Cell-Mediated Immunity during Natural Measles Infection

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ABSTRACT Natural measles causes prolonged depression of cell-mediated immunity yet little is known as to how the infection influences lymphocyte function. Therefore, we studied the properties and function of lymphocytes during and after measles. The number and proportion of circulating thymus-derived lymphocytes was low during the acute stage of measles, and at this time 37% of these cells showed positive immunofluorescent staining for measles virus after stimulation with phytohemagglutinin. 7% of B cells were shown to contain virus but their numbers did not alter during the infection. Acute-phase lymphocytes, when stimulated, yielded infective virus and half were killed on incubation with autologous serum and complement. In acute measles the increase in [^{3}H]-thymidine uptake of lymphocytes when stimulated with an optimal dose of PHA was normal in media with 10% fetal calf serum and low in media containing 10% autologous serum: the mean values were 56.8±34.1 and 23.7±25.9 cpm × 10^3 per 10^6 lymphocytes, respectively. Stimulation of acute-phase lymphocytes by *Candida* antigen was also low in media containing autologous serum averaging 1.2 × 10^3 cpm per 10^6 lymphocytes. On recovery 4–6 wk later this rose significantly to 18.9±19.8. The mean migration index of leukocytes to heat-killed candida cells in acute measles was 0.84±SD 0.08, and this fell significantly to 0.75±SD 0.08 4 wk later. Thus, depletion of T cells, an inhibitor of lymphocyte proliferation in the serum and a possible defect in antigen processing, interacts to depress cell-mediated immunity in measles.

INTRODUCTION

Natural measles infection suppresses both cell-mediated and humoral immune responses (1), and this, coupled with malnutrition, leads to the death of many children from secondary infection (2). Von Pirquet in 1908 (3), first noticed that the skin test to old tuberculin became negative during measles, and since then many scientists have investigated this phenomenon.

Smithwick and Berkovich (4) infected lymphocytes from tuberculous children with measles virus and showed a diminished blastogenic response of these cells to tuberculin. Osunkoya et al. (5, 6) studied lymphocyte transformation to phytohemagglutinin (PHA)[1] during natural measles infection: tritiated thymidine incorporation and blast-cell counts were normal despite the appearance of many giant cells infected with measles virus. However, Finkel and Dent (7) noted impairment of lymphocyte response to suboptimal doses of PHA but were unable to show that this was because of serum inhibitory factors that are known to occur in some viral infections (8). Experiments by Zweiman and Miller (9–11) demonstrated that both live and killed measles virus could suppress the response of human lymphocytes to PHA and purified protein derivative (PPD). Sullivan et al. (12) extended this work and postulated that depression by live virus of lymphocyte stimulation by PHA resulted from direct viral inhibition of host cell DNA synthesis.

Other hypotheses for the depression of cell-mediated immune responses following measles have been advanced. Osunkoya et al. (6) suggested this was because of a transient reduction in number of T lymphocytes as a result of cytopathic destruction, and this idea has been supported by the work of Joseph et al. (13) who have shown immune lysis of measles-infected HeLa cells by antibody and complement. They also showed that in vitro both T and B lymphocytes

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[1] *Abbreviations used in this paper*: FCS, fetal calf serum; HBSS, Hanks' balanced salt solution; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; PWM, pokeweed mitogen; RPMI, Roswell Park Memorial Institute medium; SRBC, sheep erythrocytes.

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and monocytes can be infected by measles virus (14). Finkel and Dent (7) suggested that phagocytic cells responsible for antigen processing are primarily affected by the virus, and Kantor raised the possibility that the virus might stimulate some lymphocytes to release a suppressor of cell-mediated immunity (15).

In an attempt to gain further knowledge and to advance treatment of the natural infection in the environment of tropical Africa we have tested some of these theories by investigating patients during and after measles. Virus was identified in different types of lymphocytes, T and B cells were counted, and PHA function was studied. Leukocyte migration and lymphocyte stimulation with *Candida* antigen was examined and, in addition, pooled measles sera which might contain inhibitory factors have been tested on lymphocytes when stimulated with PHA and *Candida* antigen.

