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CELL-MEDIATED IMMUNE REACTIONS IN MEASLES

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Summary. — Cell-mediated reactions in measles cases, in direct contacts and healthy children were tested. Indices of phytohaemagglutinin-stimulated blastic transformation and leukocyte migration inhibition were evaluated. Positive reactions were found in the first and second weeks after manifestation of clinical symptoms. The application of glucocorticosteroids in clinical complications resulted in delayed and reduced leukocyte migration inhibition. Studies on seronegative contacts suggested that positive cell-mediated reactions may appear before manifestation of clinical symptoms.

Key words: *measles*; *cell-mediated reaction*; *glucocorticosteroids*

Introduction

Recent suggestions of several authors on the application of cell-mediated immune reactions for investigation of virus immunogenicity and antiviral immunity seem to be of particular interest (Burns and Allison, 1975; Black, 1976; Fraser and Martin, 1978), especially since the diagnosis of such virus infections as measles has so far been based on clinical symptoms and supported only by serological tests.

In this study we attempted to assess the diagnostic usefulness of the cellular reactions in measles. Sick and healthy children as well as children from close contacts were examined. The effect of glucocorticosteroids on cell-mediated immunity in children with complicated measles infections (otitis, pneumonia, laryngitis) was also investigated by the leukocyte migration inhibition assay and phytohaemagglutinin-stimulated blastic transformation.

Materials and Methods

Children. A total of 127 children aged 2-7 years were investigated. They were divided into four groups: I — 34 children with typical measles; II - 31 children with clinical complications treated with glucocorticosteroids (prednisolonum — Polfa; ultracorten-H — Ciba): 3-10 mg/kg

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body weight in the first weeks of disease; in the subsequent weeks the doses were diminished, depending on the symptoms of illness; III — 41 healthy children from the environment of children with measles (contacts); and IV — 21 healthy children (control group) with or without measles antibodies. Blood samples from the control group were taken only once and from sick children — inacute stage of the disease and during convalescence.

Migration inhibition assay. The leukocyte migration inhibition test (micromethod) was a modification of the method of Ketchel and Favour (1955). Heparinized whole blood was centrifuged at 1500 rev/min for 10 min. The plasma was removed and centrifuged at 3000 rev/min for 10 min to remove blood platelets. All cells were washed twice in medium 199. The purified plasma and cell pellets were mixed with medium with or without antigen (Edmonston strain — purified and concentrated according to Ahmed et al., 1974) in a proportion of 0.45 ml autologous plasma, 0.30 ml blood cells ($1.5-2.5 \times 10^6$ leukocytes) and 0.15 ml medium 199. Medium 199 was supplemented with 30 μg/ml L-glutamine and 1% calf albumin. After incubation for 30 min at room temperature, the mixture, of blood was drawn into capillary tubes. Fifteen capillaries were prepared for control and test samples, centrifuged at 1500 rev/min and incubated at 37 °C in an atmosphere containing 5 per cent CO_2. After 18 hr the leukocyte migration zone was measured by an ocular micrometer. The leukocyte migration inhibition test was performed by the macromethod of Maini *et al.* (1973) as modified by Kańtoch *et al.* (1977). The results of the migration inhibition tests were expressed as per cent of migration calculated by the following formula:

$$\% \text{ of migration} = \frac{\text{migration area in the presence of antigen}}{\text{migration area in the absence of antigen}} \times 100$$

Migration inhibition test was accepted as positive, when the migration area (zone) in the presence of antigen was equal to or less than 80% [according to Bergstrand and Käller (1973) and to our previous observations (Kańtoch *et al.*, 1978)].

Lymphocyte transformation assay. Peripheral blood lymphocyte (PBL) stimulation with phytohaemagglutinin (PHA) was performed by an assay utilizing whole blood (according to Junge *et al.*, 1970): 0.2 ml of whole blood was diluted in 3 ml of medium 199 containing 10% calf serum and 30 μg/ml L-glutamine. PHA (40 μg/ml of Wellcome PHA) or no stimulant (control) was added to the cultures which were incubated for 3 days at 37 °C. Twenty-four hours before the termination of culture, 2 μCi of ^{3}H-thymidine (ÚVVVR, Prague) were added to each tube. The results were expressed as a stimulation index (SI), representing the ratio obtained by dividing the mean count/min of triplicate cultures of the stimulated lymphocytes by the mean of the unstimulated lymphocytes.

Serology. Measles antibody was determined by the haemagglutination inhibition (HI) test.

