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T-Lymphocyte Subpopulations in Relation to Immunosuppression in Measles and Varicella

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Patients with measles or varicella were studied during the acute phase (first week) of illness and, after recovery, by lymphocyte stimulation tests and determination of T-lymphocyte subpopulations, using the monoclonal antibodies Leu 2a and Leu 3a directed at the suppressor/cytotoxic and the helper T-cell subsets, respectively. Low proliferative responses to phytohemagglutinin were found during the acute phase of both diseases. The response to purified protein derivate of tuberculin was also low in all measles patients tested and in some of the varicella patients. In both infections increased spontaneous DNA synthesis was demonstrated. In the acute phase of measles there was T lymphocytopenia but no change of the ratio between T lymphocytes of helper and suppressor/cytotoxic cell phenotypes. In the acute phase of varicella the percentage and the absolute number of Leu 2-positive (suppressor/cytotoxic) T cells were increased. Measurement of the size of the lymphocytes indicated activation of this subset. Cryopreserved blood mononuclear cells from the acute phase of varicella could suppress the phytohemagglutinin response of autologous convalescent-phase cells. This was not seen when cells from measles patients were tested. The suppression of the lymphocyte stimulation response in varicella is probably in part caused by activation of suppressor cells, whereas the suppression of the stimulation response in measles seems to be due mainly to other mechanisms.

Measles is the classical example of an infection causing impairment of delayed skin reactivity (19, 33) and suppression of the lymphocyte stimulation response to mitogens and antigens in vitro (12, 34). Many other viral infections have also been shown to be associated with transient suppression of cell-mediated immune reactions in vivo and in vitro (2, 8, 19, 23, 27). One of several possible explanations for the immunosuppression is that the infection causes activation of suppressor cells.

Human T-lymphocyte subpopulations can now be determined by use of monoclonal antibodies directed at suppressor/cytotoxic or helper T-cell subsets (for review, see 24). It has recently been shown that immunosuppression in infectious mononucleosis is associated with a pronounced increase of T lymphocytes of the suppressor/cytotoxic cell phenotype (OKT 5^+ or OKT 8^+ cells) (10, 11, 23) and with activation of suppressor cells (23). Increased proportions of OKT 5^+ or OKT 8^+ lymphocytes have also been demonstrated in cytomegalovirus (8) and hepatitis B virus infections (29), suggesting that such a change in T-lymphocyte subpopulations might be a general phenomenon in viral infections (3).

We have previously shown that vaccination of healthy subjects with live rubella virus vaccine leads to suppression of the lymphocyte stimulation response to phytohemagglutinin (PHA) and purified protein derivative of tuberculin (PPD) and of the pokeweed mitogen-induced immunoglobulin production in vitro without causing any change in T-lymphocyte subpopulations defined by monoclonal antibodies (1a). Thus, virus-induced immunosuppression is not always associated with a relative increase of lymphocytes of the suppressor/cytotoxic cell phenotype.

Since the effect on T-lymphocyte subpopulations might differ between natural viral infections and vaccine-induced viral infections, we wanted to study T-lymphocyte subpopulations in a natural viral infection similar to rubella. We chose to study measles and, for comparison, included cases of varicella, which is caused by a herpesvirus like Epstein-Barr virus and cytomegalovirus infections.

We here report that, although both measles and varicella infections are associated with suppression of the lymphocyte stimulation response

to PHA and PPD, only varicella causes an increase in the proportion of T lymphocytes of the suppressor/cytotoxic cell phenotype.

MATERIALS AND METHODS

Patients. Heparinized blood was obtained from eight patients, 12 to 29 years old, in the acute phase of measles (1 to 5 days after the debut of the rash) and from six patients, 23 to 36 years old, in the acute phase of varicella (3 to 6 days after the debut of the rash). Seven of the patients with measles and all of the patients with varicella were also examined after recovery ("convalescent" phase: more than 4 weeks after the debut of symptoms). Some of the patients were also examined 1 and 2 weeks after the first bleeding. All patients had typical symptoms of measles or varicella without complications. The measles patients had not received measles virus vaccine in the past. The diagnoses were verified in all cases by the demonstration of significant rises of specific immunoglobulin G antibodies to measles or varicella antigens from the acute to the convalescent phase. All patients also had specific immunoglobulin M antibodies in the acute phase. Antibodies were measured by an enzyme-linked immunosorbent assay (4). Fourteen healthy persons, 19 to 36 years old, from the hospital staff served as controls. They were studied on the same occasions as the patients, each patient having his/her own control. One control was lost to follow-up.

