

Bronchoalveolar lavage cell analysis in measles viral pneumonia

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ABSTRACT: Although the immunological changes due to measles virus infection, such as suppression of delayed skin reactivity and increase in soluble CD8 in peripheral blood, have been demonstrated, the immunological changes in the lung during measles viral pneumonia (MVP) have not been reported. The aim of this study was to clarify the intrapulmonary immunological changes in MVP.

We analysed cell differentials and lymphocyte surface antigens of bronchoalveolar lavage (BAL) cells, both in the acute and the convalescent phase of MVP by flow-cytometry, using CD4+, CD8+, CD8+CD11b+ and CD8+CD11b- monoclonal antibodies in five patients and six healthy control subjects.

The absolute numbers of CD8+ and CD8+CD11b- cells were significantly greater both in the acute and the convalescent phase compared with those in controls. The percentages of CD8+ and CD8+CD11b- cells in the acute phase were significantly greater than those in controls and the convalescent phase. The CD4/CD8 ratios in the acute phase were significantly smaller than those in the convalescent phase and controls.

In conclusion, the intrapulmonary immunological changes with an increase in CD8+ and CD8+CD11b- cells in BAL fluid of MVP are thought to be the primary reaction in measles virus-infected lungs.

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Measles is still a common illness in the world [1, 2]. The most common complication of this disease is pneumonia which is classified as primary measles viral pneumonia (MVP) or secondary bacterial pneumonia [3, 4].

A number of studies have demonstrated the immunological changes due to measles virus infection. These changes include the suppression of delayed skin reactivity [5–9] and abnormally low mitogen responses [10, 11]. In peripheral blood, spontaneous proliferation of mononuclear cells [5], and increases in soluble CD8 [12], soluble interleukin-2 receptor [12], and gamma-interferon production [13] have been described.

Human T-lymphocyte subpopulations have been defined, as CD4+ (helper/inducer) and CD8+ (suppressor/cytotoxic) cells by using monoclonal antibodies [14–17]. Furthermore, the functional characteristics of two-colour-defined T-cells have been examined by the sequential cell sorter technique [18]. It has been reported that CD8+CD11b+ and CD8+CD11b- cells show suppressor [19, 20] and cytotoxic [21, 22] activity, respectively. In addition, increased proportions of the suppressor/cytotoxic cell phenotype have been demonstrated in cytomegalovirus [23], and hepatitis B virus [24] infections, suggesting that such a change in T-lymphocyte subpopulations might be a general phenomenon in viral infections [25].

To our knowledge, there has been no study on the immunological changes in the lung during MVP. The present study was designed to clarify the intrapulmonary immunological changes in MVP. We analysed cell differentials and lymphocyte surface antigens of bronchoalveolar lavage (BAL) cells, both in the acute and the convalescent phase of MVP, by flow-cytometry using anti-CD4, anti-CD8 and anti-CD11b antibodies.

Subjects

Five patients with measles, 14–20 yrs of age, were admitted to Asanogawa General Hospital, from April 15 to June 9, 1991 (table 1). The diagnosis of measles was established by a history of a prodrome and physical signs including macropapular rash, conjunctivitis, Koplik's spots and elevated immunoglobulin M (IgM) antibody level to measles virus in the serum by enzyme immunoassay. The diagnosis of coexisting MVP was made, based on the following findings: 1) dry cough; 2) decrease in arterial oxygen pressure (increase in alveolar-arterial oxygen pressure gradient); 3) no increase in white blood cell counts in peripheral blood; 4) no detection of bacteria, mycobacteria or fungus from BAL fluid (BALF);

