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SOME IMMUNOLOGICAL CHARACTERISTICS OF SUBJECTS SUFFERING FROM FREQUENT HERPES SIMPLEX VIRUS RECRUDESCENCES

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Summary. — Some parameters of specific and non-specific immunity were tested in a group of 44 subjects suffering from frequent herpes simplex type 1 (HSV-1) or herpes simplex type 2 (HSV-2) recrudescences. The tests performed included determinations of (i) HSV complement-independent and complement-dependent neutralizing antibodies, (ii) antibodies to glycoprotein C of HSV-1 and glycoprotein G of HSV-2, (iii) antibodies to viral capsid and early antigens of Epstein-Barr virus, (iv) antibodies to tetanus toxoid, (v) serum levels of IgM, IgG, IgA, transferrin, prealbumin and C'3 and C'4 components of complement, (vi) active and total T lymphocytes, (vii) phagocyting activity of polymorphonuclear neutrophils, eosinophils and mononuclear cells, (viii) skin reactivity to tuberculin, toxoplasmin, candidin, tetanus and diphtheria toxoids. In the patients the following deviations from the control groups were noted: (i) Antibody levels to homotypic but not to heterotypic HSV were enhanced, (ii) serum IgM levels were elevated, (iii) percentages and numbers of active and total T lymphocytes were decreased, (iv) phagocyting activity of neutrophils was depressed but that of eosionofils was increased.

Introduction

Herpes simplex virus (HSV) infections are an annoying medical problem. More than 90 % of the population experience the infection during their lifetime and recurrent eruptions of the latent infection occur in more than 30 % of these subjects. Herpetic recrudescences are associated with pronounced physical discomfort and often also with serious psychological distress.

Reasons for the repeated attacks are not clear. In the recent years interest has been focused on the immune system and its reactivity. However, immuno-

logical mechanisms that control acute and latent HSV infection, restrict spread of the virus in the infected host and modify the frequency and severity of recrudescences, are poorly understood. Although there is evidence that various immune responses contribute to host defence against the infection, cell-mediated immunity seems to be of primary importance (Shillitoe *et al.*, 1977; Kohl, 1985).

An increasing number of patients suffering from recurrent HSV lesions, a lack of antiviral drugs capable of eradicating an established latent infection and a suspected oncogenicity of HSV were the main stimuli for our efforts to develop a subviral HSV-1 vaccine (Kutinová *et al.*, 1988). For its evaluation a group of adult volunteers suffering over prolonged periods of time from at least 4 herpetic eruptions per year were selected. In the preparatory phase of the vaccination trial several immunological parameters — some of them repeatedly — were examined. There were two reasons for doing this. First, the study was planned as double-blind and we wanted to take the various parameters of specific and non-specific immunity into consideration when pairing the vaccine- and placebo-given subjects. In addition, we hoped to get some information on differences in these reactivities between subjects suffering from frequent herpetic recrudescences and healthy subjects, and to see whether patients suffering from type 1 and type 2 disease differ in this respect. The results of this vaccine trial have been presented elsewhere (Kutinová *et al.*, 1988). It this report we summarize the immunological findings obtained in the patients prior to vaccination.

Materials and Methods

Subjects. A total of 44 patients (28 females and 16 males) aged 18—65 yrs (mean for females 31, for males 38 yrs) were studied. All of them had sufferred from herpetic recrudescences at least four times a year for extended periods of time; nearly two thirds of them had recrudescences at least once a month. From all patients viruses were isolated and typed. Out of the 19 HSV-1 isolates from patients with facial herpes, 18 were typed as HSV-1 and 1 as HSV-2, while all isolates from areas below the waist were typed as HSV-2 viruses. At enrollment into the study all patients underwent a complex clinical check-up (including ECG, X-ray examination, laboratory examination of blood and urine). In two patients mild eosinophilia was detected; the other findings were within the norm.

Materials for tests on the various parameters of specific and non-specific humoral and cell-mediated immunity were collected during the asymptomatic phase of the HSV disease. Determinations of specific antibodies against HSV, EBV and tetanus toxoid were complemented by determinations of immunoglobulins and some other plasma proteins (transferrin, prealbumin, C'3 and C'4 fractions of complement). The state of non-specific cellular immunity was evaluated by determination of peripheral T lymphocytes, by blood cells phagocytic function and by skin tests.

Control group for serological tests was selected among surgical patients of Bulovka Hospital, employees of Prague hospitals and laboratory workers. None of these subjects suffered from recurrent herpetic lesions. They were matched with the patients by sex and age (±3 years). Each control serum was examined with the corresponding patient serum in the same test. Control groups for tests on cellular immunity were selected in a similar way.