Leukocyte (WBC) counts and differential counts were done in connection with the drawing of blood for lymphocyte studies.

Methods. Most of the methods have been described in detail before (1; Arneborn et al., in press) and are given only briefly below.

Cell preparation. Mononuclear cell suspensions were prepared by centrifugation of the blood on Ficoll-Isopaque (Ficoll-Paque; Pharmacia, Uppsala, Sweden) (5). The resulting suspension contained 5 to 10% polymorphonuclear cells and 3 to 30% monocytes (median value 13%). The monocytes were identified with a "nonspecific esterase stain" (31). There was no significant difference in the proportions of monocytes between acute- and convalescent-phase cell suspensions. A portion of the cell suspension obtained by Ficoll-Isopaque centrifugation was used for cell cultures and another portion was cryopreserved (see below). The remaining cells were treated with carbonyl iron, and phagocytic cells were removed with a magnet. We have previously found that the resulting suspension contains $\leq$0.5% polymorphonuclear cells and $\leq$0.5% monocytes. The phagocyte-depleted cell suspension was used for cell cultures and for lymphocyte subset studies.

Cryopreservation of cells. Cells were suspended in RPMI 1640 medium (GIBCO, Biocult, Paisley, Scotland) supplemented with 10% fetal calf serum (GIBCO, Biocult) and 10% dimethyl sulfoxide and stored at −196°C in liquid nitrogen. After thawing and washing, the viability was 70 to 90% as judged by trypan blue exclusion (6). The viability was similar for cryopreserved cells from both patients and controls.

Culture conditions. Cultures were set up in triplicate in tubes containing 5×10^5 mononuclear cells in 1 ml of RPMI 1640 supplemented with L-glutamine, penicillin, streptomycin, and 10% AB serum pool or in microplates (3040 Microtest II; Falcon Plastics, Oxnard, Calif.) with 2×10^5 mononuclear cells per well in 0.2 ml of the same medium. The cultures were incubated at 37°C in humidified air with 5% CO_2.

Antigens and mitogens. Cultures were stimulated with (i) PHA (Wellcome Research Laboratories, Beckenham, England) at final concentrations of 0.25, 2.5 and 25 μg/ml in microplates (cultures incubated for 72 h) and (ii) PPD (Statens Serum Institute, Copenhagen, Denmark) at final concentrations of 1 and 10 μg/ml in tubes (cultures incubated for 5 days). Most people in Sweden show a lymphocyte stimulation response to PPD since it was common practice until 1975 to vaccinate newborns with bacillus Calmette-Guérin.

Assay for DNA synthesis. Twenty-four hours before termination of culture, 0.1 μCi of [^{14}C]thymidine (Radiochemical Centre, Amersham, England) was added to each tube and 0.05 μCi was added to each microplate well. The cultures were frozen, and after thawing the cells were harvested on membrane filters (Millipore Corp., Bedford, Mass.). Radioactivity was measured in a liquid scintillation counter and expressed as counts per minute. The mean of triplicates was calculated.

To study spontaneous DNA synthesis, [^{14}C]thymidine was added on the day of cell preparation to cultures containing 5×10^5 mononuclear cells, and the cultures were incubated for 24 h.

Test for active suppression. To test whether cells from the acute phase of measles and varicella could mediate active suppression, cryopreserved blood mononuclear cells from the acute and convalescent phases were used. Each patient was tested in parallel with his/her own control. The cells of the control had been cryopreserved at the same time as those of the patient. The proliferative response to PHA and PPD of acute- and convalescent-phase cells was compared with the response of a mixture (1:1) of cells from the two occasions. (The total number of cells per culture was kept constant.) The percentage of active suppression was calculated according to the formula: percent active suppression = [(cpm expected − cpm observed)/cpm expected] × 100, where "cpm expected" equals (cpm of acute cells + cpm of convalescent cells)/2 and "cpm observed" is the response of the mixed culture.

