle & Greenwood, 1977; Orren *et al.*, 1979). Measles infection suppresses cell-mediated immunity (CMI) particularly in children with malnutrition (Coovadia, Wesley & Brain 1978; Whittle *et al.*, 1978, Orren *et al.*, 1981). Secondary infection with Herpes virus and adenovirus is commonly found in such children (Kipps & Kaschula 1976; Orren *et al.*, 1981).

Thymic humoral factor (THF), a polypeptide hormone from calf thymus, is an immunostimulating agent acting on thymus derived cells and T lymphocytes in their earlier stages of differentiation (Trainin, Pecht & Handzel 1983). In humans THF has therapeutic potential in patients with immune deficiency of primary or secondary origin, and is free from toxic effects (Zaizov *et al.*, 1977, 1979). It has led to improvement of T cell function and in some cases to a substantial clinical benefit (Handzel *et al.*, 1981).

It would be expected, therefore, that THF would either enhance recovery or prevent secondary infection in children with measles particularly if malnourished by reversing defects in acquired CMI. In this paper we present the results of a clinical trial of THF treatment in children with severe measles.

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MATERIALS AND METHODS

We studied 20 children admitted to the Cape Town City Hospital for Infectious Diseases or to the Intensive Care Unit (ICU) of Red Cross War Memorial Childrens' Hospital with complicated acute measles infection. Informed written consent for admission to the study was obtained from the children's parents and the study was undertaken according to a protocol approved by the Ethics and Research Committee of the University of Cape Town, and the Medicines Control Council. The severity of the measles infection and the presence of complications was assessed using a scoring system as outlined in the appendix. Children with onset of a measles rash in the preceeding 2 days and a score of 7 or over at presentation were admitted to the trial and allocated at random by pre-coded sealed letter draw to the treatment and non-treatment groups by the clinical assessor who alone was aware of this allocation. Children less or greater than 80% of expected weight for age were allocated separately as above to ensure comparability of nutrition in the two groups.

THF was prepared by described methods (Kook, Yakir & Trainin 1975) and was diluted in saline to a concentration of 5·0 μg/ml and stored at −20 °C in 0·5 ml aliquots. It was injected in four rotating sites daily to the treatment group in the following dosage:

Weight up to 5 kg	0·5 ml (2·5 μg THF)
Weight between 5 and 10 kg	1·0 ml (5μg THF)
Weight above 10 kg	1·5 ml (7·5 μg THF)

THF was given for 7 days or until death or until the temperature had returned to normal for at least 2 days during which there was no extension of existing, or appearance of new complications, whichever was the longer.

Each child was examined daily. Chest X-rays , ECG, EEG and other ancillary investigations were performed as clinically indicated. Antibiotics, intravenous fluids, oxygen and supplementary treatment was prescribed as required. Admission to the ICU, the clinical diagnosis of Herpes lesions and overall management of the patients remained the responsibility of the hospital staff who were not directly involved in this trial.

Full blood counts, erythrocyte sedimentation rate, serum albumin, SGPT, alkaline phosphatase and serum immunoglobulin assays were performed in the Pathology Department of the Red Cross Childrens' Hospital using standard techniques. C-reactive protein (CRP) levels were measured by radial immunodiffusion (Behring Diagnostics).

Blood mononuclear cells were isolated on Ficoll-Isopaque gradients as previously described (Beatty & Dowdle, 1978). T cells were counted by rosette formation with AET treated sheep red blood cells (Melvin 1979). T cells and T cell subsets were counted using monoclonal antibodies (MoAbs) OKT3 (total T cells), OKT4 (helper/inducer T cells) and OKT8 (suppressor/cytotoxic T cells) (Ortho Diagnostics, New Jersey, USA) as follows: 1×10^6 mononuclear cells resuspended in 200 μl of phosphate-buffered saline (PBS) were incubated for 30 min in an ice bath with 10 μl (0·05 μg) of MoAb. The cells were washed at 4 °C and 20 μl of 1/40 fluorescent labelled rabbit anti-mouse IgG (Miles-Yeda, Rehovot, Israel) was added; they were incubated on ice for 30 min, washed, resuspended in 30% glycerol in PBS and counted using fluorescent microscopy.

Lymphocyte proliferative responses to phytohaemagglutinin (PHA) (Wellcome Diagnostics) and concanavalin A (Con A) (Miles-Yeda) in AB or autologous serum in microtitre plates were performed as previously described (Beatty & Dowdle, 1979). Normal control adult lymphocytes were cultured in parallel. Results shown are the radioactivity incorporated by the patients lymphocytes as a percentage of the normal control lymphocytes.