Table 1. – Clinical features of patients with measles viral pneumonia

Patient No.	Age yrs	Sex	WBC cells·μl ⁻¹	Pao ₂		Paco ₂		FVC	FEV ₁	FEV ₁ /FVC	Days after rash onset n
Acute phase											
1	20	M	2100	68.8	9.2	36.8	4.9	95	77	78	4
2	14	F	3200	73.8	9.8	37.7	5.0	99	93	90	5
3	16	F	3500	83.2	11.1	43.7	5.8	114	85	74	7
4	20	M	5200	76.8	10.2	43.7	5.8	ND	ND	ND	13
5	18	M	5400	73.3	9.8	45.9	6.1	97	88	83	5
Mean±SEM			3900±600	75.2±2.4	10.0±0.3	41.8±2.0	5.6±0.3	101±4.5	86±3.3	81±3.4	6.8±1.6
Convalescent phase											
1			5300	100.3	13.3	36.2	4.8	94	80	81	29
2			3800	92.2	12.3	48.1	6.4	122	116	90	30
3			7000	99.8	13.3	45.7	6.1	111	94	84	37
4			5400	96.2	12.8	42.3	5.6	ND	ND	ND	67
5			5100	99.1	13.2	48.2	6.4	106	89	81	67
Mean±SEM			5300±500	97.5±1.5**	13.0±0.2**	44.1±2.2	5.9±0.3	108±5.9	95±7.8	84±2.2	46.0±8.7*

*: p<0.01; **: p<0.005: compared with acute phase. WBC: white blood cells; Pao₂: arterial oxygen tension; Paco₂: arterial carbon dioxide tension; FVC: forced vital capacity; FEV₁: forced expiratory volume in one second; ND: not determined.



Fig. 1. – Chest radiograph in the acute phase of measles viral pneumonia demonstrates linear-reticular infiltrate in bilateral lower zone, particularly on the left side (patient No. 2).

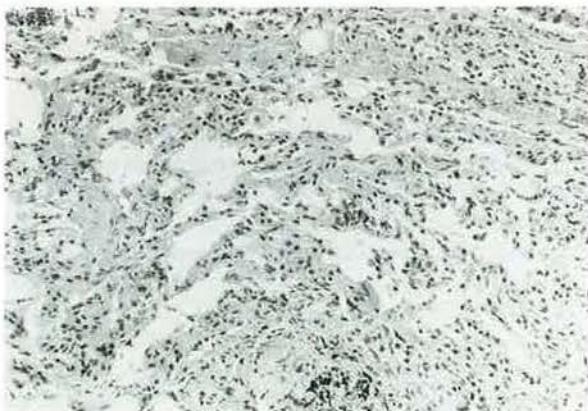


Fig. 2. – Transbronchial biopsy specimen showing mild alveolar thickness with mononuclear cells in the alveolar walls. (Haematoxylin-eosin stain; original magnification ×400) (patient No. 1).

5) linear-reticular infiltrate on chest roentgenogram (fig. 1); and 6) histologically documented alveolitis (fig. 2) on transbronchial lung biopsy specimens. The control population consisted of six healthy volunteers without history of previous pulmonary disease. All subjects were lifetime nonsmokers. Informed consent was obtained from all the subjects and their families. This study was approved by the Ethics Committee of our hospital.

Methods

Procedure of bronchoalveolar lavage

Fibreoptic bronchoscopy was performed through the transoral route, after topical anaesthesia with 2% lidocaine. Premedication with atropine (0.5 mg) and hydroxyzine pamoate (25 mg) was routinely performed, and a further sedation with 2.5–5.0 mg of intravenous diazepam was given when required. The tip of the instrument was advanced and wedged into a segmental or subsegmental bronchus in the middle or lingular lobe. Sterile 0.9% saline solution was then infused in 50 ml aliquots to a total of 150 ml. The fluid was recovered by gentle aspiration after each aliquot. The volume of fluid recovered was always above 50% of fluid instilled.

The procedure of BAL was performed in both the acute (6.8±1.6 days after the onset of the rash) and the convalescent (46.0±8.7 days after the onset of the rash) phases of MVP.

Analysis of bronchoalveolar lavage cells

Total cell number and differentials. Cellular analyses were carried out as described previously [26]. After filtering the fluid through a double layer of Dacron nets, cells were centrifuged at 1,500 rpm for 10 min and resuspended in 10 ml of RPMI-1640 (Grand Island Biological Co., Grand Island, NY, USA). Following dilution with

an equal volume of Turk solution, the number of cells was counted in a Bürker chamber. The fluid was then resuspended with RPMI-1640 to make a cell suspension containing 2×10^5 cells·ml $^{-1}$. Smears for differential counts of the cells were prepared by cyt centrifugation (Cytospin Shandon 2) at 1,000 rpm for 10 min. After staining with May-Grünwald-Giemsa stain, 300 cells were counted.