Tests on humoral immunity. Microneutralization test (MNT). MNT was performed as described (Suchánková *et al.*, 1984). In brief, sera were diluted in two-fold steps from 1 : 10 to 1 : 320 in microneutralization plates by means of automatic diluter (Dynatech). Approximately 100 $TCID_{50}$ of HSV-1 (strain KOS) or HSV-2 (strain 196) (both obtained from J. L. Melnick) in 50 μ

of medium were added to 25 μl of the diluted sera. When testing complement dependent antibodies, we added 50 μl of guinea-pig complement diluted 1 : 12 in cultivation medium instead of medium alone. After one hour incubation at room temperature 30,000 human lung fibroblasts (LEP cells) in 175 μl of medium were added to each well, plates were sealed and incubated at 37 °C for 6 days. Each serum dilution was tested against each virus type in four parallels. Titres were determined according to Kärber (1931). When calculating GMTs sera non-reactive at the 1 : 10 dilution were considered positive at the dilution of 1 : 5.

Solid-phase RIA (SPRIA) for type specific HSV antibodies. The antigens were prepared and SPRIA was performed and evaluated as described (Suchánková *et al.*,1984).The antigen contained essentially gC-1 in the case of HSV-1 preparation and purified gG-2 in the case of HSV-2 preparation. Sera were diluted 1 : 10 to 1 : 320.

Anti-Epstein-Barr virus (EBV) antibody. Indirect immunofluorescence test was used for the determination of EBV antibodies. Viral capsid antigen (VCA) antibody was determined in P3HR-1 cells and early antigen (EA) antibody in Raji cells induced by TPA and n-butyrate as described previously (Boguszáková *et al.*, 1983).

Antitetanic antibodies were tested by hemagglutination method, using freeze-dried sheep erythrocytes sensitized with purified tetanus toxoid (TETAHEM Sevac) as described by Kyselová *et al.* (1970).

Determination of plasma proteins. In patients' sera the contents of IgA, IgM, IgG, C'3 and C'4 complement components, transferin and prealbumin were determined. Antibodies used for these determinations were obtained from sera of pigs immunized with plasma proteins. Unwanted antibodies were removed from these sera with the aid of appropriate human plasma fractions immobilized on suitable polymers (copolymers of polyethylene glycol and tereftalic acid). Immunoglobulin (Ig) fractions were then separated by precipitation with caprylic acid, according to the method of Steinbuch and Audran (1967). Ig fractions were dissolved in 0.02 M phosphate buffer (pH 7.2) containing 0.15 mol/l NaCl, 40 g of polyethylene glycol 6000 and 4 g of Tween-20 per litre. The solutions were filtered through membrane filters (Sartorius, 0.3 μm) before use. In the tests 0.1 % solutions of Igs were used, except for anti-IgA, IgM and transferrin Igs, for which the respective concentrations were 0.15, 0.08 and 0.2 %. In the reactions, performed in borosilicate glass tubes, 1 ml of Ig solution was mixed with 0.5 μl of patient's serum for the determination of IgA, with 0.2 μl of serum for IgG, with 2.5 μl of serum for IgM, with 0.5 μl at serum for transferrin and 5.0 μl of serum for prealbumin and C'4, and with 2.0 μl of serum for C'3. All the measurements were performed on laser nephelometer (PDQ-TM Hyland, Travenol Inc) equipped with Nc-discharged lamp at 632.8 nm. Light-scattering by antigen-antibody complexes was monitored by means of a photomultiplier under an angle of 32 degrees.

The pool of sera from 150 healthy volunteers (preserved with 1 g of sodium azide and 4.4 g of epsilon-aminocaproic acid per litre) delipidated with Freon 113, freeze-dried under nitrogen and calibrated to Human Standard Serum (Behring-Hoechst) was used as a standard.

Tests on cellular immunity. Lymphocyte isolation. Freshly drawn venous blood (2 ml) was mixed with EDTA dipotassium salt (Lachema Brno) solution (0.2 ml) and diluted with equal volume of phosphate-buffered saline (PBS). Lymphocytes were separated by the method of Böyum (1968) on a Ficoll-Pague (Pharmacia, Upsalla) gradient. After centrifugation at 400 g for 30 minutes at room temperature the lymphocytes were washed twice with PBS and once with Medium 199 (Sevac, Prague) supplemented with 1 % of polyvinyl-pyrrolidone 10 and adjusted to a final concentration of 2×10^6/ml in the same medium.

