

## CELL-MEDIATED IMMUNITY TO VARICELLA-ZOSTER VIRUS AS ASSESSED BY THE MIGRATION INHIBITION TEST

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*Summary.* — The migration inhibition method was used to test cell-mediated immunity to varicella-zoster (VZ) virus in 10 varicella and 11 herpes zoster patients. Control groups consisted of eight children susceptible to VZ infection on serological evidence and 49 normal persons of different age categories. Depending on the positivity criterion adopted, positive results during disease were obtained in 43 % or 90 % of all tests performed in varicella patients and 47 % or 74 % in herpes zoster patients. Irrespective of which criterion of positivity was applied, a high rate of positive results was obtained in the normal control groups; in the age range from 20—44 years it was comparable to that for patients. This finding would suggest a high activity of VZ virus among the human population. Since a positive result in migration inhibition test offers evidence of recent contact with antigen, either exogenous contact with VZ infection or endogenous contact with latent VZ virus must have been involved.

*Key words:* varicella-zoster virus; migration inhibition test; migration index

### Introduction

In recent years considerable effort has been devoted to supplement our knowledge of immunity against infections caused by the varicella-zoster virus with information on cell-mediated immunity to this virus. For this purpose various methods have been used, e.g. that of lymphocyte transformation (Russel *et al.*, 1972; Jordan and Merigan, 1974; Ruckdeschel and Mardiney, 1976), the original method of virus inactivation by human lymphocytes and monocytes (Gershon *et al.*, 1976) and the technique of determining interferon level in the vesicular fluid. The formation of cutaneous interferon in herpes zoster was in a correlation with the dynamics of the disease (Armstrong *et al.*, 1970; Stevens and Merigan, 1972; Stevens *et al.*, 1975) and the ability to produce a high interferon level, as an expression of the leukocyte activity, seemed to be an important factor controlling the course of VZ infection.

This paper presents the results of a study on cell-mediated immunity to VZ virus by means of the migration inhibition test in varicella and herpes zoster patients and control subjects of different age groups.

### *Materials and Methods*

*Varicella-zoster (VZ) antigen*, for the complement-fixation reaction (CFR) prepared according to Schmidt *et al.* (1964) and a control cellular antigen prepared in the same manner from a human embryo fibroblast cell strain (LEP USOL Praha) were used. The entire study was performed with a single batch of either antigen at an optimum CFR dilution of 1:32.

*The complement-fixation reaction (CFR)* was performed in the usual manner with the optimum antigen dilution (1:32), 1.5 units of complement and the optimum haemolysin dilution.

*The indirect haemagglutination reaction*, including antigen preparation, was described by Trlifajová *et al.* (1973).

*Migration inhibition test.* Cellular immunity was assessed by the indirect test of macrophage migration inhibition in a semimicromodification (Švejar *et al.*, 1971). Peripheral blood was collected into 6 % dextran with heparin to separate lymphocytes. The cells, washed and suspended at a concentration of  $5-10^6$  live cells per ml, were incubated at 37 °C for 24 hr in an antigen-free medium (medium 199 with 5 % calf serum), or in this medium with control antigen (CA) or VZ virus antigen. Before used in the present experiment, the efficacy of the antigens, was tested in a pilot experiment on rabbits. Chinchilla rabbits were sensitized with VZ antigen administered with complete Freund's adjuvant into both hind pads. Twenty-five days after the immunization the rabbits were killed and their spleens used for the migration inhibition test. The addition of VZ antigen (but not CA) elicited specific migration inhibition in most of the animals. This experiment confirmed that VZ antigen was capable of provoking an immunologically specific reaction. Furthermore, the optimum antigen dose was titrated in this experiment. In another experiment with spleen fragments from normal, nonimmunized rabbits, an antigen dose had been titrated that was nontoxic (i.e. did not produce inhibition of cell migration from spleen fragments of normal, nonsensitized rabbits) and thus represented the maximum dose usable in the migration inhibition test proper with spleen fragments of immunized rabbits, or with human lymphocyte cultures from persons tested for their cell-mediated immunity to VZ antigen.