Lymphocyte subsets. The cell suspension was adjusted to a concentration of 2×10^7 cells·ml $^{-1}$. Then, 10 µl of monoclonal antibody at the proper dilution was added to 100 µl of this cell suspension. The cells were incubated at 4°C for 30 min in the dark, washed twice in phosphate-buffered saline, and subsequently resuspended in 0.3 ml of RPMI-1640. The suspended cells were then analysed for CD4+, CD8+, CD8+CD11b+ and CD8+CD11b- lymphocytes, using a flow cytometer (FACScan, Becton Dickinson). Monoclonal antibodies recognizing CD8 (fluorescein isothiocyanate (FITC)-conjugated Leu2), CD4 (FITC-conjugated Leu3), CD11b (phycoerythrin (PE)-conjugated Leu15) and determinants were provided by Becton Dickinson. In each analysis, cells stained by FITC- and PE-conjugated nonreactive mouse IgG (Becton Dickinson) were employed as negative control. Flow cytometric analyses were processed by SimulSET software in a FACScan. Appropriate fractions of lymphocyte were selected by gating on two dimension display, on forward scatter and side scatter.

Statistical analysis

Data were expressed as mean±SEM. Differences between the values in the acute and the convalescent phases of MVP were analysed by the Wilcoxon signed-rank test. Differences between the patients with MVP and the control subjects were analysed by the Mann-Whitney U-test. A p value of ≤ 0.05 was considered to be significant.

Results

Total cells and differentials

The total cell number in BALF obtained from the patients in the acute phase of MVP was significantly greater than that in the control subjects ($p < 0.05$) (fig. 3).

The percentage of macrophages in both the acute and the convalescent phase was smaller than that in the control subjects ($p < 0.02$ and $p < 0.05$, respectively). On the other hand, there was no significant difference in the absolute number between the three groups (fig. 3).

The lymphocyte population in both the acute and the convalescent phase of MVP was significantly greater than that in the control subjects, both in percentage ($p < 0.02$ and $p < 0.05$, respectively) and in absolute number ($p < 0.01$ and $p < 0.02$, respectively) (fig. 3).

The percentage of neutrophils in the acute phase of MVP was significantly greater than that in the convalescent phase of MVP ($p < 0.02$). The absolute number of neutrophils in the acute phase was significantly greater than that in the control subjects ($p < 0.05$) (fig. 3).

There was no significant difference in the percentage or the absolute number of eosinophils between the three groups (fig. 3).

Lymphocyte subsets and CD4/CD8 ratio

The percentage of CD4+ cells was significantly smaller in the acute phase of MVP than in the control subjects and the patients with the convalescent phase of MVP ($p < 0.05$ and $p < 0.01$, respectively). However, there were no significant differences between them in the absolute number (fig. 4).

Figure 5 is an example of expression of CD8 and CD11b by lymphocytes obtained from BALF in a two-colour FACScan analysis (patient No. 1). The