In 10 experiments with cryopreserved blood mononuclear cells from five healthy controls, the response to PHA (2.5 μg/ml), expressed as counts per minute, was on the average 52% lower in cultures with 10^5 cells than in cultures with 2×10^5 cells. Mean counts per minute ± standard error were 6.1 ± 1.1 in cultures with 10^5 cells and 12.6 ± 2.1 in those with 2×10^5 cells.

Monoclonal antibodies. Monoclonal antibodies, termed Leu 2a, Leu 3a, and Leu 4, were obtained as a gift from L. E. Evans, Memorial Sloan-Kettering Cancer Center, New York, N.Y. These antibodies can be purchased from Becton, Dickinson & Co. (Sunnyvale, Calif.) Leu 2a is directed at the suppressor/cytotoxic T-cell subset (equivalent to OKT 5^+, 8^+ cells); Leu 3a, at the inducer/helper T-cell subset (equivalent to OKT 4^+ cells); and Leu 4, at total T cells (for review, see 24).

Immunofluorescence test. Lymphocyte subpopulations were studied in cell suspensions by an indirect immunofluorescence technique as described in detail

TABLE 1. Lymphocyte subpopulations in percentage of total lymphocytes in the acute and convalescent phases of measles (eight cases) and varicella (six cases) and in 12 healthy controls (numbers 1, 4 to 14)[a]

Patient	Acute phase (% positive cells)				Convalescent phase (% positive cells)			
	Leu 2	Leu 3	Leu 4	Ratio, Leu 3/Leu 2	Leu 2	Leu 3	Leu 4	Ratio, Leu 3/Leu 2
Measles								
1	15	28	44	1.9	19	50	69	2.6
2	14	49	69	3.5	21	67	86	3.2
3	21	42	58	2.0				
4	25	42	64	1.7	22	39	74	1.8
5	34	40	68	1.2	32	34	75	1.1
6	23	32	51	1.4	34	46	77	1.4
7	28	41	61	1.5	28	38	70	1.4
8	17	45	63	2.6	20	44	73	2.2
Median[b]	23	41	63	1.7	22	44	74	1.8
Varicella								
9	37	36	77	1.0	28	43	79	1.5
10	33	41	66	1.2	24	48	67	2.0
11	39	36	85	0.9	34	49	78	1.4
12	58	28	81	0.5	26	40	74	1.5
13	33	26	59	0.8	33	37	78	1.1
14	42	26	64	0.6	30	46	65	1.5
Median	38	32	72	0.9	29	45	76	1.5
Controls								
Median	25	45	79	2.1	25	47	74	2.1
Range	16–38	38–59	66–83	1.0–3.1	15–36	30–60	59–82	0.8–3.3

[a] Phagocyte-depleted lymphocytes were tested for reactivity with monoclonal antibodies Leu 2a, Leu 3a, and Leu 4 by indirect immunofluorescence. Leu 2a identifies the suppressor/cytotoxic and Leu 3a identifies the helper subset of T lymphocytes. Leu 4 identifies the total T cells.

[b] Patient 3 not included.

previously (Arneborn et al., in press), using phagocyte-depleted lymphocytes, monoclonal antibodies Leu 2, Leu 3, Leu 4, or normal mouse serum, and a fluorescein-isothiocyanate-conjugated sheep anti-mouse immunoglobulin (SBL 111610) absorbed with polymerized human serum. This conjugate was shown not to react with human B lymphocytes. At least 200 cells from each test tube were examined and counted in a Leitz Dialux fluorescence microscope.

Immunoperoxidase staining (28) of smeared cells. Peripheral blood mononuclear cells were suspended in 0.05 ml of 0.034 M sodium citrate, smeared, dried, and fixed in dry acetone at −20°C. Smears were incubated first with monoclonal antibodies Leu 2 or Leu 3 for 30 min at room temperature and, after washings, with a peroxidase-conjugated anti-mouse immunoglobulin (DAKO Immunoglobulins, Copenhagen, Denmark) absorbed with polymerized human serum. As enzyme substrate, 0.02% 3-amino-9-ethyl carbazole in 5% *N,N*-dimethylformamide in 0.14% acetate buffer (pH 5.2) and 0.03% H_2O_2 was used (30). The slides were studied in a phase-contrast microscope, and the diameters of the stained cells were measured. At least 100 cells in each subpopulation were measured.