Statistics. The significance of differences between the results of tests was calculated by the Student's test ($P = 0.01$) and nonparametric χ^2 test ($P = 0.05$).

Results

Measles cases

Lymphocyte transformation tests were performed in controls and in children with measles. The range of normal response to PHA stimulation, to which all the results obtained were related, was estimated on 21 healthy blood donors (Fig. 1). After the appearance of clinical symptoms, i.e. 1-3 days after hospitalization, a significant reduction of PBL response to PHA stimulation was found in 62% of cases with typical measles (group I) and 53% of cases with complications (group II). In the second week of the disease the impaired response of PBL to PHA stimulation was observed in 49% of cases (group I) and 62% of cases (group II). No significant differences in the mean values of the SI in the first and second weeks of the disease were observed.

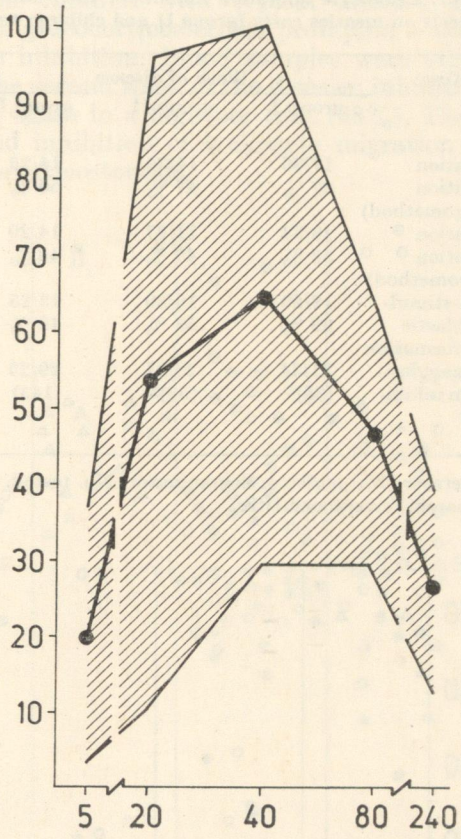

Fig. 1.
Leukocyte response to different doses of PHA in healthy children
Abscissa: μg/ml PHA; ordinate: stimulation ratio

Leukocyte migration inhibition tests were made simultaneously by the micro- and macro-method. The mean values of migration obtained by both methods showed no statistically significant differences (Table 1 and Figs 2 and 3). In acute stage of the disease positive results were observed in about 50% of patients in both groups I and II.

However, as illustrated by Figs 2 and 3 and Table 1, significant differences were observed in convalescent blood samples from children of groups I and II. The macromethod yielded positive results in 25% of the glucocorticosteroid-treated children (group II) compared to 27% for the micromethod. In the same period of the disease, the results of the leukocyte migration inhibition test in group I children (no glucocorticosteroids) were positive in 56% and 48% by the macromethod and micromethod, respectively.

Seven (group I) and three (group II) children showed inhibition of migration in both the first and second week of the disease. The absence of any cellular response during the observation period was noted in 6 (group I) and 11 (group II) children.

Table 1. Leukocyte migration inhibition, PHA-stimulated blastic transformation and serological tests on measles cases (group I) and children treated with glucocorticosteroids (group II)

Test	Week of disease			Total		
	group I	group II	group I	group II	group I	group II
Migration inhibition (macromethod)	17/30 57 %	14/30 47 %	14/25 56 %	7/28 25 %	24/30 80 %	19/30 63 %
Migration inhibition (micromethod)	18/34 53 %	11/27 47 %	14/29 48 %	7/26 27 %	26/34 77 %	17/27 63 %
PHA-stimulated blastic transformation	18/29 62 %	16/30 53 %	13/25 49 %	18/29 62 %	24/29 83 %	24/30 80 %
Haemagglutination inhibition*	31/34 (36)	28/31 (52)	29/29 (143)	31/31 (123)	34/34	31/31

Numerator: No. positive; denominator: No. tested.
*In parentheses: mean titre.

