Lymphocyte Subsets in Measles

DEPRESSED HELPER/INDUCER SUBPOPULATION REVERSED BY IN VITRO TREATMENT WITH LEVAMISOLE AND ASCORBIC ACID

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ABSTRACT Lymphocyte subsets were assessed in patients with measles using the OKT range of monoclonal antibodies. A significant decrease in cells reacting with the OKT3 and OKT4 monoclonal antibodies was observed. When the tests were repeated 3 wk after the acute infection, significant recovery of these subsets was observed. The abnormality in lymphocyte subsets could be reproduced by treating normal lymphocytes with measles virus in vitro. When lymphocytes from patients with measles or when normal cells infected with measles virus in vitro were treated with either levamisole or L-ascorbic acid for 15 min and then retested with the OKT antisera, restoration of the previously depleted $OKT3^+$ and $OKT4^+$ cell population was observed. Ascorbic-acid treatment also, to a certain extent, reversed the inability of measles mononuclear cells to produce lymphocyte mitogenic factor (helper factor for B cells) after pokeweed mitogen activation. This abnormality, however, could not be reversed by in vitro treatment with levamisole. Measles patients treated with L-ascorbic acid demonstrated no accelerated recovery in either their lymphocyte subset profile or their ability to produce lymphocyte mitogenic factor. Although the cause of the depressed $OKT3^+$ and $OKT4^+$ lymphocyte subpopulations in patients with measles is not clear, the results suggest that the effect is not due to an aberration of protein synthesis, but rather to a blocking or steric change produced by the virus on the cell membrane. It is likely that both ascorbic acid and levamisole have the ability to reverse this effect by virtue of their antioxidant properties.

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INTRODUCTION

There has been widespread interest in the effects of measles virus infection on immune function because of the long-recognized depression of cell-mediated immune responses that accompanies infection with this virus (1). Since measles virus has been repeatedly detected in circulating blood lymphocytes (2) and has been recovered from lymphocytes stimulated by phytohemagglutinin (PHA)[1] in vitro (3), it has been inferred that measles affects immune responses by infecting certain lymphocyte subpopulations. Measles virus replicates equally well in all major human mononuclear (MN) cell subpopulations, including T and B lymphocytes and monocytes (4). Furthermore, the virus can productively infect both T cells with IgG (T_G) or IgM (T_M) receptors on their surface membranes (5). It has been previously shown, however, that it is primarily helper-cell function that is defective in measles infection (6, 7) and the finding of infective virus in all subpopulations does not necessarily imply defective function of all those subpopulations.

Monoclonal antibodies directed against specific human subsets provide a tool for the analysis of T cell subpopulations in man (8). In particular, the OKT3 antiserum defines the entire peripheral T cell population, the OKT4 antiserum defines 55–60% of human T cells of the so-called inducer/helper subset, whereas the OKT8 antiserum is directed against suppressor/cytotoxic T cells (9). This study investigated lymphocyte subsets in patients with acute measles infection

[1] *Abbreviations used in this paper:* LMF, lymphocyte mitogenic factor; LMS, levamisole, MN cell, mononuclear cell; PHA, phytohemagglutinin; PWM, pokeweed mitogen; SRBC, sheep erythrocytes.

and indicated depression of total T cells as shown by reactivity with the OKT3 antibody and by E rosettes. The $OKT4^+$ subset was particularly involved. Because the antihelminthic drug levamisole (LMS) has been described as having immunostimulatory activities (10, 11), its effect on these abnormal lymphocyte subsets was assessed in vitro. The results indicated that LMS produced a rapid and remarkable increase in the $OKT3^+$ and $OKT4^+$ lymphocyte subsets. To observe whether this response was specific to LMS, the effect of ascorbic acid, another antioxidant agent, was similarly assessed and it too produced restoration of cell membrane markers. In vivo use of high doses of ascorbic acid, however, did not reverse the abnormality of lymphocyte subsets in patients with measles infection. Because the finding of abnormal lymphocyte subsets does not always correlate with functional lymphocyte activity, the production of a T cell-derived helper factor (lymphocyte mitogenic factor [LMF]) by measles-infected cells was assessed before and after treatment with the antioxidant drugs.

METHODS

Patients. Studies werre carried out on 42 well-nourished African children ranging in age from 4 mo to 5 yr (the majority being 1.5 to 3 yr old). All children had uncomplicated measles, as assessed by clinical investigation, roentgenogram, and absence of pyrexia after the rash had reached its maximum intensity. Children were treated with only a mild analgesic (paracetemol) although some received 250 mg vitamin C/d (Propan Laboratories, Johannesburg, South Africa). Tests were performed in the acute phase of the disease, usually on the day of the appearance of the rash or 1 d thereafter. Normal adult laboratory workers served as daily controls. In previous studies from this laboratory (unpublished data), it has been shown that T cell subsets are similar in distribution in adults and children.