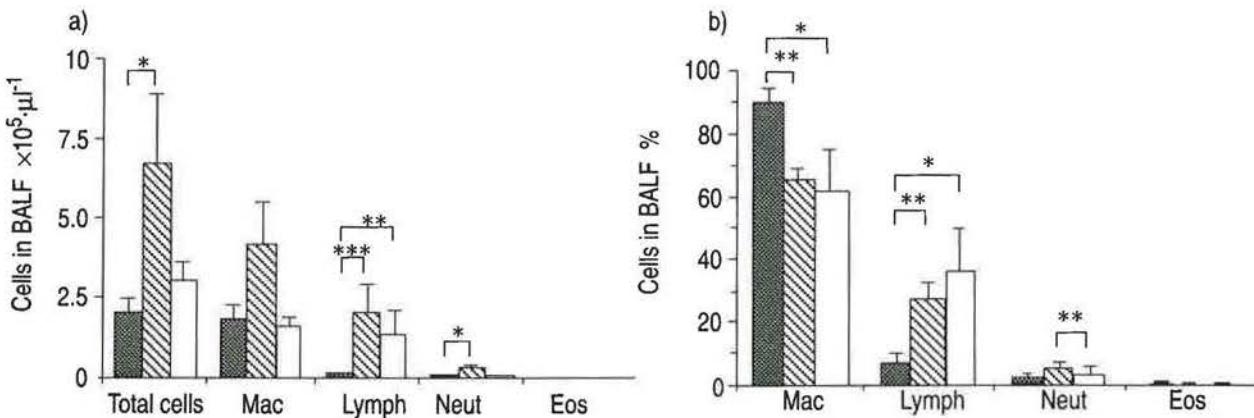


Fig. 3. – Absolute number (a) and percentage (b) of differential cells in BALF in measles viral pneumonia (mean±SEM). BALF: bronchoalveolar lavage fluid; Mac: macrophages; Lymph: lymphocytes; Neut: neutrophils; Eos: eosinophils. ■■■: control (n=6); ▨▨: acute phase (n=5); □□: convalescence (n=5). *: $p < 0.05$; **: $p < 0.02$; ***: $p < 0.01$.

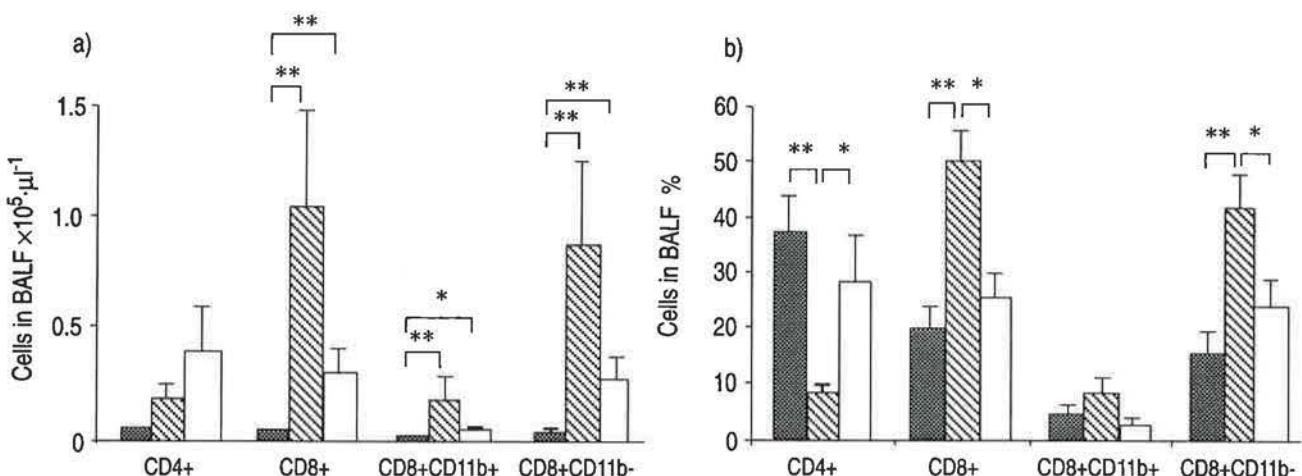


Fig. 4. – Absolute number (a) and percentage (b) of lymphocyte subsets in BALF in measles viral pneumonia (mean \pm SEM). BALF: bronchoalveolar lavage fluid. ■■■: control (n=6); ▨▨▨: acute phase (n=5); □□□: convalescence (n=5); *: p<0.05; **: p<0.01.