METHODS

Children with acute measles diagnosed by the presence of Koplik's spots and(or) a typical rash, were studied as outpatients at the Ahmadu Bello University Hospital, Zaria, Kaduna State, Nigeria. Only well-nourished children (weight for age greater than 80% of Harvard standard) (16) with a rash less than 4 days old were selected. The mean age of the patients was 20 mo (range 9–60 mo).

10 ml of blood were taken by venipuncture; this procedure was repeated 4–6 wk later when the child had completely recovered. At this time an intradermal skin test with *Candida* antigen was made and the result was read 48 h later.

Controls were children of similar age who had completely recovered from an attack of *Plasmodium falciparum* malaria 4–6 wk earlier and who were having blood taken in the course of another study. The study was approved by the ethical committee of the Faculty of Medicine, Ahmadu Bello University.

Separation of lymphocytes. 10 ml of blood were collected in 100 IU of preservative-free heparin (Boots Pure Drug Co., Nottingham, England) and the total and differential leukocyte count was determined. Blood was diluted 1:2 with Hank's balanced salt solution (HBSS, Oxoid, London, England) and layered onto Ficoll-sodium metrizoate (Lymphoprep, Nyegaard, Oslo, Norway) at a ratio of 3:1 in 100 × 16-mm plastic tissue culture tubes (Sterilin, Teddington, England). The tubes were centrifuged for 30 min at 400 *g* at 20°C, the supernate removed, the cells collected with a pasteur pipette, and the suspension diluted more than 5 times with HBSS before centrifugation at 400 *g* for 10 min at 10°C. After two washes in HBSS the tests were performed.

Identification of T and B lymphocytes. 0.25 ml of a 0.5% suspension of washed sheep erythrocytes (SRBC) was added to 1×10^6 lymphocytes in 0.25 ml HBSS containing 0.15 ml of fetal calf serum (FCS) which has been previously absorbed with sheep erythrocytes. After incubation at 37°C for 5 min the mixture was spun at 200 *g* for 5 min and then left overnight at 4°C. Cells were then resuspended by flicking the tube, and the number of lymphocytes binding 3 or more erythrocytes were counted by phase-contrast microscopy. B lymphocytes were counted by darkfield UV microscopy (Zeiss Illuminator, Carl Zeiss, Inc., New York, exciter filter BG 3, barrier filter 440) after staining with fluorescein isothiocyanate-labeled sheep antihuman immunoglobulin (Wellcome Reagents, Wellcome Research Laboratories, Beckenham, England) at a concentration of 1:4. This conjugate was centrifuged at 30,000 *g* filtered with a microsyringe filter holder and a 0.22-μm filter (Millipore Corp., Bedford, Mass.), and stored in small aliquots which were only used on a few occasions.

Separation of T lymphocytes. Nylon fiber (Fenwal Laboratories, Morton Grove, Ill.) was washed in 0.2 N HCL then washed thoroughly with distilled water and dried at 37°C. The washed nylon was loosely packed to the 0.5-ml mark in 1-ml plastic syringes (Argyle, Feltham, Middlesex, England) washed through with Roswell Park Memorial Institute medium (RPMI 1640, Flow, Irvine, Scotland) which was buffered with 20 mM/liter Hepes and 20 mM/liter sodium bicarbonate and contained penicillin 100 IU/ml and streptomycin 100 μg/ml. Before adding lymphocytes the column was washed with RPMI/10% FCS prewarmed to 37°C. Lymphocytes, about 10×10^6 in 0.2 ml RPMI/10% FCS were added to the column which was kept at 37°C for 30 min before eluting with RPMI at 37°C. Eluted lymphocytes were washed twice with RPMI and adjusted to 10^6 lymphocytes/ml in RPMI/10% FCS. Contamination with B lymphocytes averaged less than 2%.