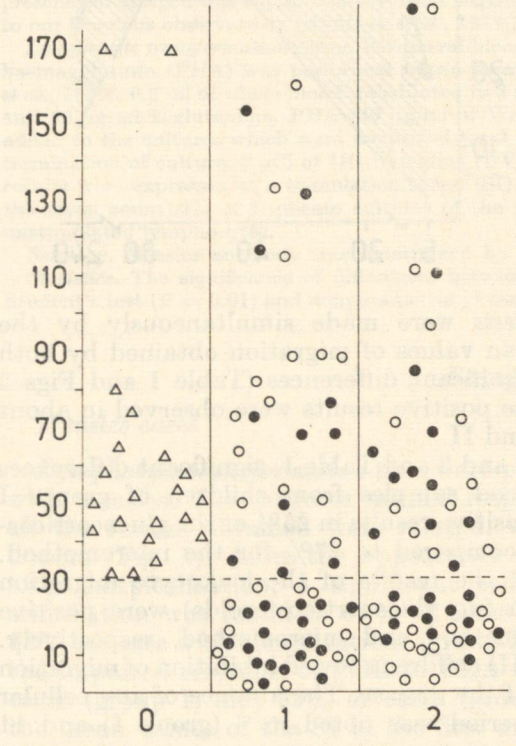

Fig. 2.
Leukocyte response to PHA
△ — control group; ● — measles cases; ○ — children treated with glucocorticosteroids.
Abscissa: weeks after onset of rash; ordinate: stimulation ratio. The values above the horizontal broken line are statistically significant.

The significant difference between children with typical measles and those with complications treated with glucocorticosteroids prompted a third measurement of leukocyte migration inhibition. Blood samples were taken from 19 steroids-treated children in the second week of the disease; inhibition of leukocyte migration was observed again in a high per cent (68%). These results suggested delayed and reduced inhibition of leukocyte migration in the measles cases treated with glucocorticosteroids.

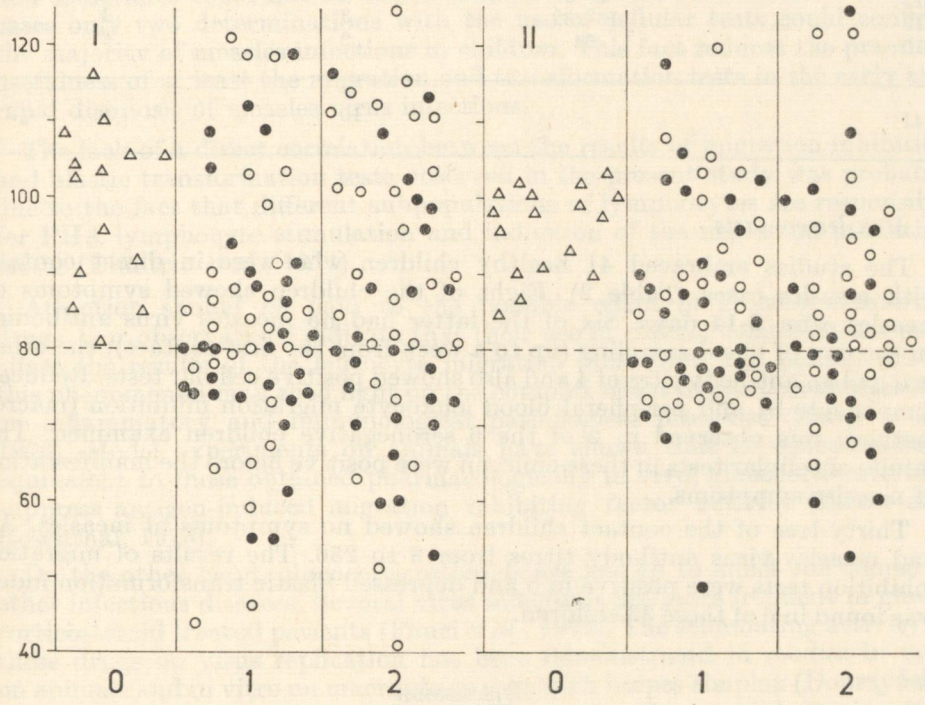

Fig. 3.

Inhibition of migration of peripheral blood leukocytes (I — macromethod; II — micromethod △ — control group; ● — children with typical measles infection; ○ — children treated with glucocorticosteroids.

Abscissa: weeks after onset of rash; ordinate: % of migration

In the acute phase of the disease HI antibodies reached titres from 4—128 in 59 cases. No measles virus antibodies were found in 6 children (9%) in spite of positive cellular results. In the period of convalescence, antibodies in titres from 64 to 256 were found in all the sera examined. The sera of 50 children (77%) showed a four fold increase in titre in convalescence. No differences were observed in the antibody titres of glucocorticosteroids-treated (group II) and untreated (group I) children.