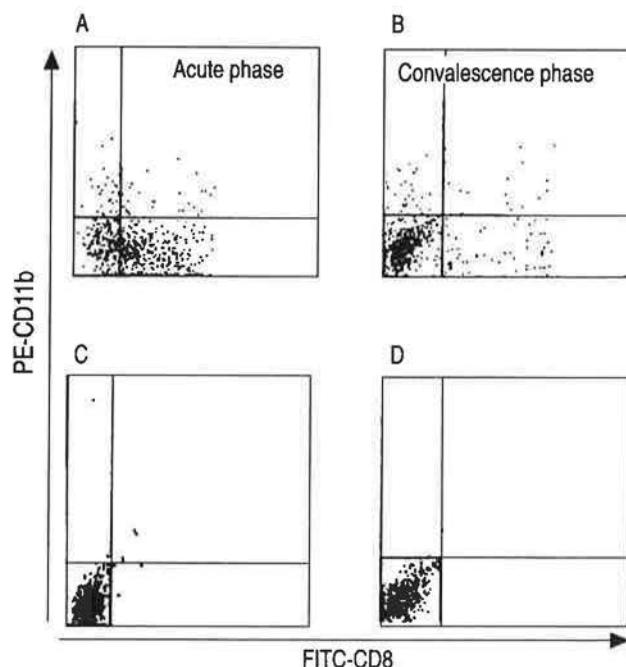


Fig. 5. – Expression of CD8 and CD11b by lymphocytes obtained from BALF in a two colour FACScan analysis. A: Acute phase in patient No. 1; B: Convalescent phase in Patient No. 1; C: Nonspecific bindings of the antibody to CD8 and CD11b in A; D: Nonspecific bindings of the antibody to CD8 and CD11b in B. BALF: bronchiodilator lavage; FITC: fluorescein isothiocyanate; PE: phycoerythrin; IgG: immunoglobulin G.

percentages of CD8+ and CD8+CD11b- cells in the patients in the acute phase were significantly greater than those in the patients in the convalescent phase and in the control subjects ($p<0.05$ and $p<0.01$, respectively). The absolute numbers of CD8+ and CD8+CD11b- cells both in the acute and the convalescent phase were significantly greater than those in the control subjects ($p<0.01$ and $p<0.01$, respectively) (fig. 4).

The absolute number of CD8+CD11b+ cells in the patients in the acute and the convalescent phase of MVP

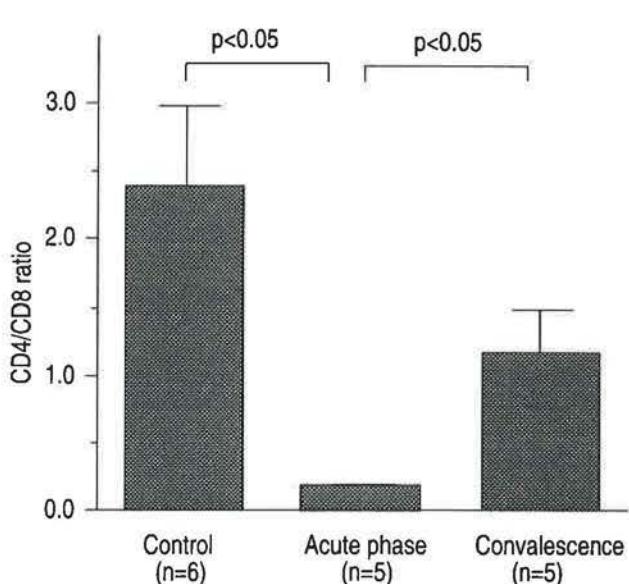


Fig. 6. – CD4/CD8 ratio in BALF in measles viral pneumonia.

was significantly greater than those in the control subjects ($p<0.01$ and $p<0.05$, respectively). However, the percentages were no significantly different (fig. 4).

The CD4/CD8 ratio in the acute phase of MVP was significantly less than in the convalescent phase and in the control subjects ($p<0.05$ and $p<0.05$, respectively) (fig. 6).

Discussion

Primary measles virus infection is immunologically characterized both by the development of a strong anti-viral immune response [27, 28] and multiple abnormalities of immune regulation [5–11]. Regarding the anti-viral response, BECH [27] reported the development

of complement fixing antibodies in measles patients. GRAVES *et al.* [28] showed that large amounts of antibody to nucleocapsid protein of measles virus developed in the patients by day one of the rash. Concerning abnormalities of immune regulation, it has been well established that skin test reaction to tuberculin decreases during measles infection [5–9], and that lymphoproliferative responses to mitogens are depressed in measles patients [10, 11]. On the other hand, in spite of lymphocytopenia, no change in the ratio of helper T-lymphocytes to suppressor cytotoxic cells has been shown in peripheral blood of measles patients [11, 29, 30].