The production of leucocyte inhibitory factor (LIF) following PHA stimulation of the patients lymphocytes was assayed by determining the inhibition of migration of normal control purified leucocytes under agarose (Clausen, 1971). Results were expressed as the percentage of migration inhibition by supernates from PHA stimulated lymphocyte cultures as follows:

$$\left(1 - \frac{\text{Migration with PHA supernate}}{\text{Migration with unstimulated supernates}}\right) \times 100.$$

Nasopharyngeal swabs for the isolation of Herpes Simplex virus and serum antibody titres to Herpes Simplex were processed as previously described (Orren *et al.*, 1981).

Results were analysed by the Mann–Whitney U-test, and the Fisher exact probability test (Siegel, 1956).

RESULTS

The clinical findings in the two groups were similar on day 0 (Table 1). In the untreated group six children had an uncomplicated course, four children were admitted to the ICU of whom two died on days 6 and 11, because of extension of pneumonia and its complications in spite of intensive supportive treatment (including assisted ventilation). The two other children in this group who developed complications—in the one case extension of pneumonia and in the other encephalitis and convulsions—recovered completely.

In the treated group the average duration of THF administration was 9 days; no adverse effects were noted. Seven children made an uneventful recovery and two were admitted to the ICU because of extension of pneumonia. The one child died at 14 days after having received respiratory support for 12 days. The other child developed an empyaema requiring closed chest drainage, and myocarditis but recovered fully. One other child in the treated group developed severe diarrhoea and lactose intolerance which responded to appropriate treatment.

Table 1. Clinical data

	Untreated		THF treated	
	Median	Range	Median	Range
No. of patients	10		10	
Age (months)	13·5	(7–28)	10·5	(5–66)
Sex	5M/5F		8M/2F	
Measles score (see appendix)	8·0	(7–13)	8·0	(7–11)
Temp settled (day)	4·0	(3–9)	4·5	(2–14)
ESR (mm/h)				
Day 0	36·0	(10–85)	32·5	(10–97)
Day 14	42·5	(10–65)	28·0*	(10–66)
CRP (mg/dl)				
Day 0	2·4	(0·1–9·8)	4·4	(0·1–13·0)
Day 7	0·6	(0·1–12·8)	0·1†	(0·1–1·6)
ICU treatment	4		2	
Complicated course	2		2	
Deaths	2		1	
Herpes Simplex clinical lesions	4		0‡	
Herpes Simplex culture positive	5 (1 disseminated)		3	
Herpex Simplex positive antibody titre day 0	3		3	
Herpes Simplex rise in antibody titre	3		3	

* $P < 0{\cdot}05$ (Mann–Whitney U-test).
† $P < 0{\cdot}01$ (Mann–Whitney U-test).
‡ $P = 0{\cdot}05$ Fisher exact probability test.

Table 2. Nutritional and biochemical data

	Untreated		THF treated	
	Median	Range	Median	Range
No. of patients	10		10	
% EWA*	88	60–111	83·5	68–125
<80% EWA*	3		3	
Albumin g/l (day 0)	32·35	20·3–40·6	27·60	20·2–29·5
Albumin g/l (day 14)	33·25	29·1–37·1	31·90	26·4–35·0
IgG g/l (day 0)	9·98	8·0–20·0	9·13	6·73–1·46
IgM g/l (day 0)	1·46	0·51–2·03	1·20	0·59–2·59
IgA g/l (day 0)	0·82	0·31–1·42	0·69	0·36–2·28
SGPT u/l (day 0)	25·5	5–40	17·0	11–32
Alk PO4 u/l (day 0)	164·5	32–281	187·0	24–478
Hb g/100 ml (day 0)	10·5	5·6–11·6	10·35	6·8–11·9

* % EWA = percentage expected weight for age.

The erythrocyte sedimentation rate at the end of the 14 day study period ($P < 0{\cdot}05$, Mann–Whitney U-test), and CRP levels after 7 days ($P < 0{\cdot}01$) were lower in the group treated with THF. None of the patients treated with THF developed herpetic lesions, but four of the untreated group developed typical fever blister type lesions ($P = 0{\cdot}05$, Fisher exact probability test). Herpes Simplex isolates were non-significantly fewer in the treated group. The number of patients with pre-existing anti-Herpes Simplex antibody who showed a rise in antibody levels was similar in the two groups.