Preparation of peripheral blood (MN cells). MN cells from patients and controls were isolated from heparinized (preservative-free heparin, 10 U/ml) venous blood by Hypaque-Ficoll (Pharmacia Fine Chemicals, Uppsala, Sweden) density centrifugation. MN cells in RPMI 1640 (Gibco Laboratories, Grand Island, NY) plus 10% pooled human AB serum were exposed to 0.2 mg/ml carbonyl-iron powder (GAF Corp., New York) The cells and iron were gently rotated and incubated for 30 min at 37°C. The carbonyl-iron was then removed with a magnet and the cell suspensions transferred to new tissue culture tubes and washed twice in Hanks' balanced salt solution (HBSS, Wellcome Reagents, Beckenham, Kent, England). The residual remaining monocyte population ranged from 1 to 4% as assessed by yeast phagocytosis, the rest of the cells being lymphocytes.

Analysis of lymphocyte subpopulations by means of monoclonal antibodies. Production and characterization of monoclonal antibodies OKT3, OKT4, and OKT8 were subjects of prior reports (9, 12). Since these antibodies were of the IgG_2 subclass and fixed complement, they were used for complement-mediated lysis. Phagocyte-depleted MN cells (1.0×10^6) were treated with 5 μl of the different reconstituted monoclonal antibodies (Orthoclone, Ortho Pharmaceutical, Raritan, NJ) at room temperature for 45 min. Subsequently, cells were incubated with a 1:5 dilution of fresh rabbit complement for 1 h at 37°C. Background cytotoxicity was determined with MN cells incubated with rabbit complement alone, and spontaneous cell death was in the range of 0 to 2% of cells. Cell death after incubation with antibody and complement was determined by trypan blue staining and was expressed as a percentage of $OKT3^+$, $OKT4^+$, and $OKT8^+$ cells. In some experiments, lymphocyte subsets were assessed with an indirect immunofluorescence technique (12) and were enumerated under a Leitz fluorescence microscope (E. Leitz, Inc., Rockleigh, NJ).

Enumeration of various types of MN cells. Macrophages, B lymphocytes, T lymphocytes, and null cells were determined with the Quantigen T and B cell assay kit (Bio-Rad Laboratories, Richmond, CA). Briefly, MN cells from Hypaque-Ficoll gradients are incubated with Immunobeads at 37°C for 1 h. Yellow-brown immunobeads possessing polyclonal anti-human antibodies bind to and rosette B cells. Colorless immunobeads possessing a monoclonal anti-T antibody (T101), which correlate with E rosette-positive lymphocytes, bind to and rosette T lymphocytes. Monocytes were identified by their ability to ingest both types of beads and the remaining unlabeled cells represented the null cell population.

Enumeration of E rosette-forming cells. E rosette-forming cells were quantitated by spontaneous rosette formation with sheep erythrocytes (SRBC). Briefly, 1×10^6 lymphocytes were placed in round-bottomed tubes and were incubated with an equal volume of 1% SRBC in the presence of 10% fetal calf serum (Gibco Laboratories) at 37°C for 15 min, centrifuged at 100 *g* for 5 min, and placed on ice for a minimum of 1 h. After gentle resuspension of the pelleted cells, the percentage of lymphocytes binding three or more SRBC was enumerated. In some experiments, E rosette results were confirmed with the OKT11 antibody that is reactive with >95% of E rosette-positive cells.

In vitro effects of antioxidant agents on lymphocyte subsets. The effects of the following drugs were assessed: L-2,3,5,6-tetra-hydro-6-phenylimidazothiazole (LMS) (Ethnor laboratories, Johannesburg, South Africa) and L-ascorbic acid, (BDH Chemicals Ltd., Poole, England). These agents were dissolved in RPMI 1640 and added in varying concentrations to adherent cell-depleted MN cells at 37°C. At different time periods, cells were washed three times in HBSS and enumerated for cell surface receptors by the above-mentioned techniques. In some experiments, cells were treated for 16 h with 10 μg/ml cycloheximide (Sigma Chemical Co., St. Louis, MO), after which they were washed and then exposed to the above pharmacological agents.

Production and assay of LMF. MN cells were cultured in RPMI 1640 plus 10% heat-inactivated human AB serum supplemented with 200 mM L-glutamine (Merck AG, Darmstadt, West Germany), 100 U/ml penicillin, and 100 μg/ml streptomycin in the presence (active) and absence (control) of a 1:100 dilution of pokeweed mitogen (PWM, Gibco Laboratories) Cultures were incubated for 24 h at 37°C in 5% CO_2 in humidified air. Supernatants were then collected by centrifugation at 200 *g* for 10 min, after which control culture supernatants were supplemented with PWM, and stored at −20°C until tested for helper activity In some experiments, MN cells were treated for 2 h with different antioxidant agents, washed, and recultured with PWM for 24 h and supernatants harvested as described above.