Measles is often complicated by primary or secondary pneumonia, otitis media, encephalitis, for example [1–4]. Although it has been reported that soluble CD8 increases in cerebrospinal fluid of measles patients with encephalomyelitis [12], it has never been determined whether inflammatory cells and lymphocyte subpopulation in the lungs change in MVP. Therefore, we analysed bronchoalveolar lavage cells in five patients with MVP and compared the data between the acute and the convalescent phases.

Our study showed that lymphocytes in BALF significantly increased in parallel with the increment in CD8+ and CD8+CD11b- cells, which resulted in a decreased CD4/CD8 ratio, in the acute phase of MVP. CD8 is a 33-kDa transmembrane protein, present as an associative recognition molecule in the CD3+ cell receptor complex on the cytotoxic or suppressor subpopulation of T cells [31, 32]. The interaction of the CD8+ cell with a target cell bearing histocompatibility locus antigens (HLA) Class 1 [33], such as a virus-infected cell, results in the activation of the CD8+ cell and phosphorylation of CD8 coincidentally with the cytotoxic response [34]. The precursor and effector cytotoxic T-lymphocytes express the CD8+CD11b- phenotype [21]. It has been suggested that CD8+ and CD8+CD11b- cells may be important in the clearance of measles virus from the lungs in the acute phase of the pneumonia. This reaction seems to be a primary, but not a secondary, reaction in the infected lungs, because it has been shown that CD8+ cells in the peripheral blood do not increase in measles patients with or without pneumonia [11, 29, 30].

However, McFARLAND *et al.* [35] have recently reported that CD8+CD11b+ cells are cytotoxic memory cells, and that the percentage of CD8+CD11b+ splenocytes in mice gradually increased to a maximum level of expression at 8–9 days after lymphocyte choriomeningitis virus infection, and then declined to near naive levels (<3% of naive mouse splenocytes) by day 19–20 postinfection. In our study, in contrast to CD8+CD11b- cells, the percentage of CD8+CD11b+ cells did not significantly increase. The main reason for this discrepancy between CD8+CD11b- cells and CD8+CD11b+ cells may be that we performed BAL too late to observe the increase in the percentage of CD8+CD11b+ cells. BAL in the acute phase of MVP was performed at 6.8 ± 1.6 days after the onset of the rash. Since the time from the virus exposure to the appearance of rash is about 2 weeks [36],

BAL in acute phase was performed at about 20 days after measles virus infection.

Absolute numbers of CD8+ cells and CD8+CD11b- cells in BALF remained raised in the convalescent phase, when the pneumonia had completely resolved. Infectious virus titres rapidly decline as VOLLE *et al.* [37] demonstrated, whereas viral antigen remains elevated in the lungs for at least 60 days. It has been suggested that the persistent viral antigen is a chronic stimulus, which may maintain a raised level of CD8+ cells and CD8+CD11b- cells in the convalescent phase.

In addition, the decrease in CD4+/CD8+ ratio in BALF seen in the patients with MVP is also observed in patients with acquired immune deficiency syndrome complicated with cytomegalovirus pneumonia [38]. In contrast, it has been reported that the CD4+/CD8+ ratio in BALF increases in pneumonia caused by *Mycoplasma pneumoniae* [39] and *Chlamydia psittaci* [40]. From these findings, it is suggested that examination of lymphocyte subpopulations in BALF may be clinically useful to distinguish viral pneumonia from any other infectious pneumonia.