Separation of B lymphocytes. Lymphocytes at a concentration of 5×10^6 ml in RPMI were dispensed in 0.5-ml aliquots in 12 × 75-mm plastic tubes (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). 0.25 ml of a 2% suspension of SRBC and 0.4 ml of heat-inactivated FCS (56°C for 0.5 h) previously absorbed with SRBC was added to each tube. The tube was mixed thoroughly, incubated for 5 min at 37°C, spun at 200 *g* for 7 min at 4°C, and incubated for a further 3–4 h in crushed ice. The pellets were then gently resuspended by flicking the tube, pooled, layered onto Lymphoprep, and spun at 400 *g* for 20 min at 20°C. B lymphocytes were removed from the interface, washed thrice in RPMI, and suspended at 1×10^6 lymphocytes/ml in RPMI/10% FCS. Contamination with T lymphocytes averaged less than 2%.

Identification and isolation of measles virus from lymphocytes. Blood was defibrinated with glass beads; the erythrocytes were sedimented with an equal volume of 3% Dextran (Pharmacia, Inc., Uppsala, Sweden) in phosphate-buffered saline (PBS), pH 7.2, and the supernate was removed. The sedimented leukocytes were washed twice with medium and cultured at a concentration of 1×10^6 lymphocyte per ml in RPMI 1640, prepared as above, 10% decomplemented FCS, and 5 μg per ml of purified PHA (Wellcome Reagents). The cells were dispensed in 0.2-ml amounts in flat bottom microtiter plates (Cooke Laboratory Products, Div. Dynatech Laboratories, Inc., Alexandria, Va.), and after incubation at 37°C for 3 days in 5% CO_2 in air they were washed with PBS and stained by direct immunofluorescence, with a high titer fluorescein or rhodamine isothiocyanate-labeled, measles immune human serum at a concentration of 1:8. This serum had been absorbed against pig liver powder and then spun at 30,000 *g* for 30 min. The successful absorption of antibody with measles virus showed that this fluoresceinated antiserum was specific for measles. It also did not stain normal PHA-stimulated lymphocytes which were used as a control. To determine if B cells were infected with virus, stimulated cells were first stained with fluoresceinated antihuman immunoglobulin (see identification of B lymphocytes), washed, and then counterstained with the rhodamine isothiocyanate-labeled measles immune serum. The remaining cells and the supernate were frozen at −20°C for up to 1 mo. After thawing, 1 ml was inoculated onto a layer of Vero cells which has been grown in 25-cm^2 tissue culture flasks (Corning Glass Works, Corning, N. Y.) con-

taining Eagle's minimum essential medium (Autopow, Flow), 10% FCS, penicillin 100 U/ml, streptomycin, 100 μg/ml and amphotericin B 2.5 μg/ml. After 1 h, 10 ml of medium was added and after incubation at 37°C for 14–21 days the virus was identified by its cytopathic effects and by immunofluorescence. Purified T and B lymphocytes were cultured as above in U microtiter plates (Linbro Scientific Co., New Haven, Conn.) with either 5 μg/ml PHA or a 1:100 dilution of Pokeweed mitogen (PWM, Grand Island Biological Co., Grand Island, N. Y.) before identification of measles virus by immunofluorescence and culture.

Cytotoxicity studies. Lymphocytes separated on Lymphoprep were grown for 3 days with or without PHA in U well microtiter plates in RPMI 1640/10% FCS. After 3 days the cells were pooled, centrifuged at 250 *g* for 5 min at 10°C, the supernate was removed, and the cells were washed with 10 ml RPMI/10% FCS. The lymphocytes were then suspended at 1×10^6/ml in RPMI/10% FCS and dispensed in 0.2-ml amounts in a V well microtiter plate, centrifuged, and the supernate was removed with a pasteur pipette. 0.1 ml of serum was added to the button of cells and the suspension was incubated for 90 min at 37°C before adding 0.5% trypan blue and assessing viability. The number of cells excluding trypan blue was counted at ×320 magnification by phase-contrast microscopy and expressed as a percentage of the total counted. The sera used for these experiments with the exception of the FCS, was prepared by clotting blood in glass bottles at room temperature for 2 h. It was then separated, and stored for no longer than 3 days at −25°C. Specimens were coded and all viabilities were read by the same observer.