The mitogenic activity of the culture supernatants were tested on purified tonsillar B lymphocytes as described previously (6). Briefly, 0.1 ml of control and active supernatants were added in triplicate to microtiter wells containing 1

$\times 10^5$ B cells obtained by differential rosetting and centrifugation procedures. The cultures were incubated at 37°C in 5% CO_2 in humidified air for 96 h, after which DNA synthesis was assayed by adding 0.2 μCi/well tritiated thymidine ([*methyl*-^{3}H]thymidine, sp act 24 Ci/mmol, Radiochemical Centre, Amersham, England) for the last 18 h of culture. Cells were harvested by a multiple harvester, filter disks were dried, Insta-fluor (Chemlab, Johannesburg, South Africa) was added (5 ml), and radioactivity measured in a Packard Tricarb liquid scintillation counter (Packard Instrument Co., Downers Grove, IL). Results were expressed as net counts per minute (counts in the presence of active supernatant minus control supernatant).

Because of the extreme variability in tonsillar B cell responses, batches of LMF-containing supernatants were accumulated and tested on one particular tonsillar preparation. This difference in response is reflected in Tables III and VII.

In vitro effects of measles virus on lymphocyte subsets. 2×10^6 MN cells/ml were cultured in RPMI 1640 with 10% fetal calf serum and 10 μg/ml PHA for 24 h in Falcon tissue-culture flasks (Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, CA). The cells were then washed and 0.5×10^6 viable cells were resuspended in 12×75-mm tissue-culture tubes to which was added 1.5×10^6 plaque-forming units (pfu) of live or inactivated (56°C for 1 h) measles virus (Edmonston strain, National Institute of Virology, Johannesburg, South Africa). The cells were cultured with the virus at 37°C for varying time intervals, but usually for 16 h, after which they were washed and lymphocyte subtypes assessed. In some experiments, MN cells were treated with L-ascorbic acid for 30 min before subtype estimation.

To assess whether measles virus preferentially infected any particular lymphocyte subpopulation, $OKT4^+$- and $OKT8^+$-enriched lymphocytes were preactivated with PHA and then exposed to live measles virus or inactivated measles virus. After 24–48 h, the cells were washed, exposed to hyperimmune human antimeasles immunoglobulin (National Blood-Fractionation Centre, Durban, South Africa), and washed three times and surface fluorescence detected by treatment with a sheep anti-human fluorescein-labeled immunoglobulin (Wellcome Reagents). To obtain enriched $OKT4^+$ and $OKT8^+$ populations, lymphocytes were treated with these monoclonal antibodies and complement and the remaining viable cells were recovered after thorough washing and repetition of this cycle.

Statistical evaluation was by Student's *t* test.

RESULTS

Characterisation of lymphocyte subsets. A significant decrease in those cells reacting with the OKT3 and OKT4 monoclonal antibodies was observed in every measles patient examined. The normal lymphocyte population is composed of 62.6±7.0% $OKT3^+$ cells, of which 40.5±6.2% react with the OKT4 antiserum and 22.3±5.1% react with the OKT8 antiserum. In contrast, however, in a group of 42 measles patients, the mean lymphocyte population is composed of 48.3±9.0% $OKT3^+$ cells, of which 22.0±6.3% were $OKT4^+$ and 23.2±7.9% were $OKT8^+$ cells.

In 10 patients of this group, lymphocyte subsets were reexamined 1 wk after the acute illness, when little change in the lymphocyte subset profile was noted (Table I [*a*]). 3 wk after the acute infection, however, considerable recovery in both the total T cell numbers and in those cells reacting with the OKT4 antiserum was observed, the ratio of $OKT4^+$ to $OKT8^+$ tending to normalize significantly ($P < 0.05$).

In view of the finding (see below) that ascorbate corrected the abnormal subset profile in vitro, seven patients were treated with 250 mg L-ascorbate/d, and lymphocyte subsets tested before initiation of therapy and 1 and 3 wk later. As is indicated in Table I (*b*), ascorbate treatment did not improve the recovery of the measles patients lymphocyte subsets and in fact, recovery of $OKT3^+$ and $OKT4^+$ subsets was less in the ascorbate-treated group than in untreated patients. Ascorbate treatment in vivo, therefore, did not hasten the normalization of the lymphocyte subset profile in patients with measles infection.

Enumeration of various MN cell populations. By the Quantigen immunobead method, T cells from the measles patients were noted to be depressed in number. Even more striking was the significant increase in the percentage of cells (null cells) that failed to

TABLE I

Characterization of T Lymphocyte Subsets in 10 Patients with Acute Measles Infection and during Convalescence

	Percentage of reactivity			
	$OKT3^+$	$OKT4^+$	$OKT8^+$	OKT4:OKT8
Normal controls	62.6±7.00	40.50±6.20	22.03±5.10	1.94±0.66
(*a*) Acute infection	45.5±7.7	20.64±6.02	24.10±8.90	0.91±0.42
1 wk later	46.2±7.10 (NS)	22.73±3.72 (NS)	22.82±6.70 (NS)	1.12±0.42 (NS)
3 wk later	61.3±4.16 ($P < 0.01$)	38.33±8.00 ($P < 0.01$)	19.00±4.00 ($P < 0.5$)	2.14±0.91 ($P < 0.05$)
(*b*) Acute infection	47.4±7.3	21.6±6 7	21.7±8.9	1.00±0.51
1 wk later	39.4±4.8 (NS)	22.7±5.9 (NS)	17.1±5.1 (NS)	1.46±0.57 (NS)
3 wk later	52.1±12.6 ($P < 0.5$)	30.4±9.3 ($P < 0.1$)	19.4±5.4 (NS)	1.66±0.72 ($P < 0.5$)

Some patients (*a*) were untreated, whereas others (*b*) received 250 mg L-ascorbic acid/d. Mean±SD.