The supernatant fluids obtained after centrifugation and filtration (Millipore 0.22  $\mu\text{m}$ ) of the blood-cell cultures were tested for presence of the migration inhibition factor (MIF) by determining the migration activity of normal rabbit-spleen fragment cells. As a rule, they were used in the migration inhibition test in series containing 20-30 samples after storage at -25 °C. Each experimental value represented a mean of six fragments tested under identical conditions. The results were expressed in terms of the migration index (MI), i.e. the ratio of the migration activity in the supernatant tested to that in antigen-free control medium. Difference values in the range from 0.90-1.10 were considered normal, MI values below or above these limits were considered evidence of inhibition and stimulation, respectively. As a standard criterion, a difference of at least 10 % in MI values between culture with CA and culture with VZ virus antigen was taken as evidence of MIF release and hence of cellular hypersensitivity to the virus. Differences in MI values of 8 and 9 % were considered plus-minus. An alternative criterion was based on a statistical approach (see below).

### *Results*

Table 1 presents migration inhibition tests on 10 patients with chickenpox. In the course of hospitalization, MI values tended to increase, decrease or stayed at approximately the same level in different patients. In some of them high MI values were detected during the first days of illness, at a time when CF antibodies were still at a very low level.

The results of tests performed on herpes zoster patients are presented in Table 2. Here also a variation in MI values in the course of hospitalization was found and there was no parallel with serological results.

Table 1. The results of migration inhibition tests on varicella patients

Patient No.	Age (years)	Day* of exanthem	CFR titre	Migration CA	inhibition test VZ	difference × 100
1	9	3	8			
		6	16	0.91	0.87	- 4
		13	128	0.93	0.78	-15
2	8	8	8	0.96	0.89	- 7
		15	128	0.94	0.80	-14
3	13	7	512	0.96	0.87	- 9
		14	512	0.92	0.85	- 7
		21	512	0.92	0.83	- 9
4	4	8	8	0.94	0.86	- 8
		15	128	0.94	0.83	-11
5	9	8	256	0.92	0.84	- 8
		15	512	0.91	0.83	- 8
6	14	3	8	0.98	0.82	-16
		10	64	0.95	0.85	-10
		23	64	0.93	0.85	- 8
7	6	3	8	0.92	0.80	-12
		16	256	0.96	0.91	- 5
8	9	4	8	0.93	0.84	- 9
		10	64	0.90	0.83	- 7
9	6	6	64	0.93	0.83	-10
		13	256			
10		3	8	0.95	0.85	-10
		17		0.91	0.74	-17
11	50	3				
		30	512			
		11 mo	8	0.92	0.84	- 8

\* mo = months.

Cell-mediated immunity to VZ virus was also determined by the migration inhibition test in normal control persons of different age (Table 3) and in eight children demonstrated serologically as susceptible to VZ infection (Fig. 1).

The standard way of positivity evaluation (see Materials and Methods) was to consider a difference in MI values of  $-0.10$  (10 %) or greater as positive and values of  $-0.08$  and  $-0.09$  as plus-minus.

Using this criterion, cell-mediated immunity was found in seven of the 10 varicella patients; 43 % of all tests performed on them were positive. If considering plus-minus values as positive, a positive result was obtained in each varicella patient during at least a certain period of his (her) hospitaliza-

Table 2. The results of migration inhibition tests on zoster patients

Patient No.	Age (years)	Day* of exanthem	CFR titre	Migration inhibition test		
				CA	VZ	Difference × 100
1	14	3	8	0.95	0.88	- 7
		10	128	0.87	0.79	- 8
		19	64			
2	55	8	1024	0.92	0.77	-15
		22	1024			
		29	1024	1.00	0.89	-11
3	62	6	8	0.92	0.81	-11
		13	256	0.91	0.85	- 6
		5 mo	32	0.94	0.89	- 5
4	71	3	128			
		8	512	0.93	0.78	-15
		15	512	0.96	0.83	-13
5	75	6	8	0.93	0.83	-10
		13	128			
		19	128	0.91	0.80	-11
6	45	10	512	0.93	0.89	- 4
		17	512	0.92	0.85	- 7
7	3	11	128	0.92	0.88	- 4
		48	128	0.93	0.88	- 5
8	61	18	512			
		25	256	0.68	0.66	- 2
9	67	7	512			
		14	256	0.83	0.69	-14
10	73	6	256			
		13	1024	0.71	0.64	- 7
11	77	7	512			
		27	512	0.83	0.72	-11