Lymphocyte culture with PHA. Lymphocytes separated on Lymphoprep were cultured at 5×10^5/ml in RPMI 1640 in microtiter U plates containing either 10% decomplemented FCS or 10% autologous serum which was not decomplemented. After incubation in 5% CO_2 at 37°C for ≅54 h, with the appropriate dilution of PHA 0.8 μc of [^{3}H]-thymidine (Radiochemical Center, Amersham, England) was added to each well. After another 18 h the cells were pooled, washed in 10 ml PBS and harvested on 2.5-cm glass fiber disks (GFC, Whatman, Inc., Clifton, N. J. and Reeve Angel, London) with a sampling manifold (Millipore Corp.). Each disk was first rinsed with 10% trichloracetic acid followed by 95% ethanol. After drying at 100°C for 0.5 h in hot air, the disks were placed in 3 ml scintillation fluid (NE 233, Nuclear Enterprises Ltd., Reading, England), with 10% Hyamine (Nuclear Enterprises Ltd.), kept overnight at 4°C in the dark and counted for 100 s under the same conditions in a hand-operated β scintillation counter (NE 5503, Nuclear Enterprises Ltd.). Results were expressed in counts per minute per 10^6 lymphocytes, and the increase in counts was calculated by subtracting the counts for the culture not containing PHA from that of the PHA cultures. The dose of the PHA that gave optimal stimulation in this system was 5 μg/ml.

Lymphocyte culture with Candida antigen. Lymphocytes were separated by Dextran sedimentation and thereafter the procedure was the same as for the PHA cultures except that *Candida* antigen at a final concentration of 20 μg/ml was added to each well and the cells were incubated for 6 days before harvesting.

Candida antigen was prepared from the same strain as that used in the leukocyte migration test (see below) by disrupting whole cells in a Dynomill disintegrator for 30 min. This antigen gave optimal lymphocyte stimulation at a final concentration of 20 μg/ml.

Skin tests with Candida antigen. 0.1 μg of the disrupted *Candida* antigen in 0.1 ml of PBS was injected intradermally into the volar surface of the forearm. After 48 h the mean diameter of the indurated area was measured with a ruler. Positive responses varied from 3 to 15 mm in diameter.

Study of serum factors. Pools of five or six sera were stored in aliquots at −20°C for up to 3 mo before testing.

Leukocyte migration test. A standard method was used (17). Leukocytes were separated from heparinized blood by sedimentation in 100 × 16-mm tissue culture tubes (Sterilin) for 0.5–1 h. After washing in Eagle's minimum essential medium they were suspended at a concentration of 1×10^8/ml in this medium containing 10% FCS and used to fill 12.5-μl capillaries (half of a 25-μl micro capillary, Drummond Scientific Co., Broomall, Pa.). The tubes were plugged with paraffin wax (BDH Chemicals, Poole, England) spun at 800 *g* for 5 min, cut with a diamond pen, and the cell buttons were placed in plastic migration chambers (Sterilin) containing medium and 10% FCS with or without antigen, sealed with coverslips and incubated at 37°C for 18 h. All operations were carried out at room temperature before the final incubation. The area of cell migration was projected by a prism attachment to a standard microscope, drawn on paper, and measured by weighing the cut-out area. Results have been expressed as a migration index which is the ratio of the average area of migration in the presence of antigen to the average area of migration in the absence of antigen. Each of these averages were calculated from four observations.