Statistics. The difference between the acute-phase and the convalescent-phase values were calculated and analyzed by the Wilcoxon rank sum test. In the DNA synthesis tests the logarithms of the results were used for statistical analysis.

RESULTS

WBC and differential counts. In the acute phase of measles there was a significant decrease of the WBC ($P < 0.01$) and of the absolute number of lymphocytes ($P < 0.02$). (Median values $\times 10^9$ per liter of blood were as follows: WBC—acute phase, 4.1; convalescent phase, 5.7; lymphocytes—acute phase, 1.1; convalescent phase, 2.6.) During varicella infection the WBC was not significantly changed but the proportion of lymphocytes was increased ($P < 0.02$; median values, 52% for acute phase and 37% for convalescent phase), as was the absolute level of lymphocytes in most cases (not significant). In both diseases increased proportions of atypical lymphocytes were found ($P < 0.01$ for both diseases), with the following median values: measles—acute phase, 6%; convalescent phase, 0.5%; varicella—acute phase, 12%; convalescent phase, <0.5%.

Lymphocyte subpopulations. In patients with measles (Tables 1 and 2) the relative and absolute levels of T lymphocytes identified by monoclonal antibody Leu 4 were significantly decreased ($P < 0.01$ and < 0.05, respectively).

TABLE 2. Absolute numbers of lymphocyte subpopulations in acute and convalescent phases of measles (6 cases; patients 1, 4 to 8) and varicella (six cases) and in 12 healthy controls (patients 1, 4 to 14)[a]

Disease phase	Absolute no. × 10^9 per liter of blood			
	Leu 2	Leu 3	Leu 4	Non-Leu 4
Measles				
Acute phase	0.3 (0.1–0.6)	0.5 (0.2–1.0)	0.7 (0.3–1.5)	0.4 (0.3–0.8)
Convalescence	0.6 (0.5–0.9)	1.1 (0.6–1.3)	1.8 (1.3–2.2)	0.7 (0.4–1.0)
Varicella				
Acute phase	1.1 (0.6–2.4)	0.9 (0.6–1.5)	2.1 (1.2–3.6)	0.8 (0.4–2.1)
Convalescence	0.7 (0.3–1.1)	0.9 (0.6–1.6)	1.7 (0.9–2.5)	0.6 (0.4–1.1)
Controls				
1st bleeding	0.6 (0.2–1.0)	1.1 (0.4–1.8)	1.9 (0.9–2.4)	0.5 (0.3–0.7)
2nd bleeding	0.7 (0.3–0.9)	1.1 (0.5–1.9)	1.6 (1.2–2.6)	0.6 (0.3–1.3)

[a] Lymphocyte subpopulations were identified by the use of monoclonal antibodies Leu 2a directed at suppressor/cytotoxic T cells, Leu 3a directed at helper T cells, and Leu 4 directed at total T cells. Non-Leu 4, Lymphocytes not reacting with Leu 4. Values are median values (range given in parentheses).

Non-T lymphocytes were not significantly reduced. The percentages and absolute levels of Leu 2^+ and Leu 3^+ lymphocytes showed a tendency to decrease in parallel with the T lymphocytes. As a result, the ratio between Leu 3^+ and Leu 2^+ cells was about the same in the acute and convalescent phases of measles and approximately the same as in the healthy controls (Table 1).

In the two measles patients also studied 1 and 2 weeks after the first test, there was a tendency towards a normalization of the T-lymphocyte levels on these occasions, but no major changes of the ratio of Leu 3^+ to Leu 2^+ cells were found (data not shown).

In varicella (Tables 1 and 2) the percentage of T lymphocytes was mostly unchanged, but the absolute level was often increased (not significant) as a result of the lymphocytosis. The absolute level of Leu 4-negative (= non-T) lymphocytes was also increased ($P < 0.05$). There was a significant increase of the percentage of Leu 2^+ lymphocytes ($P < 0.02$) and a significant decrease of the percentage of Leu 3^+ cells ($P < 0.01$). As a result, the Leu 3^+/Leu 2^+ ratio was decreased ($P < 0.02$). The absolute level of Leu 2^+ cells was also increased ($P < 0.01$), but the absolute number of Leu 3^+ cells was approximately the same in the acute and convalescent phases of varicella.