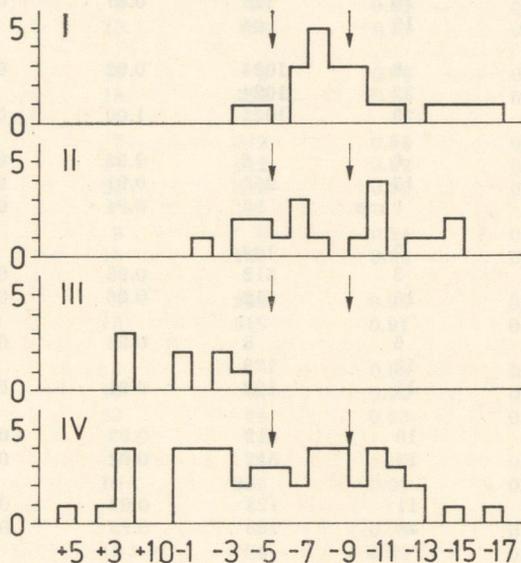
\* mo = months.

tion and 76 % of all tests were positive. If considering plus-minus values as negative, cell-mediated immunity was not found in three varicella patients (patients Nos 3, 5 and 8).

In herpes zoster patients, 47% of tests were positive and six of 11 patients displayed cell-mediated immunity to VZ virus in the course of disease (by the standard criterion). If taking plus-minus values for positive, a positive result was obtained in seven patients and 53 % of all tests were positive. If taking plus-minus values as negative, a positive value was not obtained in five of 11 patients at any testing (patients Nos 1, 6, 7, 8 and 10).

Among the normal control group a positive result was obtained in 27 % of persons and, assuming plus-minus values to be positive, in 43 % of subjects. Positivity rates were lowest in the eldest and youngest persons (Table 3).

Very small differences in MI values were obtained in eight VZ-susceptible children so that cellular immunity could not be demonstrated in any of them.



**Fig. 1.**

Distribution of migration-inhibition test results according to groups of patients and normal subjects

Abscissa: difference ( $\times 100$ ) between migration indices in cultures with CA and VZ virus antigen  
Ordinate: number of persons

Arrows indicate the borderline between positive and negative values; according to the statistical criterion used (see text), values from  $-6$  to  $-17$  were considered positive; according to the usual criterion, values from  $-10$  to  $-17$  were taken as positive

Groups: I — varicella patients; II — zoster patients; III — susceptible children; IV — normal controls

Logically, the MI difference values found in susceptible children (Fig. 1) should be taken as a basis for the evaluation of negativity limits.

Fig. 1 presents a comparison of the values obtained by the migration inhibition tests in all the patient and control groups. It is obvious that the standard positivity criterion (see above) is excessively rigid for determining cell-mediated immunity to VZ virus under the conditions used. Accordingly, a borderline between positive and negative results was established statistically on the basis of the standard deviation of the measurement. Differences in MI values equal to or greater than  $-0.06$  were considered positive and differences equal to or less than  $\pm 0.05$  were considered negative (Table 3). This

Table 3. Cell-mediated immunity to VZ virus determined by migration inhibition tests in normal persons

Migration-inhibition test Difference CA-VZ $\times$ 100	Age groups					Total
	7-9	20-23	40-44	61-64	81-85	
+ 5				1		1
+ 4						
+ 3					1	1
+ 2	2		1	1		4
+ 1						
0						
- 1	2			1	1	4
- 2		1		2	1	4
- 3	1	1	2			4
- 4					2	2
- 5		1		1	1	3
- 6		1	1		1	3
- 7			1		1	2
- 8	1	1	1		1	4
- 9	1		2		1	4
-10		2				2
-11	1	1		2		4
-12		1	1	1		3
-13	1			1		2
-14						
-15			1			1
-16						
-17			1			1
Total	9	9	11	10	10	49

criterion of positivity corresponded to the values determined in eight children with negative varicella history (Fig. 1).

According to this statistical positivity criterion, positive results were obtained in all varicella patients at least at some stage of hospitalization with 90 % of all tests performed in them having been positive; cell-mediated immunity was not detected in only two of 11 herpes zoster patients. In one of these negatives, only a single test was performed during hospitalization (patient No. 8), while the other negative patient (No. 7) represented a rare case of herpes zoster in a child aged 3 years. Seventy-four per cent of all tests performed on the herpes zoster patients were positive. Cell-mediated immunity was furthermore found in 53 % of the normal control persons. The highest positivity rates were obtained for age groups in the range from 20 to 44 years.