The antigen used was heat-killed *Candida albicans* cells (70°C for 0.5 h, kindly supplied by Dr. D. W. R. McKenzie, Mycological Reference Laboratory, London School of Hygiene and Tropical Medicine) at a concentration of 1×10^7 cells per ml.

Statistics. Student's *t* test or Student's paired *t* test, or the Mann-Whitney U tests were used to test the significance of the difference between means. A P value of <0.05 was regarded as significant.

Tests were done in mixed batches containing samples from patients with acute measles, patients who had recovered from measles, and from controls.

RESULTS

T and B lymphocytes. Table I shows the number of lymphocytes and the percentage of T, B and null lymphocytes in 15 children with acute measles, in 10 children 4–6 wk after measles and in 19 control children. Both the children with measles and those

TABLE I

Number of Lymphocytes and Mean Percentage of Lymphocyte Subpopulations during Measles

Group	No. lymphocytes	T Cells	B Cells	Null cells
	10⁹/liter	%	%	%
Acute measles (15)	3.8±1.5	38.7±13.8	32.7±8.4	26.7±16.5
4 wk later (10)	5.4±2.5	42.2±7.1	29.9±6.0	27.9±9.5
Controls (19)	4.1±1.6	53.3±10.0	32.3±9.0	14.4±10.3

All values are ±SD.

who had recovered had a significantly lower percentage of T lymphocytes in their blood than the controls ($P < 0.01$ in both cases). They also had a higher percentage of null cells ($P < 0.01$ in both cases).

Fig. 1 shows the absolute number of T cells of the children during measles and of the controls. The number of T lymphocytes was low in the acute phase but rose to control levels by 4 wk. ($P < 0.05$ acute vs. controls and $P < 0.05$ acute vs. convalescent, Mann-Whitney U test used). There was no significant difference between patients and controls in the number of B cells.

Measles virus in lymphocytes. Measles virus was not detected by immunofluorescence on the surface of unstimulated lymphocytes from eight patients with acute measles. After culture with PHA lymphocytes showed positive staining for measles in all of 10 patients with acute measles. The average proportion of positive cells was 14% (range 4–38%), and in all preparations giant cells which stained brightly were seen. Double immunofluorescent staining of lymphocytes from four patients stimulated with PWM showed that a small proportion (<5%) of the immunoglobulin bearing cells were also positive for measles virus.

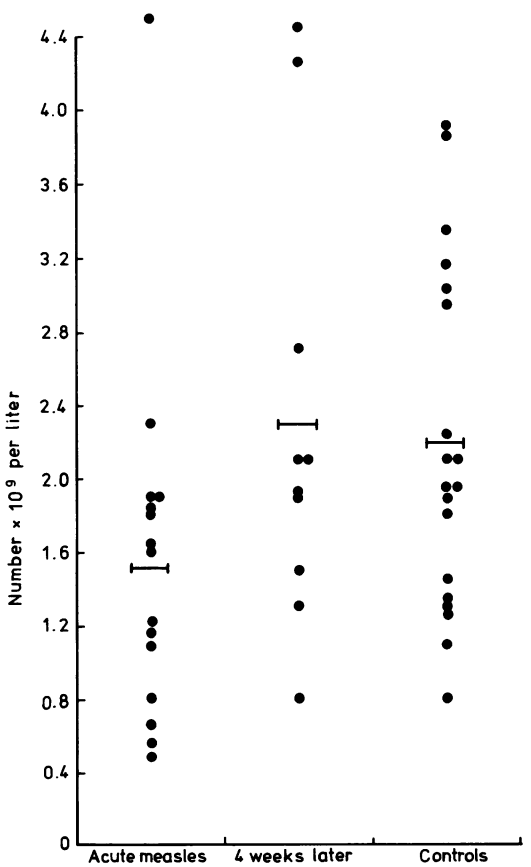

FIGURE 1 Absolute number of T cells of children during measles and of controls. Bar indicates mean.