References

1. Weinstein L, Franklin W. – The pneumonia of measles. *Am J Med Sci* 1949; 217: 314–324.
2. Assaad F. – Measles: summary of worldwide impact. *Rev Infect Dis* 1983; 5: 452–459.
3. Reimann HM. – The Pneumonias: A Monograph in Modern Concepts of Pulmonary Diseases. In: Myers JA, ed. St. Louis, Warren H Green Inc., 1971; pp. 115–116.
4. Miller DL. – Frequency of complications of measles, 1963: report on a national inquiry by the Public Health Laboratory Service in collaboration with the Society of Medical Officers of Health. *Br Med J* 1964; 2: 75–78.
5. Tamashiro VG, Perez HH, Griffin DE. – Prospective study of the magnitude and duration of changes in tuberculin reactivity during uncomplicated and complicated measles. *Pediatr Infect Dis J* 1987; 6: 451–454.
6. Von Pirput C. – Das Verhalten der kutanen tuberkulinreaktion während der Masern. *Deutsch Med Wochenschr* 1908; 34: 1297–1300.
7. Christensen P. – Epidemic of measles in Southern Greenland, 1951: measles in virgin soil. Measles and tuberculosis. *Acta Med Scand* 1953; 144: 450–454.
8. Helms S, Helms P. – Tuberculin sensitivity during measles. *Acta Tuberc Scand* 1956; 35: 166–171.
9. Starr S, Berkovich S. – Effects of measles: gamma-globulin-modified measles and vaccine measles on the tuberculin test. *N Engl J Med* 1964; 270: 386–391.
10. Finkel A, Dent PB. – Abnormalities in lymphocyte proliferation in classical and atypical measles infection. *Cell Immunol* 1973; 6: 41–48.
11. Hirsch RL, Griffin DE, Johnson RT, *et al.* – Cellular immune responses during complicated and uncomplicated measles virus infections of man. *Clin Immunol Immunopathol* 1984; 31: 1–12.
12. Griffin DE, Ward BJ, Jaurequi E, Johnson RT, Vaisberg A. – Immune activation in measles. *N Engl J Med* 1989; 320: 1667–1672.
13. Griffin DE, Ward BJ, Jaurequi E, Johnson RT, Vaisberg A. – Immune activation during measles: interferon-gamma and neopterin in plasma and cerebrospinal fluid in complicated and uncomplicated disease. *J Infect Dis* 1990; 161: 449–453.