In the immunofluorescence studies increased numbers of large lymphocytes were observed during the acute phase of both measles and varicella. To find out which subpopulation these cells belonged to, the sizes of the individual cells in the two T-lymphocyte subsets were measured on smeared cells stained by indirect immunoperoxidase (Fig. 1). In three measles patients studied, a slight increase of the proportions of medium-sized and large lymphocytes in both the Leu 2^+ and the Leu 3^+ subsets was found. In varicella patients, on the other hand, there was a marked increase of medium-sized and large Leu 2^+ cells, whereas in the Leu 3^+ subset only a slightly increased proportion of medium-sized lymphocytes was found. Large non-T cells were also seen in both infections, but the size of B cells was not measured due to lack of a suitable B-cell marker in the immunoperoxidase test.

Spontaneous DNA synthesis. The [^{14}C]thymidine uptake during the first 24 h of lymphocyte culture was greatly increased in the acute phase of both measles and varicella. The median value for measles (seven cases) was 5,600 cpm (range, 2,000 to 11,800 cpm) and that for varicella (five cases) was 2,900 cpm (range, 1,000 to 9,400 cpm). The patients in convalescent phase and the controls taken together (23 cases) had a median value of 400 cpm (range, 100 to 1,100 cpm). The difference between the acute and convalescent phases is highly significant in both diseases ($P < 0.01$).

In unstimulated cultures where [^{14}C]thymidine was not added until after 48 or 96 h, the uptake was always low and approximately the same in the acute and convalescent phases and in the controls. The median values in the acute phase of measles and varicella were 200 cpm

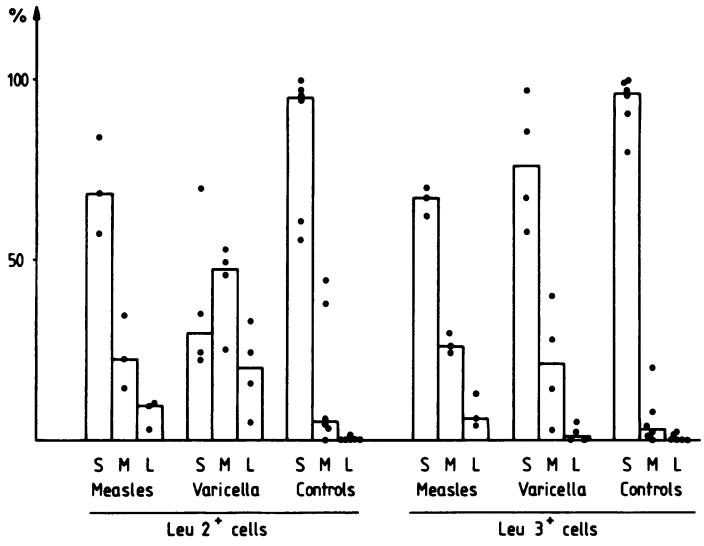

FIG. 1. Percentage of small, medium, and large cells within the Leu 2^+ and the Leu 3^+ T-lymphocyte subsets in the acute phase of measles and varicella and in healthy controls. S, Small (cell diameter, <7.5 μm); M, medium (cell diameter, 7.6 to 9.0 μm); L, large (cell diameter, >9.0 μm). Bars show means.

(range, 100 to 400 cpm) in microplates (12 cases) and 300 cpm (range, 200 to 700 cpm) in tubes (8 cases). The patients in convalescent phase and the controls taken together had a median value of 100 cpm (range, 100 to 1,600 cpm) in microplates (33 tests) and 400 cpm (range, 200 to 1,700 cpm) in tubes (27 tests).

PHA stimulation of DNA synthesis. In the acute phase of measles and varicella there were significant decreases in the proliferative responses of blood mononuclear cells to PHA at 0.25 μg/ml ($P < 0.01$ in measles; $P < 0.02$ in varicella) and 2.5 μg/ml ($P < 0.01$ in measles and varicella) but not to PHA at 25 μg/ml (which generally gave very low responses). The results obtained with 2.5 μg of PHA per ml are shown in Table 3.

Phagocyte-depleted lymphocytes also showed a decreased response to PHA in most patients in the acute phase of measles as well as of varicella (measles: PHA at 0.25 μg/ml, $P < 0.05$, and at 2.5 μg/ml, not significant; varicella: PHA at 0.25 μg/ml, $P < 0.025$, and at 2.5 μg/ml, $P < 0.05$; single-sided tests).