Measles virus was isolated from five out of seven PHA-stimulated lymphocyte cultures. 1 wk after the onset of the rash measles virus could not be detected in 10 patients by either immunofluorescence or by culture.

The lymphocytes from nine children with acute measles were separated into both T and B subpopulations and were stimulated for 3 days with PHA or PWM in FCS and then were examined for measles antigen on their surface by immunofluorescent microscopy. Table II shows that both T and B lymphocytes contained measles virus and that T lymphocytes when stimulated by PHA showed a greater percentage of positive cells than T or B lymphocytes stimulated with PWM ($P < 0.01$). There was no significance difference between the percentage of positive T and B lymphocytes when stimulated with PWM. Giant cells were seen in both T- and B-lymphocyte preparations, the number increasing with the percentage of positive cells.

The mean [^{3}H]thymidine uptake for T lymphocytes stimulated by PHA was 53.1±SD 16.4 cpm × 10^3 per 10^6 lymphocytes. The values for T and B lymphocytes stimulated by PWM were 8.5±SD 5.5 and 6.3±SD 3.6 cpm × 10^3 per 10^6 lymphocytes, respectively.

Cytoxicity studies on stimulated lymphocytes. Table III shows that lymphocytes from patients with acute measles, after stimulation with PHA, are killed on addition of autologous serum and complement ($P < 0.001$, for differences between FCS and autologous serum) and that inactivation of complement decreased the killing power of the autologous sera ($P < 0.01$ for difference between active and inactive autologous sera). Sera from adult donors also killed the patients'

TABLE II

T and B Lymphocytes Showing Positive Immunofluorescent Staining for Measles Antigen after Stimulation with PHA or Pokeweed

Patient	T Lymphocytes		B Lymphocytes
	PHA	PWM	PWM
	%		%
1	42	—	24
2	20	9	8
3	34	11	—
4	11	0	0
5	47	1	1
6	16	0	0
7	14	0	4
8	82	0	9
9	74	0	10
Mean±SD	37±26	3±4	7±11

TABLE III

Viable Lymphocytes after Stimulation with PHA and Incubation in Various Sera

Donor	FCS	Autologous serum + C	Autologous serum − C	Adult serum + C	Measles serum + C
			%		
Acute measles (9)	60.7±8.2	29.4±8.5	45.4±10.4	46.7±8.4	—
Adult (8)	58.3±7.1	59.7±8.1	—	—	53.8±7.2

All percentages are ±SD.

stimulated lymphocytes ($P < 0.001$ for difference from FCS), but the effect was not so great as when the patients' own serum was used. Stimulated lymphocytes from healthy adult volunteers were not affected by their own serum but showed a slight and insignificant reduction in viability when incubated in acute-phase measles sera.

Unstimulated lymphocytes from the same patients with measles were not killed on incubation with their own sera and complement. The viability counts for these cells were 67.3%±SD 11.8 in FCS and 66.3% ±SD 8.9 in autologous serum.

PHA stimulation. The results of culturing lymphocytes of patients and controls with a suboptimal dose of PHA (0.1 μg/ml) or an optimal dose of PHA (5 μg/ml) are shown in Table IV. In the acute stage of measles the increase in [^{3}H]thymidine uptake was significantly higher in medium containing FCS than in autologous serum at both concentrations of PHA, ($P < 0.05$ for 0.1 μg, $P < 0.025$ for 5 μg/ml PHA). Control children had a significantly higher increase in [^{3}H]thymidine uptake than children with acute measles in both types of media at the low concentration of PHA ($P < 0.05$), but at optimal concentration only the increase in 10% autologous serum was significantly higher ($P < 0.025$).

[^{3}H]Thymidine uptake in unstimulated cultures did not differ significantly between groups or sera. It averaged 0.8±SD 0.5 × 10^3 cpm per 10^6 lymphocytes. There was also no significant difference in viabilities of stimulated or unstimulated lymphocytes in the various cultures. This averaged 66.0±12.8%.