"Active" E rosette test. The assay was done by the method described by Wybran and Fudenberg (1971) in our modification (Kamínková, 1981). Sheep red blood cells (SRBC) stored in Alsaver's solution at 4 °C were washed three times with PBS and resuspended in the same medium as used for the isolation of lymphocytes. Fresh suspension of SRBC was prepared before every testing. Hundred μl of lymphocyte suspension (2×10^5 cells) were mixed with the same volume of SRBC to give a lymphocyte to SRBC ratio of 1 : 8. Tubes were centrifuged for 5 minutes at 200 g at room temperature. Immediately after centrifugation they were slightly tilted, a drop of solution was placed on a microscope slide and the number of rosettes was immediately determined. Positive E rosettes (3 or more SRBC attached to a lymphocyte) were determined on the count of 200 lymphocytes in duplicate.

Total E rosette test. The assay was done by the method described by Hoffman and Kunkel (1976) in our modification (Kamínková, 1981). Hundred μl of lymphocyte suspension (2×10^5) was mixed with 100 μl of 0.5 % SRBC suspension to give a lymphocyte to SRBC ratio of 1 : 50.

The tubes were centrifuged for 10 minutes at 100 g at room temperature and subsequently incubated at 4 °C overnight. The number of positive E rosettes was counted as described for "active" E rosette test.

Phagocytic activity of peripheral blood leucocytes. The assay was done by a simple micromethod described by Větvička *et al.* (1982) using synthetic hydrophilic particles based on 2-hydroxyethylmethacrylate. Hundred μl of heparinized fresh blood (5 IU of heparine per ml) were mixed with 50 μl of particle suspension in PBS adjusted to give a final concentration of $4-5 \times 10^8$/ml. The mixture was incubated for 60 minutes at 37 °C. After incubation the cell smears were prepared and stained using the May-Grünwald-Giemsa procedure. Leukocytes with at least 3 engulfed particles were considered to be phagocyting cells. Four hundred leukocytes were counted. Final results were expressed as percentage of phagocyting cells.

Skin tests. Immunoskintest SEVAC (Doutlík *et al.*, 1984*a*) — a set of 5 recall antigens (tuberculin, toxoplasmin, candida, tetanus and diphtheria antigens) — was used. A dose of 0.1 ml of each antigen was administered i.d. into the fore-arm (3 antigens into the left and 2 antigens plus saline as a negative control into the right forearm). The test was read after 48 hr. An infiltrate (with or without erythema) up to the diameter of 5 mm was considered negative, an infiltrate with a diameter equal to or bigger than 6 mm positive (6—10 mm = +, 11—15 mm = ++, 16—20 mm = +++ and more than 20 mm = ++++). The final skin test index (STI) was calculated as the sum of crosses (+) divided by the number of antigens.

Statistical analysis. Data were analyzed by Student's test or non-parametric χ^2 test.

Results

Anti-HSV-1 and anti-HSV-2 neutralizing antibody status in patients and controls is shown in Table 1. Patients with HSV-1 disease show significantly higher antibody titres against this virus. Similarly HSV-2 patients possessed significantly more antibodies reactive with HSV-2 than the control subjects. On the other hand, in either group the antibody titres against the heterotypic virus were not significantly increased in the patients over those determined in the control subjects. Complement-dependent antibodies reached higher titres than complement-independent antibodies and the differences between the patients and the control subjects were more marked in the case of the former than the latter antibodies.

Table 1. Complement-dependent and complement-independent antibodies against HSV-1 and HSV-2 in patients and healthy controls as determined by MNT

Patients with isolate	Test virus	Complement	Geometric mean titre (GMT)		Ratio GMT patients to controls	Significance
			Patients	Controls		
HSV-1	HSV-1	—	28.6	19.6	1.5	$p < 0.01$
	HSV-2	—	12.6	10.5	1.2	NS*
	HSV-1	+	97.0	36.3	2.7	$p < 0.001$
	HSV-2	+	22.2	15.2	1.5	NS
HSV-2	HSV-1	—	25.9	24.6	1.1	NS
	HSV-2	—	23.5	11.8	2.0	$p < 0.001$
	HSV-1	+	66.8	65.4	1.0	NS
	HSV-2	+	51.6	19.7	2.6	$p < 0.001$

* NS, not significant

Table 2. Antibodies against gC-1 (HSV-1) and gG-2 (HSV-2) in patients and healthy controls as determined by SPRIA

Patients with isolate	Test antigen	Geometric mean titre (GMT) Patients	Controls	Significance
HSV-1	gC-1	501	142	$p < 0.05$
	gG-2	≤ 5	≤ 5	NS
HSV-2	gC-1	381	288	NS
	gG-2	17.3	6.4	$p < 0.001$

The results of SPRIA with isolated gC-1 and gG-2 antigens are shown in Table 2. The patients with HSV-1 isolates had significantly higher titres of gC-1 antibodies than the controls. Similarly, patients infected with HSV-2 had significantly higher anti-gG-2 antibody titres than the corresponding controls.