Three children in each group were classified as undernourished (less than 80% of expected weight for age). The median weight for age, albumin levels, immunoglobulin levels and SGPT were all non-significantly lower at the outset in the group receiving THF (Table 2). One undernourished

Table 3. Cellular immune function (median values)

	Day 0		Day 7		Day 14		Median control values
	Untreated	THF treated	Untreated	THF treated	Untreated	THF treated	
Total lymph count mm/^{3}	1,941	1,796	3,729	3,767	3,663	3,600	
E rosettes % (T cells)	44·5	47·0	51·0	39·0	49·0	35·0	60·5
OKT3 % (T cells)	54·0	64·0	60·0	60·0	62·5	60·0	68·0
OKT4 % (T helper cells)	28·0	36·0	40·0	37·0	41·5	49·0	50·0
OKT8 % (T suppressor cells)	31·5	22·0	23·0	19·0	27·0	23·0	29·0
OKT4/OKT8 ratio	1·5	1·32	1·09	2·83	1·69	2·17	1·83
PHA AB% of control†	64·0	77·0	69·0	100·0*	92·0	99·0	
PHA auto % of control†	56·0	54·5	56·0	72·5	84·5	88·0	
CON A AB % of control†	60·5	80·5	73·0	129·5	93·0	107·0	
CON A auto % of control†	32·0	45·0	44·0	59·0	67·5	62·0	
LIF (% migration inhibition)	9·0	12·0	10·0	10·0	10·0	7·0	15·0

* P value $< 0{\cdot}05$ (Mann–Whitney U-test).

† AB and auto refer to cultures supplemented with either AB or autologous serum.