TABLE II

Absolute Lymphocyte Counts and Percentage of Various Cell Types Observed in MN Preparations from Measles Patients and Controls

	Lymphocytes*	B cells	T cells	Null cells	Monocytes	E rosettes	OKT11$^+$	OKT3$^+$	OKT4$^+$	OKT8$^+$
Patient										
1	4,400	4	42	46	8	41	36	38	28	20
2	1,240	6	50	34	10	52	50	48	20	30
3	4,030	6	48	36	10	44	43	44	29	20
4	1,350	8	49	34	9	50	48	43	22	26
5	1,100	7	30	55	8	32	33	34	18	17
Control										
1	2,400	7	68	14	11	70	68	66	38	26
2	3,750	7	59	22	12	57	59	62	40	19
3	2,850	4	68	14	14	65	63	62	44	21
4	3,800	7	75	11	7	68	67	65	46	26
5	2,950	8	64	16	12	60	61	68	40	21

B cells, T cells, null cells, and monocytes were enumerated with Quantigen beads.
* Lymphocytes per cubic millimeter.

rosette with either the T or the B cell marker beads. These results together with absolute lymphocyte counts and percentage cell subsets are indicated in Table II.

Functional assay of helper activity. To relate the functional activity of measles MN cells with their lymphocyte subset profiles, LMF production after PWM stimulation was assessed. Supernatants from these cultures were assayed for helper activity by assessing blastogenesis of purified tonsillar B lymphocytes. As is shown in Table III, LMF production, which was initially depressed, improved after 1 wk and remained essentially the same at 3 wk. This finding did not correlate with the subtype profile, which remained abnormal at 1 wk, although showing some normalization at 3 wk. Because subset markers could be improved by *in vitro* treatment with ascorbate, children with measles were treated with 250 mg L-ascorbate/d, and LMF production assessed after 1 and 3 wk. As can be seen in Table III (*b*), LMF production was improved by 1 wk, but not to a greater extent than in untreated children.

TABLE III

Production of LMF by Measles MN Cells during Acute and Convalescent Phase of the Illness

Patient		Acute phase	1 wk (Percent change)	3 wk (Percent change)
(*a*)	1	6,111	8,881 (45)	7,796 (−14)
	2	5,235	13,070 (150)	13,770 (5)
	3	3,898	14,496 (272)	16,206 (12)
	4	2,330	16,297 (599)	18,101 (11)
	5	2,128	10,211 (380)	12,064 (18)
(*b*)	6	3,205	10,667 (233)	10,821 (1)
	7	5,314	16,591 (212)	11,799 (−41)
	8	4,393	6,555 (49)	13,567 (107)
	9	9,350	17,116 (83)	17,281 (1)
	10	2,962	7,766 (162)	ND (ND)

Results expressed as net counts per minute of [^{3}H]thymidine incorporation. Mean of 10 normals, 23,400±8,050. Patients 1–5 were untreated (*a*), whereas patients 6–10 received 250 mg L-ascorbic acid/d (*b*).

Effects of in vitro LMS and ascorbate treatment on lymphocyte subsets. When MN cells from patients with acute measles infection were treated in vitro for 15 min with 60 μg/ml of LMS (a dose previously shown to have an optimal effect), a significant increase in the number of OKT3$^+$, OKT4$^+$, and OKT8$^+$ cells was observed. In most cases the trend was toward normalization of the ratio between OKT4$^+$ and OKT8$^+$ cells (Table IV).

When cells from patients with acute measles were pulsed in vitro with 10^{-4} M L-ascorbic acid, considerable increase in the OKT3$^+$ and OKT4$^+$ cell populations was observed, with minimal improvement in the OKT8$^+$ population. Once again, a significant normalization of the ratio between OKT4$^+$ and OKT8$^+$ cells was found (Table V). Because the possibility existed that ascorbate might enhance the capacity of the measles-infected cells to antibody- and complement-mediated lysis, these experiments were repeated using indirect fluorescence microscopy to assess subtypes before and after ascorbate treatment. As can be seen in Table VI, virtually identical results were obtained with both cytotoxicity and fluorescence techniques. In five experiments, MN cells from patients with measles