A comparison of the results evaluated according to the two positivity criteria used is presented in Table 4.

The mean positive values obtained for varicella and herpes zoster patients did not differ from each other. Nor was there any significant difference between the mean positive values for patients with VZ infection and control normal persons (Table 5).

**Table 4. A comparison of results of migration inhibition tests evaluated according to the two positivity criteria used**

Groups tested	Age	Neg.		Plus-minus		Pos.					
		+ 5 to	- 7	- 8 and	- 9	- 10 +	+ 5 to	- 5	Neg.	Pos.	
Normal population	7-9	5		2		2 = 22 %		5		4 = 44 %	
	20-23	4		1		4 = 44 %		3		6 = 67 %	
	40-44	5		3		3 = 27 %		3		8 = 73 %	
	61-64	6		0		4 = 40 %		6		4 = 40 %	
	81-85	8		2		0 = 0 %		6		4 = 40 %	
Susceptible children		8		0		0 = 0 %		8		0 = 0 %	
Patients											
Varicella		5		8		9 = 41 %		2		20 = 91 %	
Herpes zoster		9		1		9 = 47 %		5		14 = 74 %	

Calculation of the parametric coefficient of linear correlation failed to show any relationship between cell-mediated immunity values and duration of disease (considered from the onset of exanthem). Moreover, no correlation was found between the results of migration inhibition tests and the course of disease in either varicella or herpes zoster patients. As Table 1 and 2 show, there was no agreement between the results of migration inhibition and serological tests in the course of disease (CF and indirect-haemagglutination antibodies).

### Discussion

None of the eight children susceptible to VZ infection (see Fig. 1) were found positive by MIT under the test conditions employed. One of our specificity criteria for positive findings was based on this fact in the present work. As mentioned above, this criterion appears to be most reasonable and accurate.

The results of migration inhibition tests performed repeatedly in the course of varicella or herpes zoster were in no correlation with the dynamics of disease.

**Table 5. Mean positive values obtained for varicella and herpes zoster patients and control normal persons**

Groups tested	Positivity criterion	No. of tests	Mean value	Standard deviation
Varicella	- 6+	20	10.15	3.083
Herpes zoster		14	10.43	3.081
Controls		26	9.92	2.785
Varicella	- 10+	9	12.83	1.936
Herpes zoster		9	12.78	2.774
Controls		13	12.15	1.994

Russel *et al.* (1972), using the method of lymphocyte transformation, found lower values of cell-mediated immunity during the first three days of exanthem in herpes zoster patients than in control adult persons. These authors presented no data on the later course of the disease. Among our group of hospitalized patients only one was tested on the third day of exanthem (patient No. 1, Table 2). Other reported studies were based on determination of cell-mediated immunity at different stages of convalescence after VZ infection. Jordan and Merigan (1974) stated that they found no correlation between the degree of lymphocyte transformation and disease activity and that this was in accord with other authors' experience with vaccinia, measles, herpes simplex and Epstein-Barr viruses. However, Patel *et al.* (1979) found the development of VZ-specific cell-mediated response in normal persons by intense proliferative activity eight to ten days after the onset of illness with significant decline 70 to 80 days later. According to Gershon and Steinberg (1979), ongoing VZ infection was associated with minimal, and recovery from VZ infection with vigorous cellular immune response. The method of lymphocyte transformation (Patel *et al.*) and VZ virus inactivation (Gershon and Steinberg) were used.

In our conditions we found no correlation between cell-mediated immunity as determined by the migration inhibition test and the dynamics of the disease.

The positive findings in the control group of normal persons of different ages signifies contact with VZ virus antigen either in the form of recent disease, as a "booster experience" on repeated contact with disease, or in the form of activation of latent virus which presumably may persist in the organism after primary infection for life. Most probably, the positive results in the younger age groups were an aftermath of primary infection (varicella) or the result of contact with a patient, while in the higher age groups they rather reflected latent virus activation; in the aged, the low values were influenced by the natural deterioration of the ability to respond by cell-mediated immunity.

Irrespective of which criterion of positivity was applied, the relatively high percentage of positive results in controls came as a surprise. Among the age groups in the range from 20–44 years it was comparable to the positivity rates in patients suffering from VZ infection. This finding suggests a high activity of VZ virus among the human population.

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