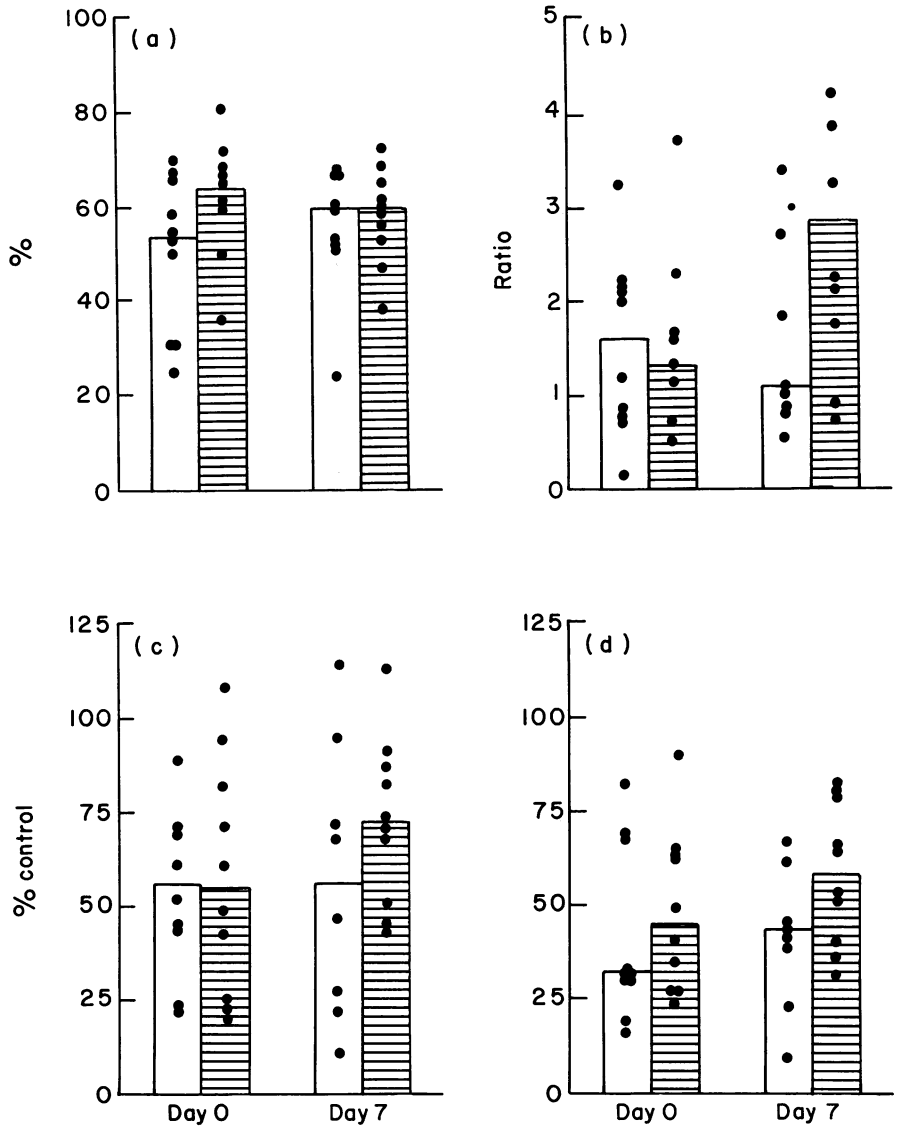

Fig. 1. Comparison of individual and median results (bars) obtained in patients treated with THF (■) and untreated (□) patients at various time intervals. (a) T cell percentages (OKT3) and (b) T helper suppressor ratios (OKT4/OKT8) were measured by fluorescent microscopy. The lymphocyte transformation responses to (c) PHA and (d) Con A were measured in cultures supplemented with autologous serum. The results are expressed as the percentage of normal control lymphocyte cultures done in parallel.

child in the untreated group died on day 11 from pneumonia and one undernourished child in the treated group developed diarrhoea and lactose intolerance.

On admission total lymphocyte counts, T cell numbers as measured by E rosettes and MoAb, T helper cells and LIF production were low (Table 3 & Fig. 1). Lymphocyte responses to PHA and Con A were also low particularly in cultures supplemented with the patient's serum.

The differences in the median values for these immunological parameters on admission (Fig. 1), especially of mitogen responses were not statistically significant, but they complicate the

interpretation of the higher lymphocyte blastogenic responses to PHA and Con A in the treated group which was statistically significant for PHA cultures in AB serum. There was also lower numbers of T suppressor cells and total T cells and a higher helper to suppressor T cell ratio in the treated group. The LIF results were similar in the two groups.

DISCUSSION

Active immunization programmes have dramatically reduced the incidence of measles infection in highly developed countries (Measles prevention, 1982). Until this goal has been achieved severe complicated and sometimes fatal measles infection will continue in developing countries, especially in malnourished children under 1 year old. Our patients showed low lymphocyte counts which reflects a poor prognosis (Coovadia *et al.*, 1978) and a moderate to severe depression of CMI function (Whittle *et al.*, 1978; Orren *et al.*, 1981). In addition lymphocytes transform poorly in the serum of patients with acute measles (Orren *et al.*, 1981). The frequency of secondary infection, especially Herpes Simplex in our study shows the importance of this immunodeficiency.

It should be noted that although efforts to include an equal proportion of malnourished children in each group was undertaken by means of clinical criteria (percentage expected weight for age) the serum albumin concentration shows that the group which received THF were more severely malnourished. This may have influenced the results and further cases in the trial would permit a better evaluation. The effects of a measles immunization campaign undertaken in the Western Cape was apparent in that the number of children who fulfilled the criteria for inclusion in this study were fewer than anticipated and precluded a larger series.

Our results show that treatment with THF, an immunostimulant particularly of CMI, resulted in objective clinical benefits as evidenced by the fall in the ESR and CRP levels and the lesser incidence of severe complications including Herpes virus infection. There were suggestions of improved T cell function especially of lymphocyte mitogenic responses but because of a pre-treatment difference the statistically significant differences of lymphocyte responses to PHA in cultures supplemented with control AB serum at 7 days must be interpreted with caution.

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APPENDIX

Clinical measles score. For admission to trial patients score 7 or more points

Rash	
Mild	1
Intermediate	2
Confluent	3
Mucous membranes	
Kopliks only	1
Kopliks and/or mucosal inflamation	2
Diffuse Kopliks and/or diffuse mucosal inflamation	3
Pneumonia (clinical + X-ray)	
Tachypnoea plus bronchial changes	1
Tachypnoea recession and adventitious sounds plus bronchial changes	2
As above plus patchy consolidation or emphysema or bronchial breathing or diffuse crepitations	3
As above plus dense consolidation or effusion or atelectasis	4
Laryngotracheobronchitis	
Mild	1
Moderate	2
Severe	3
GIT	
Diarrhoea (> 5 stools/day)	1
Diarrhoea and Dehydration	2
Encephalitis	
Depressed level of consciousness	2
Coma	3
Coma and/or convulsions	4
Myocarditis	
ECG + pulse only	3
plus cardiac failure	4
Malnutrition	
Less than 80% EWA	1