TABLE IV
Lymphocyte Subsets After In Vitro LMS Treatment

	Percentage of reactivity							
	Before treatment				After treatment			
Patient	OKT3$^+$	OKT4$^+$	OKT8$^+$	OKT4:OKT8	OKT3$^+$	OKT4$^+$	OKT8$^+$	OKT4:OKT8
Normals	69.1±3.0	42.2±2.8	25.4±4.6	1.66±.24	67.2±3.1	40.8±3.2	24.7±2.2	1.65±.23
1	42	17	22	0.77	75	50	26	1.92
2	50	20	30	0.67	78	38	40	0.95
3	55	32	20	1.60	84	55	38	1.45
4	38	20	19	1.05	51	30	23	1.30
5	44	24	19	1.37	85	58	33	1.76
6	45	18	28	0.64	76	53	28	1.89
7	56	23	33	0.70	69	36	46	0.78
8	45	19	27	0.70	68	44	27	1.63
Mean±SD	46.9±6.3	21.9±5.0	24.8±5.4	0.94±.36	73.3±10.9	45.5±10.1	32.6±8.0	1.34±0.51
P value	—	—	—	—	<0.005	<0.005	<0.05	<0.1

were treated with 10 μg/ml cycloheximide before treatment with ascorbic acid. No effect on conversion of OKT3$^+$ and OKT4$^+$ cells by ascorbate could be shown by prior treatment with cycloheximide. Whereas the mean percentage of subsets before treatment were OKT3$^+$, 48.4; OKT4$^+$, 22.1; and OKT8$^+$, 25.3, the figures after ascorbate treatment and after cycloheximide followed by ascorbate (in brackets) were as follows: OKT3$^+$, 68.4 (67.9); OKT4$^+$, 46.5 (43.5); and OKT8$^+$, 27.2 (27.3).

Neither of these agents had any significant effect on normal lymphocyte subsets.

Effects of in vitro LMS and ascorbate treatment on E rosette formation and on the OKT11 antigen. MN cells from 10 patients were treated with LMS and a similar group with L-ascorbic acid for 60 min at 37°C, washed, and rosetted with SRBC, and the number of E rosettes compared with those obtained after treatment with medium alone. Neither LMS nor ascorbate influenced the low E rosette numbers observed in the measles patients. In the patient group, 41.5±7.3% of MN cells formed rosettes (controls, 63.4±4.2%) and after LMS and L-ascorbic acid treatment these figures remained at 41.8±8.4 (61.0±3.8) and 40.8±8.0 (62.2±2.8), respectively. To confirm that the defect in E rosette formation could not be corrected by the pharmacological agents, the percentage of MN cells marked with OKT11 monoclonal antibody was assessed before

TABLE V
Lymphocyte Subsets After In Vitro Treatment with L-Ascorbic Acid

	Percentage of reactivity							
	Before treatment				After treatment			
Patient	OKT3$^+$	OKT4$^+$	OKT8$^+$	OKT4:OKT8	OKT3$^+$	OKT4$^+$	OKT8$^+$	OKT4:OKT8
Normals	62.6±7.0	40.5±6.7	21.3±5.2	1.94±0.66	63.8±6.1	41.4±5.9	20.8±5.8	1.99±0.74
1	44	26	19	1.37	75	52	26	2.00
2	65	38	28	1.36	82	58	30	1.93
3	64	29	18	1.61	75	48	28	1.71
4	47	24	24	1.00	78	40	43	0.93
5	39	15	23	0.65	69	46	23	2.00
6	48	22	25	0.88	67	42	25	1.68
7	54	26	24	1.08	84	48	38	1.26
8	48	25	22	1.14	67	44	22	2.00
Mean±SD	49.2±10.4	25.0±6.3	21.8±4.5	1.14±0.31	73.0±7.8	47.3±5.4	27.9±8.3	1.69±0.40
P value	—	—	—	—	<0.005	<0.005	<0.1	<0.01

TABLE VI

Lymphocyte Subsets in Patients with Measles Before and After In Vitro Treatment with L-Ascorbic Acid°

Patient	Before treatment				After treatment			
	OKT3$^+$	OKT4$^+$	OKT8$^+$	OKT11$^+$	OKT3$^+$	OKT4$^+$	OKT8$^+$	OKT11$^+$
1	44 (44)	26 (25)	14 (16)	38 (39)	64 (73)	45 (43)	17 (18)	37 (40)
2	34 (38)	16 (14)	17 (19)	39 (42)	52 (50)	36 (30)	16 (19)	41 (38)
3	45 (48)	23 (23)	20 (22)	42 (41)	70 (64)	48 (43)	22 (21)	39 (40)
4	34 (38)	17 (18)	18 (18)	36 (39)	63 (68)	45 (52)	17 (19)	39 (38)
5	49 (47)	27 (26)	20 (20)	42 (39)	56 (55)	33 (27)	20 (18)	41 (41)

° Measured by complement-mediated cytotoxicity and indirect immunofluorescence (results in brackets).

and after ascorbate treatment. This antibody binds to the same T cell surface antigen as the E rosette receptor. As is indicated in Table VI, there was no significant increase in OKT11$^+$ cells after in vitro treatment with ascorbate.