14. Kung PS, Goldstein G, Reinherz EL, Schlossman SF. — Monoclonal antibodies defining distinctive human T-cell surface antigens. *Science* 1979; 106: 347–349.
15. Poppema S, Bhan AK, Reinherz EL, McClusky RT, Schlossman SF. — Distribution of T-cell subsets in human lymph nodes. *J Exp Med* 1981; 153: 30–41.
16. Reinherz EL, Moretta L, Roper M, et al. — Human T-lymphocyte subpopulations defined by Fc receptors and monoclonal antibodies. A comparison. *J Exp Med* 1980; 151: 969–974.
17. Reinherz EL, King PC, Goldstein G, Schlossman SF. — A monoclonal antibody reactive with the human cytotoxic/suppressor T-cell subset previously defined by a heteroantisera termed TH2. *J Immunol* 1980; 124: 1301–1307.
18. Gatenby PA, Kansas GS, Xian CY. — Dissection of immunoregulatory subpopulations of T-lymphocytes within the helper and suppressor sublineages in man. *J Immunol* 1982; 129: 1997–2000.
19. Landay A, Gartland GL, Clement LT. — Characterization of a phenotypically distinct subpopulation of Leu2+ cells which suppresses T-cell proliferative responses. *J Immunol* 1983; 131: 2757–2761.
20. Takeuchi T, DiMaggio M, Levine H, Schlossman SF, Morimoto C. — CD11 molecule defines two types of suppressor cells within the T8+ population. *Cell Immunol* 1988; 111: 398–409.
21. Clement L, Dagg M, Landay A. — Characterization of human lymphocyte subpopulations: Alloreactive cytotoxic T-lymphocyte precursor and effector cells are phenotypically distinct from Leu2+ suppressor cells. *J Clin Immunol* 1984; 4: 395–402.
22. Pistoia V, Carroll AJ, Prasthofer EF, et al. — Establishment of Tac-negative, interleukin-2-dependent cytotoxic cell lines from large granular lymphocytes (LGL) of patients with expanded LGL populations. *J Clin Immunol* 1986; 6: 457–466.
23. Carney WP, Rubin RH, Hoffman RA, Hansen WP, Healy K, Hirsch MS. — Analysis of T-lymphocyte subsets in cytomegalovirus mononucleosis. *J Immunol* 1981; 126: 2114–2116.
24. Thomas HC. — T-cell subsets in patients with acute and chronic HBV infection, primary biliary cirrhosis and alcohol induced liver disease. *Int J Immunopharmacol* 1981; 3: 301–305.
25. Bach MA, Bach JF. — The use of monoclonal anti-T-cell antibodies to study T-cell imbalances in human disease. *Clin Exp Immunol* 1981; 45: 449–456.
26. Nishi K, Kanamori K, Yasui M, et al. — Two-color analysis of lymphocyte subpopulation of bronchoalveolar lavage fluid and peripheral blood in patients with sarcoidosis. *Jpn J Thorac Dis* 1989; 27: 1066–1073.
27. Bech V. — Studies on the development of complement fixing antibodies in measles patients. *J Immunol* 1959; 83: 267–275.
28. Graves M, Griffin DE, Johnson RT, et al. — Development of antibody to measles virus polypeptides during complicated and uncomplicated measles virus infections. *J Virol* 1984; 49: 409–412.
29. Arneborn P, Biberfeld G. — T-lymphocyte subpopulations in relation to immunosuppression in measles and Varicella. *Infect Immun* 1983; 39: 29–37.
30. Griffin DE, Moenich TR, Johnson RT, Lindo de Soriano I, Vaisberg A. — Peripheral blood mononuclear cells during natural measles virus infection: cell surface phenotypes and evidence for activation. *Clin Immunol Immunopathol* 1986; 40: 305–312.
31. Ledbetter JA, Evans RL, Lipinski M, Cunningham-Rundles C, Good RA, Herzenberg LA. — Evolutionary conservation of surface molecules that distinguish T-lymphocytes helper/inducer and cytotoxic/suppressor subpopulations in mouse and man. *J Exp Med* 1981; 153: 310–323.
32. Emmrich F, Kanz L, Eichmann K. — Cross-linking of the T-cell receptor complex with the subset-specific differentiation antigen stimulates interleukin-2 receptor expression in human CD4 and CD8 T-cells. *Eur J Immunol* 1987; 17: 529–534.
33. Blue M-L, Craig KA, Anderson P, Branton KR Jr, Schlossman SF. — Evidence for specific association between class I major histocompatibility antigens and the CD8 molecules of human suppressor/cytotoxic cells. *Cell* 1988; 54: 413–421.
34. Acres RB, Conlon PJ, Mochizuki DY, Gallis B. — Phosphorylation of the CD8 antigen on cytotoxic human T-cells in response to phorbol myristate acetate or antigen-presenting B-cells. *J Immunol* 1987; 139: 2268–2274.
35. McFarland HI, Nahill SR, Maciaszek JW, Welsh RM. — CD11b (Mac-1): a marker for CD8+ cytotoxic T-cell activation and memory in virus infection. *J Immunol* 1992; 149: 1326–1333.
36. Ray CG. — Disorders caused by biologic and environmental agents (continued): Measles (Rubella). In: Braunwald E, Isselbacher KJ, Petersdorf RG, Wilson JD, Martin JB, Fauci AS, eds. *Harrison's Principles of Internal Medicine*. 11th edn. 1987; pp. 682–683.
37. Voller A, Bidwell D, Bartlett A. — Microplate enzyme immunoassays for the immunodiagnosis of viral infections. In: Rose NR, Friedman M, eds. *Manual of Clinical Immunology*. Washington D.C., American Society for Microbiology, 1976; pp. 506–512.
38. Venet A, Clavel F, Israël-Biet D, et al. — Lung in acquired immune deficiency syndrome: infections and immunological status assessed by bronchoalveolar lavage. *Bull Eur Physiopathol Respir* 1985; 21: 535–543.
39. Hayashi T, Ichikawa Y, Fujino K, et al. — Analysis of lymphocyte subsets in peripheral blood and bronchoalveolar lavage fluid in patients with pneumonia due to *Mycoplasma pneumoniae*. *Jpn J Thorac Dis* 1986; 24: 162–167.
40. Kurashima K, Watanabe A, Kawamura Y, et al. — Bronchoalveolar lavage fluid findings and lung function in psittacosis. *Jpn J Thorac Dis* 1989; 27: 1288–1293.