Table 2. Cell and antibody-mediated responses to measles virus and PHA in contacts

No. of children with measles/No. of children tested	Titre of HI measles antibodies	No. of positive results	
		Migration inhibition (macromethod)	PHA-stimulated blastic tranformation
6/6	0	2	2
2/12	4—8	3	4
0/13	16—32	3	1
0/10	64	2	2
8/41		10	9

Measles contacts

The studies embraced 41 healthy children who were in direct contact with measles cases (Table 2). Eight of the children showed symptoms of measles after 3-14 days. Six of the latter had no measles virus antibodies on the day of blood sampling (up to a week from the first contact); the other two had an antibody titre of 4 and also showed positive cellular tests. Reduced lymphocyte SI and peripheral blood leukocyte migration inhibition (macromethod) was observed in 2 of the 6 seronegative children examined. The results of cellular tests in these children were positive before the manifestation of measles symptoms.

Thirty-tree of the contact children showed no symptoms of measles. All had measles virus antibody titres from 8 to 256. The results of migration inhibition tests were positive in 5 and depressed blastic transformation index was found in 6 of these 33 children.

Discussion

The reactivity of lymphocytes to measles antigen in the course of natural infection was studied by few authors (Chiba *et al.*, 1974; Rustigian *et al.*, 1975; Wesley *et al.*, 1978). The results obtained by Chiba *et al.* (1974) indicated the appearance of a cellular response several days after the manifestation of clinical measles symptoms. These studies, which involved the cytotoxic test, drew attention to the occurrence of a cellular response before the appearance of a humoral response. Our data, based on leukocyte migration inhibition and lymphocyte blastic transformation tests, confirmed this observation. On the other hand, the findings in seronegative persons after direct contact with measles cases suggest that a cellular response may occur before the manifestation of the clinical symptoms.

The results of the cellular tests indicate considerable differentiation which may be connected with both the individual properties of the infected persons

and the different doses of infecting virus. This problem was dealt with earlier in studies on blood samples from children vaccinated against measles (Kańtoch *et al.*, 1975) and model experiments on monkeys and guinea pigs immunized with measles virus (Kańtoch *et al.*, 1978). The depressed lymphocyte SI and leukocyte migration inhibition in children with measles were not always connected with the acute phase of the disease. Cellular response occurred in both the first and second week after the manifestation of clinical symptoms and sometimes could not be determined by either of the test. Thus in most cases only two determinations with the use of cellular tests could confirm the majority of measles infections in children. This fact reduces the practical usefulness of at least the migration and transformation tests in the early and rapid diagnosis of measles virus infections.

The lack of a direct correlation between the results of migration inhibition and blastic transformation tests observed in the present study was probably due to the fact that different subpopulations of lymphocytes are responsible for PHA lymphocyte stimulation and induction of the migration inhibiting factor (Dimitriu *et al.*, 1974).

Attention should be paid to the possible effect of glucocorticosteroid drugs. Our observations indicate that their use in clinical complications may affect the results of the leucocyte migration inhibition. The elucidation of this phenomenon may shed light on the complex effect of glucocorticosteroids on inflammatory and immunological pathological processes (Fauci *et al.*, 1976). Model experiments on animals have shown that at concentrations equivalent to those obtained pharmacologically in vivo, glucocorticosteroids suppress antigen-induced migration inhibiting factor activity (Balow and Rosenthal, 1973).

On the other hand, glucocorticosteroids are known to affect the course of other infectious diseases. Several virus infections are more frequent in glucocorticosteroid treated patients (Fauci *et al.*, 1976). The stimulating activity of these drugs on virus replication has been demonstrated in studies in vivo on animals and in vitro on macrophage cells with herpes simplex (Dobrzyński, 1970) and mouse hepatitis (Gallily, 1964) viruses. One possible explanation may be the interference of glucocorticosterois with interferon synthesis. Glucocorticosteroids are known to inhibit the production of interferon induced by Newcastle disease virus in patients treated after transplantation (Rytel and Balay, 1973) and to have common receptors with interferon on the cell surface (Filipic *et al.*, 1977), which may suggest blocking of the penetration of interferon into virus-infected cells. Since measles virus stimulates interferon production, it is possible that determination of the level of interferon would explain the possible role played by these mechanisms in the glucocorticosteroid treatment of measles.

On the other hand, Nishibe and Inoue (1978) reported that glucocorticosteroids inhibit a human herpesvirus (Inoue virus) replication in vitro. These data might suggest that depression of cell-mediated reactivity can be a result of the inhibition of virus replication.

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