In vitro effect of LMS and ascorbate on LMF production by measles MN cells. Because the in vitro treatment of measles MN cells with LMS and L-ascorbic acid reversed the abnormality of lymphocyte subsets, the effect of these agents on the depressed LMF production by measles MN cells was also assessed. Considerably reduced LMF was produced by MN cells from measles patients and this function was not significantly altered by in vitro treatment with LMS in the limited number of patients studied (Table VII). However, when measles MN cells were initially pulsed with L-ascorbic acid (10^{-4} M) for 2 h and then cultured for LMF production, a significant increase in the production of this lymphokine was observed (Wilcoxan matched pairs signed rank test). When normal MN cells were treated with either LMS or L-ascorbic acid, increased LMF production was not observed (results not shown).

TABLE VII

Effect of In Vitro LMS (a) or L-Ascorbic Acid (b) Treatment of Measles MN Cells on Their Production of LMF

	Patient	Untreated	Drug-treated	Percent change
(*a*)	1	1,200	1,486	(23.8)
	2	1,275	808	(−36.6)
	3	1,261	2,193	(73.9)
	4	139	106	(−23.7)
	5	3,141	4,590	(46.1)
	6	2,926	2,848	(2.7)
(*b*)	7	1,016	1,949	(91.8)
	8	170	2,545	(1,397.0)
	9	2,311	4,885	(111.4)
	10	1,481	3,883	(162.2)
	11	180	878	(387.8)
	12	917	4,329	(372.1)
	13	2,036	3,791	(86.2)

Net counts per minute percentage of increase in brackets. Mean of six normals, 14,468±6.218.

In vitro effects of measles virus on lymphocyte subsets. Normal PHA-activated MN cells incubated with live measles virus before testing with the OKT antisera showed a significant decrease in the OKT4$^+$ population ($P < 0.005$). Some decrease in both the total OKT3$^+$ subset and the OKT8$^+$ population was noted but these changes were not statistically significant (Fig. 1). A maximal effect of the measles virus on the OKT4$^+$ population could be detected after only 2 h of incubation with the virus, but cells treated with inactivated measles virus had no effect on the lymphocyte subsets. When MN cells that were infected with measles virus were then washed and pulsed with L-ascorbic acid for only 30 min (10^{-4} M), significant recovery of the OKT4 marker was detected ($P < 0.005$). Moderate increases in both the OKT3$^+$ and OKT8$^+$ subsets were also noted but these results were not statistically significant.

When enriched OKT4$^+$ and OKT8$^+$ populations were individually activated by PHA and then treated with live or inactivated measles virus, viral antigen as detected by membrane fluorescence was observed on both the OKT4- and OKT8-enriched cell populations that had been treated with live virus (Table VIII).

DISCUSSION

In all patients examined in this study, we found the percentage of T cells to be depressed, using monoclonal OKT3 antibody and Quantigen beads, as well as enumerating E rosette-forming cells. In many, but not all patients, an absolute lymphopenia was present. The finding that patients with measles have depressed numbers of circulating T cells, has been shown by others (13, 14) and it has been suggested that the virus may inactivate a population of helper T cells. Mc-