PPD stimulation of DNA synthesis. The proliferative response of freshly prepared mononuclear cells to PPD at 1 and 10 μg/ml was significantly decreased in the acute phase of measles ($P < 0.05$) (Table 3). Also, when cryopreserved cells were tested the PPD response of acute-phase cells was found to be decreased ($P < 0.02$) (Table 4).

In the varicella patients as a group no significant decrease of the PPD response was seen when freshly prepared mononuclear cells were used (Table 3). However, when cryopreserved cells were tested acute-phase cells showed a significantly lower PPD response than did convalescent-phase cells ($P < 0.05$) (Table 4). Cryopreserved cells generally had lower responses to PPD than had freshly prepared cells, but this can hardly explain the discrepant results. The discrepancy seems to be due to the results in two patients, numbers 11 and 12, who both had decreased responses in the acute phase when cryopreserved cells were used but not when freshly prepared cells were used. However, the corresponding two healthy controls had convalescent-phase responses of freshly prepared cells that were approximately 50% lower than the acute-phase responses. (The results in these two subjects are the main reason for the decreased median values in the convalescent phase of the controls in Table 3.) This makes it probable that the convalescent responses of the fresh cells of patients 11 and 12 were too low for some technical reason and that varicella actually caused suppression of the PPD response as observed in the tests with cryopreserved cells. However, it seems that varicella, compared with measles, caused greater suppression of the PHA response but less suppression of the PPD response (Table 3).

Test for active suppression. It seems that cryopreserved acute-phase cells from four of the six varicella patients suppressed the PHA response of cryopreserved autologous convalescent-phase cells (Table 4). These four patients also had the lowest PHA responses during the acute phase. Taken as a group, the varicella patients

TABLE 3. Lymphocyte stimulation response to PHA at 2.5 μg per ml of blood mononuclear cells (PBL) and of phagocyte-depleted lymphocytes (PDL) and to PPD at 10 μg per ml of PBL in the acute and convalescent phases of measles and varicella and in healthy controls studied in parallel[a]

Patient	[^{14}C]thymidine uptake (cpm × 10^3)					
	Acute phase			Convalescent phase		
	PHA		PPD (PBL)	PHA		PPD (PBL)
	PBL	PDL		PBL	PDL	
Measles						
1	15.1	12.1	7.1	23.7	29.1	11.7
2	22.3	20.0	ND[b]	28.9	30.1	ND
4	4.3	3.7	1.7	17.3	21.5	10.3
5	15.9	21.4	1.3	30.7	13.5	10.3
6	9.9	ND	ND	18.5	ND	ND
Median	15.1	16.1	1.7	23.7	25.3	10.3
Varicella						
9	2.3	1.8	ND	21.4	21.1	ND
10	7.5	13.7	18.0	9.4	15.0	19.4
11	0.7	0.2	2.2	11.5	10.2	2.4
12	1.9	ND	14.9	14.6	ND	10.9
13	9.7	13.9	14.6	15.6	9.7	8.9
14	7.7	7.0	6.0	14.2	24.4	26.4
Median	4.9	7.0	14.6	14.4	15.0	10.9
Controls						
Median	18.8	22.1	16.1	19.3	26.7	12.8
Range	10.7–26.8	12.5–34.6	4.3–35.3	8.4–26.6	5.7–35.7	2.2–30.3

[a] PBL from 10 controls and PDL from 8 controls were tested for PHA response, and PBL from 8 controls were tested for PPD response. A control to measles patient 2 is lacking.
[b] ND, Not done.

had a significantly higher percentage of "active suppression than the controls at 2.5 μg of PHA per ml ($P < 0.05$, single-sided rank sum test) but not at 0.25 μg of PHA per ml. In the PPD stimulation test three of the five varicella patients tested showed signs of active suppression. These patients also had the lowest PPD responses during the acute phase. However, the percentage of active suppression of the PPD response in the varicella patients taken together was not significantly different from that of the controls.

In the five cases of measles tested there was no indication of active suppression of the PHA or PPD responses (Table 4). This was not due to higher acute-phase responses in measles than in varicella since two measles patients had PHA responses as low and three measles patients had PPD responses as low as those varicella patients who seemed to have active suppression. The percentage of active suppression of the response to 2.5 μg per ml of PHA was significantly higher in varicella than in measles patients ($P < 0.05$, Student's t test).