In another set of experiments lymphocytes from 10 children with acute measles were cultured with an optimal dose of PHA in media containing either 15% pooled-acute, 15% pooled-recovered serum, or 15% FCS. The increase in [^{3}H]thymidine uptake was significantly lower ($P < 0.001$) in acute-phase serum: this averaged 40.7±SD 31.6 × 10^3 cpm per 10^6 lymphocytes and 80.3±SD 51.0 and 95.8±SD 54.2 in recovered and FCS, respectively.

Lymphocyte stimulation by Candida antigen. The mean uptake of [^{3}H]thymidine in 10% autologous serum in unstimulated and *Candida* containing cultures in children with measles is shown in Table V. In the acute stage uptake was low in both unstimulated and antigen cultures and on recovery it rose significantly in both types of culture ($P < 0.02$ for both cases). The rise on recovery was not obviously related to the presence of a positive skin test which was noted in 7 of the 10 patients at this time. The viabilities of lymphocytes (mean 67±SD 6.5%) did not differ significantly between any of the cultures.

In a separate set of experiments lymphocytes from nine children with acute measles were cultured with *Candida* antigen in media containing either 20% pooled-acute or 20% pooled-convalescent serum. There was no significant difference in the increase in [^{3}H]-thymidine uptake of stimulated over control cultures in these media. This was 2.7±SD 3.9 and 2.4±SD 4.6 × 10^3 cpm per 10^6 lymphocytes for acute and recovered serum, respectively.

TABLE IV

Effect of 10% Fetal Calf or Autologous Serum on PHA Response of Lymphocytes‡ during Measles*

	0.1 μg PHA/ml		5 μg PHA/ml	
	FCS	Autologous serum	FCS	Autologous serum
Acute measles (10)	3.1±3.8	0.1±0.3	56.8±34.1	23.7±25.9
4 wk later (10)	9.9±17.0	0.5±0.9	67.8±49.9	52.0±61.2
Controls (12)	17.5±20.8	12.2±19.2	37.7±19.3	56.5±33.5

* Obtained at the time of culture.

‡ Expressed as mean increase in [^{3}H]thymidine uptake in cpm × 10^3 per 10^6 lymphocytes.

TABLE V

Response of Lymphocytes in 10% Autologous Serum‡ to Candida Antigen during Measles*

Stage of disease	Unstimulated	*Candida*
Acute measles (10)	0.5±0.4	1.2±1.4
4 wk later (10)	4.0±4.3	18.9±19.8

* Expressed as mean [^{3}H]thymidine uptake in cpm × 10^3 per 10^6 lymphocytes.

‡ Obtained at the time of culture.

Leukocyte migration to Candida antigen. The mean migration index for 17 healthy adult volunteers who had a positive skin test to *Candida* antigen was 0.69±SD 0.11 which was significantly lower than the mean of 0.87±SD 0.06 in 6 adults who had a negative skin test ($P < 0.01$). This inhibition was reversible by puromycin at a concentration of 10 μg/ml.

Fig. 2 shows the migration indices to heat-killed candida cells in 12 patients during and 4–6 wk after measles. The mean index for the children in the acute stage of measles was 0.84±SD 0.08 and on recovery this fell to 0.75±SD 0.08 ($P < 0.02$). Those with positive skin tests on recovery tended to have lower migration indices.

DISCUSSION

Our work shows that natural measles lowers the number of T lymphocytes thus offering an explanation for the transient lymphopenia reported in this infection (18). A high proportion of T cells contained measles virus, and, when stimulated, lymphocytes were lysed by autologous antibody and complement. We think this is one of the mechanisms by which the T cells are depleted. In contrast, few B cells were shown to be infected and their numbers did not alter significantly during the infection. It is also of interest that the proportion of T cells remained low for over 4 wk after the acute infection, which may explain why cutaneous-delayed hypersensitivity reactions are diminished for several weeks after the acute infection (19). The proportion of T cells that contained virus varied according to the degree of mitogenic stimulation, for when weakly stimulated by PWM no more positive cells were seen than when B lymphocytes were stimulated by this mitogen. However, if in measles T cells are more powerfully stimulated than B cells, more T lymphocytes would express virus and so be more susceptible to lysis by antibody.