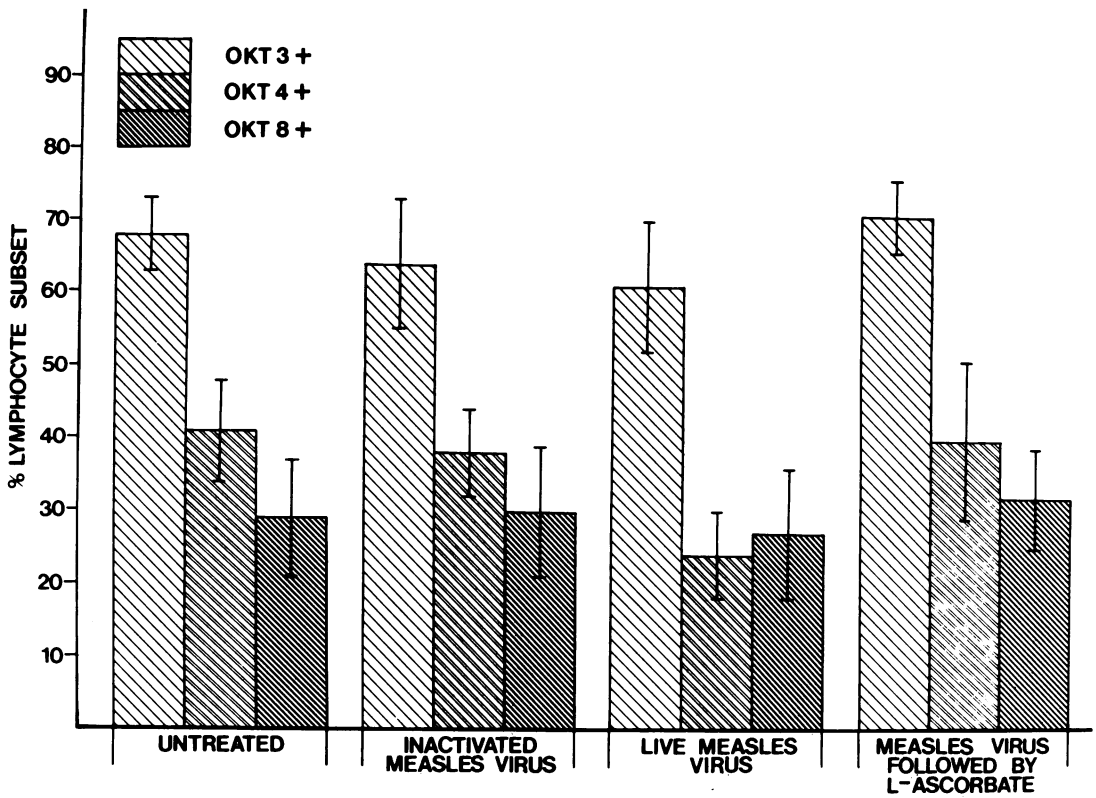

FIGURE 1 The effect of adding inactivated measles virus, measles virus alone, or measles virus followed by L-ascorbate on lymphocyte subsets. All lymphocytes were pretreated with PHA for 48 h before addition of measles virus.

Farland (7) showed that a defect of helper T cells existed in measles-infected mice, and in other studies the virus has been shown to suppress antibody synthesis when added during the first 48 h of lymphocyte culture (15). Furthermore, MN cells from a group of patients with acute measles infection were shown to develop less intracytoplasmic immunoglobulin after stimulation with PWM than controls, owing primarily to a defect in helper factor (LMF) production (6). Although it has been proposed that binding of measles virus to T cells may alter the circulating lymphocyte subpopulations (16), the results described in this study indicate that a subpopulation of helper/inducer cells ($OKT4^+$ cells) are quantitatively deficient in the peripheral blood. Although this defect remained for at least 1 wk, lymphocyte recovery was observed 3 wk after the acute infection. These results are similar to the recovery of E rosette-forming cells in children re-

TABLE VIII
Expression of Measles Antigen on OKT4- and OKT8-enriched PHA-activated Lymphocytes Treated with Live or Inactivated Measles Virus

	Percentage of positive staining cells					
	OKT4-enriched			OKT8-enriched		
Experiment	No virus	Inactivated	Live	No virus	Inactivated	Live
1	2	4	44	4	3	40
2	7	6	45	11	5	45
3	13	6	46	9	8	55
4	1	5	45	3	4	44
5	2	5	31	3	6	53

ceiving measles vaccine (13). Identical aberrations in lymphocyte subsets could be produced in vitro by infecting normal MN cells with live measles virus. This effect was extremely rapid, being detected after only 2 h incubation with the virus, a result that suggests that the effect is not due to some aberration of protein synthesis, but rather to a blocking or steric change produced by the virus on the receptor. Although the in vitro experiments use PHA-activated MN cells, we believe that these results are comparable to the situation in vivo, where a considerable number of patients' lymphocytes are in an activated form, as assessed by their ability to incorporate tritiated thymidine spontaneously (17). Furthermore, we have shown (results unpublished) that 31±9.0% of circulating lymphocytes from patients with measles express measles antigen as detected by indirect immunofluorescence, whereas in normal controls, only 5.0±2.0% of cells are reactive with a hyperimmune antimeasles immunoglobulin.

Although other workers have described aberrations of lymphocyte subsets during other acute viral infections, the finding of low $OKT4^+$ cells and normal $OKT8^+$ cells is probably unique. In infectious mononucleosis, an increased percentage of $OKT5^+$ T cells has been described (18) and a similar observation of increased numbers of $OKT8^+$ T-cells has been shown in acute viral hepatitis (19). McFarland (7) suggests that the defect of helper T cells may be related to a selective attachment of the virus to these cells, but without cell lysis. These findings are substantiated by the results of this report, which indicate that the depression in T cell numbers could be reversed by treatment with two antioxidant drugs, namely, LMS and L-ascorbic acid. Furthermore, MN cells infected in vitro by measles virus developed abnormal lymphocyte subsets that could be corrected after 30 min of L-ascorbic acid treatment. Although the number of $OKT3^+$ cells was also increased by treatment with these drugs, E rosette numbers, which are also depressed in patients with measles, did not increase. This finding was confirmed by showing that the number of cells marking with the OKT11 monoclonal antibody (positive with >95% of E rosette-positive cells) was not increased after ascorbate treatment. Presumably, the OKT3 antibody reacts with a different antigen from that responsible for E rosette formation. It is likely, therefore, that the mechanism whereby measles virus depresses E rosette formation differs from its mode of action on the OKT4 receptor. In all experiments using the monoclonal antibodies, use has been made of complement-mediated cytotoxicity to enumerate subsets. To exclude the possibility that this method might produce artefacts, a number of experiments were performed using the conventional fluorescence technique, and the same pattern of defect was observed, viz. depressed $OKT3^+$ and $OKT4^+$ cells with restoration to normal values after ascorbic acid or LMS treatment.