DISCUSSION

In the present study we demonstrated suppression of the proliferative response of blood mononuclear cells to PHA and PPD in measles and varicella. The immunosuppressive effect of measles infection is well documented (12, 19, 33, 34), although some investigators have found normal PHA responses (15, 21). Immunosuppression in varicella seems to be less well studied. Diminished delayed cutaneous reactivity to tuberculin during varicella has been reported (19, 27), but we are not aware of any previous studies of varicella-induced suppression of the lymphocyte stimulation response to mitogens in vitro.

Monocytes can have a suppressive effect on the lymphocyte stimulation response (25). However, in the present study the PHA response was still suppressed after removal of phagocytic cells, suggesting that monocytes were not the main cause of suppression. This is in agreement with our findings in rubella virus vaccine infection (1a).

WBC subpopulations showed different distribution patterns in the acute phase of measles and varicella. Measles was characterized by lymphocytopenia. The percentage and absolute number of T lymphocytes were decreased, but the ratio between the suppressor/cytotoxic and helper T-lymphocyte subsets was unchanged. Slightly increased percentages of medium-sized and large T lymphocytes were seen, indicating some T-cell activation, but the proportion of

TABLE 4. Proliferative response to PHA at 2.5 μg and PPD at 10 μg per ml of cryopreserved blood mononuclear cells (PBL) from the acute and convalescent phases of measles (five cases) and varicella (six cases) and from healthy controls[a]

Patient	[^{14}C]thymidine uptake (cpm × 10^3)								% Active suppression	
	Acute (A)		Convalescent (B)		Mixture (A + B)		Expected value			
	PHA	PPD	PHA	PPD	PHA	PPD	PHA	PPD	PHA	PPD
Measles										
1	1.6	0.3	17.6	5.4	11.5	3.0	9.6	2.9	−20	−3
2	5.7	ND[b]	4.8	ND	4.6	ND	5.3	ND	13	ND
4	1.8	0.4	6.8	3.1	5.2	1.7	4.3	1.8	−21	6
6	6.6	ND	18.9	ND	11.8	ND	12.8	ND	8	ND
8	11.6	0.4	22.4	4.9	19.0	2.5	17.0	2.7	−12	7
Median	5.7	0.4	17.6	4.9	11.5	2.5	9.6	2.7	−12	6
Varicella										
9	1.5	0.3	11.2	8.4	4.4	3.2	6.4	4.4	31	27
10	3.9	ND	24.9	ND	15.7	ND	14.4	ND	−9	ND
11	1.5	0.2	17.5	2.7	5.5	0.6	9.5	1.5	42	60
12	1.6	8.7	17.2	31.0	4.8	23.0	9.4	19.9	49	−16
13	4.0	16.8	19.0	16.7	11.4	24.3	11.5	16.8	1	−45
14	1.8	0.7	13.3	6.9	3.3	1.4	7.6	3.8	57	63
Median	1.7	0.7	17.4	8.4	5.2	3.2	9.5	4.4	37	27
Controls										
Median	14.0	4.2	14.4	3.7	13.7	4.5	14.6	3.5	4	2
Range	2.9–23.9	2.2–9.8	2.6–29.1	1.0–11.3	6.3–21.6	1.3–12.0	6.2–23.4	1.9–10.6	−40–39	−29–54

[a] Ten controls were studied for PHA responsiveness (numbers 1, 4, 6, 8 to 14) and seven of the controls were tested for PPD responsiveness (numbers 1, 4, 9, 11 to 14). To study active suppression, PBL from the acute and convalescent phases were mixed in the ratio 1:1.