As indicated in Table 3 no significant differences in antibody titres against EBV antigens examined (VCA, EA) were observed between the patients and the controls.

The presence and distribution of tetanic antibody levels are shown in Table 4. More than 90 % of subjects in each group possessed the minimal protective level of antibody, exceeding 0.01 IU per ml. The whole group of HSV patients did not differ significantly from the adult population control group investigated earlier (Doutlík *et al.*, 1984*b*) nor was the difference between HSV-1 and HSV-2 patients significant (χ^2 with Yates' corection, $p > 0.05$). However, the highest values equal to or exceding 2.56 IU/ml were less frequent in patients with HSV-1 isolates than in the control group and this difference was significant ($p < 0.025$).

Plasma proteins levels determined are summarized in Table 5. All the findings except those for IgM were within the normal limits. More than 60 %

Table 3. Antibodies against EBV antigens in patients and healthy controls as determined by immunofluorescence test

Patients with isolate	Antibodies against	Geometric mean titre (GMT) Patients	Controls	Significance
HSV-1	VCA	132.0	101.5	NS
HSV-2		95.1	90.3	NS
All		121.5	95.1	NS
HSV-1	EA	8.5	8.9	NS
HSV-2		7.8	9.1	NS
All		8.1	9.0	NS

Table 4. Antitetanic antibodies in HSV patients and healthy individuals

Level of antitetanic antibodies in IU/ml	Patients with isolate				Controls	
	HSV-1		HSV-2			
	No.	%	No.	%	No.	%
<0.01	1	5.0	2	8.3	10	5.3
0.01—0.16	5	25.0	3	12.5	21	11.2
0.32—1.28	11	55.0	7	29.2	50	26.6
≧2.56	3	15.0	12	50.0	107	56.9
Total	20	100	24	100	188	100

of both the type 1 and type 2 patients had IgM values above normal levels, whereas levels of IgA were slightly depressed in about 30% of patients.

The presence of "active" T lymphocytes in peripheral blood, is shown in Table 6. The percentages of "active" T lymphocytes were significantly lower ($p < 0.01$) in both groups of patients (HSV-1 and HSV-2) than in the healthy controls. Similar results were obtained on repeating the test. However, absolute numbers of "active" T lymphocytes were less dramatically depressed. In the repeated test (not shown) the difference between type 1 patients and the controls was not significant.

The values of total T lymphocytes displayed a similar tendency (Table 7). The percentages of total T lymphocytes were significantly depressed ($p < 0.01$) in both groups. Again, absolute numbers were less markedly depressed. Statistically significant difference ($p < 0.05$) was only found in the HSV-2 group of patients. Similar results were obtained on retesting.

Phagocyting activities of peripheral neutrophils, eosinophils and monocytes, characterized by phagocyting index (see Materials and Methods) are shown in Table 8. As can be seen the most marked difference was found

Table 5. Plasma protein contents in HSV patients

	Patients with isolate		Normal limits
	HSV-1 (g/l)	HSV-2 (g/l)	(g/l)
IgA	2.87 ± 1.06	2.73 ± 0.82	2.2— 5.2
IgG	11.8 ± 2.2	11.05 ± 2.40	8.2—13.9
IgM	2.08 ± 0.60	1.75 ± 0.84	0.75— 1.7
C 3	0.77 ± 0.10	0.83 ± 0.1	0.64— 1.15
C 4	0.24 ± 0.06	0.25 ± 0.06	0.16— 0.38
Transferrin	3.4 ± 1.27	3.7 ± 1.05	2.0— 4.0
Prealbumin	0.32 ± 0.09	0.35 ± 0.07	0.1— 0.4

Table 6. "Active" T lymphocytes in HSV patients and control subjects

Parameter	Patients with isolate		Control subjects
	HSV-1	HSV-2	
Percentage of active cells	10.6 ± 2.35 ($p < 0.01$)	9.09 ± 1.46 ($p < 0.01$)	26.47 ± 1.13
Number of active cells	246 ± 74.67 ($p < 0.05$)	184 ± 35.55 ($p < 0.05$)	420 ± 19.3

in eosinophils — their phagocyting activity was increased several times in both groups of patients over such activity seen in controls. On the other hand, phagocyting activities of neutrophils and monocytes were lower in the patients than in the control group of healthy subjects; this depression was more marked in neutrophils than in monocytes. Again, nearly identical results were obtained on retesting.