The studies reported here also show that measles virus will infect both $OKT4^+$ and $OKT8^+$ PHA-activated lymphocytes as assessed by surface membrane fluorescence, and it is unlikely that the predominant effect of the virus on $OKT4^+$ cells is due to preferential viral replication in this population. The finding that the antigenic determinants detected by the OKT4 antiserum were diminished when exposed to measles virus suggests that the virus induces an alteration (chemical or steric) in the antigenic structure of these determinants. Furthermore, this was borne out by the finding that null cells are increased in number in patients with measles infection. It is unlikely that the quantitative depression of $OKT4^+$ cells is due to an inhibition of host cell protein synthesis, as the effect can be rapidly reversed by ascorbic acid or LMS, even when the cells had been pretreated with cycloheximide.

Although the mode of action of LMS and ascorbate in correcting the abnormal lymphocyte subsets is not known, a number of possibilities exist. The finding that the effect could be observed after only a short pulse (5–15 min) suggests that the changes are due to some conformational alteration on the cell surface rather than a change in cellular function. It is likely that both these agents are producing this effect by virtue of their antioxidant properties. It is possible that viral infection of lymphocytes may lead to masking of antigenic determinants and that the two agents may cause unmasking and dissolution of the cell membrane involving new molecular configurations, thus restoring its original antigenic arrangement. If the nonexpression of the antigen is determined by proteins bound by disulfide bonds, adding ascorbate or LMS may result in release of a sulfhydryl group that could be necessary for the expression of the antigen. Preliminary experiments (not reported), indicate that another antioxidant agent, dithiothreitol, also has the ability of restoring the OKT4 marker in measles-infected cells. Ascorbic acid and LMS may restore the OKT markers by affecting the cyclic nucleotide content of cells. Both ascorbic acid (20) and LMS (21) have been shown to increase intracellular cyclic guanosine monophosphate levels, but it would be speculative to suggest that this effect restores antigenic determinants on infected cells, especially after so short a period of exposure to the drugs. A further possible mechanism of action of ascorbate and LMS is a direct antiviral effect. However, although ascorbate has been shown to inactivate a variety of enteroviruses (22), there is little evidence that it or LMS has a direct effect on paramyxoviruses.

The finding that measles-infected lymphocytes, treated with ascorbic acid recovered their OKT4 an-

tigens, suggested that the drug may have the same effect when given to patients during the acute phase of their illness. Untreated children retained abnormal lymphocyte markers 1 wk after the acute infection, but by 3 wk, some recovery was evident. Similar findings were evident in the treated group and ascorbate did not accelerate the recovery of the subset markers. In fact, recovery at 3 wk was less in the ascorbate-treated group than in the nontreated group. It is not known, however, whether the dose administered was adequate to reverse the abnormal subset markers in vivo.

Recently, considerable evidence has appeared to indicate that defects in lymphocyte subsets do not necessarily correlate with defective in vitro functional assays of help or suppression (23, 24). In this study, although lymphocyte subset markers had not recovered 1 wk after the acute episode, the production of LMF, which was initially depressed, improved considerably after 1 wk. This might suggest that the effect of virus on surface protein antigens and on helper cell function occurs by different mechanisms. When MN cells from measles patients in the acute stage of their illness were pulsed with LMS before culture for LMF production, no improvement in LMF production could be detected. The number of samples tested, however, was limited. It is noteworthy that treatment of measles MN cells with LMS resulted in a significant increase not only in the $OKT4^+$ population, but also in the $OKT8^+$ population. This could explain the lack of effect of LMS on the ability of cells to produce LMF. It also suggests that this agent has a highly nonspecific action on cell membranes. When measles MN cells were pulsed with ascorbate, significant improvement in LMF production was observed, and it has previously been shown that lymphocyte function that was depressed by in vitro exposure to influenza virus could be counteracted by ascorbic acid treatment (25).

In the present study, the complexity of measles virus infection on human lymphocytes was demonstrated. Not only does the virus inhibit the production of LMF and other lymphokines (17), but in addition it causes an aberration in lymphocyte subset profile, resulting in low $OKT3^+$, $OKT4^+$, and $OKT11^+$ cell populations. The exact mechanism of ascorbic acid activity in reversing both the defective LMF production and the abnormal subset profile is not clear, but these findings reiterate the complexity of viral interaction with cells of the immune system. We are unable to explain the disparity between LMS and ascorbate effects on LMF production, as both these compounds had a similar effect on lymphocyte subset markers and neither influenced the number of E rosette-forming cells. It is possible, therefore, that although these two agents have some effects in common, they may also be acting by different mechanisms, especially concerning helper factor production. As regards possible benefit of these drugs, it appears as though children treated with ascorbate from their time of admission to hospital demonstrated no obvious clinical benefit from this therapy.

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