[b] ND, Not done.

these activated cells was approximately the same in both subsets. Thus, the distribution of T-lymphocyte subpopulations in measles was similar to that previously found in rubella virus vaccine infection (1a). Varicella, on the other hand, caused relative lymphocytosis. The percentage of T lymphocytes was unchanged, but within the T lymphocytes the helper subset was decreased and the suppressor/cytotoxic subset increased, resulting in a low ratio between the two subsets. The proportions of medium-sized and large cells were greater in the suppressor/cytotoxic cell subset than in the helper cell subset, indicating activation preferentially of the former cell type. Thus, the alterations of T-lymphocyte subpopulations in varicella are similar to the findings in infectious mononucleosis, cytomegalovirus, and hepatitis B virus infections (8, 10, 11, 23, 29). It cannot be excluded that measles patients may have a change of T-cell subpopulations that develops at a later time after the onset of illness than it does in varicella patients (only two measles patients were studied during week 2 after the debut of the rash). However, the fact remains that the measles patients had suppressed lymphocyte stimulation responses at a time when the ratio between the T-lymphocyte subsets was normal.

Reinherz and co-workers have shown that mononuclear cells from the acute phase of infectious mononucleosis can suppress the proliferative response of convalescent-phase cells to tetanus antigen and the immunoglobulin production of pokeweed mitogen-stimulated B cells (23). We found that mononuclear cells from the acute phase of varicella could suppress the PHA response of convalescent-phase cells. Thus, it is likely that the suppression of the lymphocyte stimulation responses during varicella to some extent is caused by the activation of suppressor cells. It seems probable that the Leu 2^+ T cells are responsible for the active transferable suppression in varicella, although this has not been directly proven. In some instances suppression of the PHA and PPD responses were not transferable with acute-phase cells, indicating that there were also mechanisms other than suppressor T-cell activation causing suppression in varicella.

Mononuclear cells from the acute phase of measles did not suppress the proliferative response of autologous convalescent-phase cells, which is in agreement with results of a recent study in which lymphocytes from measles-vaccinated subjects failed to suppress the PHA response of allogeneic lymphocytes from healthy donors (14). The suppression of lymphocyte stimulation responses in measles seems to be

due mainly to mechanisms other than suppressor T-cell activation.

Preactivation of lymphocytes in vivo is one possible cause of diminished in vitro responsiveness to mitogens or antigens. It has been shown in mice that lymphocytes preactivated by allogeneic cells do not respond to PHA and concanavalin A (1). In the present work measles as well as varicella were associated with increased spontaneous DNA synthesis, indicating preactivation of the lymphocytes in vivo. This has been shown before in measles (15) and also in many other infections (9, 13, 17, 35). In infectious mononucleosis the spontaneous DNA synthesis has been localized to the atypical lymphocytes (26, 35) which, in this disease, are mainly T cells (22). As mentioned above, the size of the T lymphocytes indicated some activation of both subsets in measles and activation preferentially of the suppressor/cytotoxic subset in varicella. In both diseases we have also found an increased spontaneous in vitro production of immunoglobulins M and G, showing that the B cells were activated also (Arneborn et al., Clin. Exp. Immunol., in press).

It should be noted that in a previous study of rubella vaccinees we found no signs of increased spontaneous DNA synthesis 7 to 11 days after vaccination, when the suppression of the in vitro lymphocyte response was maximal (2).

It has been shown that virus infection of immunocompetent cells can cause suppression of lymphocyte responses. During measles, virus can be found in both T and B lymphocytes (34). Measles virus infection of human lymphocyte cultures in vitro suppresses the proliferative response to PHA without causing proliferation (36). The virus is found preferentially in OKT 4^+ (helper) T cells after in vitro infection (S. Jacobson, personal communication). In mice, defective helper cell function has been demonstrated in measles-infected lymphocytes (18), and in humans reduced mitogen-induced helper factor production has been shown in lymphocytes from measles patients and in lymphocytes of healthy donors infected in vitro with measles virus (16). After rubella vaccination a correlation in time between suppression of the PHA response and viremia has been reported (7). However, measles vaccination of immune subjects has been shown to cause suppression of lymphocyte proliferation and chemotactic factor production in the absence of detectable virus replication in blood mononuclear cells (14). Rubella vaccination also leads to suppression of the PHA response in immune vaccinees (32), in whom viral infection of blood mononuclear cells has been shown to be extremely rare (20). Thus, it seems that measles and rubella infections may have suppressive effects even in the absence of lymphoid cell infection.

In summary, we have demonstrated suppression of the lymphocyte proliferative response to PHA and PPD in the acute phase of measles and varicella. In varicella, but not in measles, there was an increase of the proportion of T lymphocytes of the suppressor/cytotoxic phenotype and signs of activation of suppressor cell activity.

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