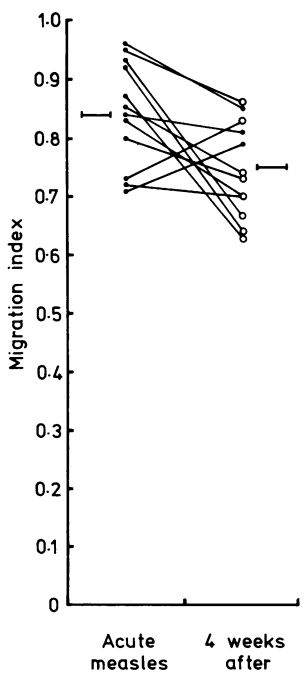

FIGURE 2 Migration indices to *Candida* antigen in children during measles. Bar indicates mean. Open circle signifies a positive skin test to *Candida* antigen.

The proliferative response of lymphocytes to PHA was also lowered during the acute phase of measles. This was shown to be the result of an inhibitory factor in acute phase serum, for the lymphocytes responded normally to an optimum dose of PHA in convalescent serum or FCS. This conclusion contradicts experimental evidence (10, 12) which suggests that the decreased [^{3}H]thymidine uptake is the result of direct viral inhibition of host-cell DNA synthesis, an effect which is only achieved with an unnaturally large dose of virus. Viability counts showed this inhibition was not a result of killing of lymphocytes. We think it is separate from the cytotoxic effect of undiluted acute-phase sera on measles-infected lymphocytes which is critically dependent on the amount of complement, over 90% of lytic activity being lost when complement is diluted to 1:4 (13). In our lymphocyte cultures sera were diluted 1:5 or 1:10 so complement-dependent lysis in this system would have been minimal or absent.

A third major finding was that the response of lymphocytes to *Candida* antigen was markedly depressed. The explanation for this, which is more complicated, lies in favor of a defect in antigen handling. We have some evidence of monocyte dysfunction in that the response to a suboptimal dose of PHA was depressed (20). Also, the proliferative response of acute-phase lymphocytes to antigen was lowered in both acute and recovered serum which suggest the cells were at fault. Still further evidence of defective lymphocyte or monocyte function was given by the abnormally high leukocyte migration indices in the acute stage of the illness. This indicates that leukocyte migration inhibitory factor, a soluble lymphocyte factor (21), was not produced at this stage. Impairments in polymorph function such as deficient random migration and chemotaxis have been found in the acute stage of measles (22), but this is an unlikely explanation for our results as we have found leukocyte migration tests are still abnormal 2 wk after the onset of the rash (23), a time when polymorph function has returned to normal (22). Perhaps the infected macrophages are unable to process antigen or perhaps antigen receptors on the surface of lymphocytes are disturbed. This possibility is raised as alterations in the cell membrane have been shown in cells

infected with Newcastle disease virus (24). In addition these defects were reinforced by the suppressive effect of acute-phase serum on lymphocyte proliferation. This was apparent even in the unstimulated cultures which showed a low [^{3}H]uptake in media containing this serum.

These three major abnormalities, T-cell depletion, a powerful inhibitor in the serum, and defective antigen handling help explain why cell-mediated immune responses are profoundly depressed during measles. This is important in Nigeria for measles affects the young who are often malnourished, and in such children the disease is severe and prolonged (23). As a result intercurrent infections such as tuberculosis, candidiasis (23), and herpes simplex,[2] which are normally controlled by cell-mediated immunity, became rampant after measles and contribute to the alarmingly high mortality of the disease (2).

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[2] Unpublished observations.