Evaluation of the skin tests disclosed a broad variation of STI values ranging from 1.2 to 4 which is similar to the distribution of STI in healthy subjects (Fig. 1). The mean STI in the HSV patients (2.47 ± 0.51) was slightly but not significantly higher than in the controls (2.26 ± 0.54). In not a single case STI signalled a marked deficiency in cell immunity.

Discussion

Although subjects suffering from recrudescent HSV have no other apparent predisposition to infectious diseases, it is broadly believed that an immunological defect is involved in the pathogenesis of the disease. Its nature is not understood, however. Most likely it is not a defect in specific humoral immunity because HSV antibody levels in the patients are enhanced over those in normal seropositive subjects (Douglas and Couch, 1970; Gange

Table 7. Total T lymphocytes in HSV patients and control subjects

Parameter	Patients with isolate		Control subjects
	HSV-1	HSV-2	
Percentage of total cells	49.16 ± 3.28 ($p < 0.01$)	50.12 ± 2.49 ($p < 0.01$)	76.71 ± 0.82
Number of total cells	1041 ± 105 (NS)	1010 ± 82.61 ($p < 0.05$)	1247 ± 52.8

Table 8. Phagocyting activity of peripheral neutrophils, eosinophils and monocytes in HSV patients and control subjects

Phagocyting cells	Patients with isolate		Control subjects
	HSV-1	HSV-2	
Neutrophils	31.12 ± 3.61*) ($p < 0.01$)	33.11 ± 3.39 ($p < 0.01$)	52.42 ± 1.56
Eosinophils	32.84 ± 10.40 ($p < 0.01$)	19.52 ± 2.08 ($p < 0.01$)	6.44 ± 1.42
Monocytes	44.90 ± 3.66 ($p < 0.05$)	45.81 ± 3.50 (NS)	55.0 ± 3.23

*) percentage of phagocyting cells

et al., 1975; Kalimo *et al.*, 1977; Russel, 1974). The changes in cell mediated immunity are less clear although extensive data on this issue have been gathered (Kohl, 1985). A variety of deviations in cell immunity have been reported in subjects suffering from frequent recrudescences. The changes demonstrated have concerned activity of NK cells (Thong *et al.*, 1975; Yasukawa and Zarling, 1983; Sheridan *et al.*, 1985), proliferative response of lymphocytes to stimulation by HSV antigens *in vitro* (Gange *et al.*, 1975; Shillitoe *et al.*, 1977; Kalimo *et al.*, 1983; El Araby *et al.*, 1975; Corey *et al.*, 1978; Weinberg *et al.*, 1985; Kirchner *et al.*, 1978), migration inhibition of neutrophils and macrophages (Rabson *et al.*, 1977; Shillitoe *et al.*, 1977) and lymphokine production (O Reilly *et al.*, 1977; Cunningham and Merigan, 1983; Sheridan *et al.*, 1985). Quite impressive have been the studies analyzing sequential changes of various cell functions in HSV patients (Thong *et al.*, 1977; Shillitoe *et al.*, 1977; Kalimo *et al.*, 1983; Sheridan *et al.*, 1985). These reports have suggested that progressively developing depression of cell functions in remission predetermines a new recrudescence. From the quoted as well as from numerous other studies it can be concluded that T lymphocytes play a key role in immune reactions involved in herpetic recrudescences, and that their altered reactivity — though poorly defined at this moment — is associated with the break-down of the system controlling HSV latency.

It has not been the aim of the present study to determine an immunological mechanism operative in HSV recurrency. The main reason for examining the various immunological parameters in the HSV patients was to include these characteristics into the pairing procedure for the placebo-controled trial with HSV subunit vaccine (Kutinová *et al.*, 1988). The present report summarizing the immunological findings in HSV patients before vaccination is a by-product of these efforts. To the best of our knowledge some of the features of the immune state of HSV patients examined in this study have not yet been reported.

In agreement with the previous reports (see above) we detected significantly increased levels of specific anti HSV antibodies in the patients than in the control subjects. It may be of interest that these increases (observed in both HSV-1 and HSV-2 patients) were limited to the homotypic viruses suggesting that primarily type specific antigens were inolved. This was de-

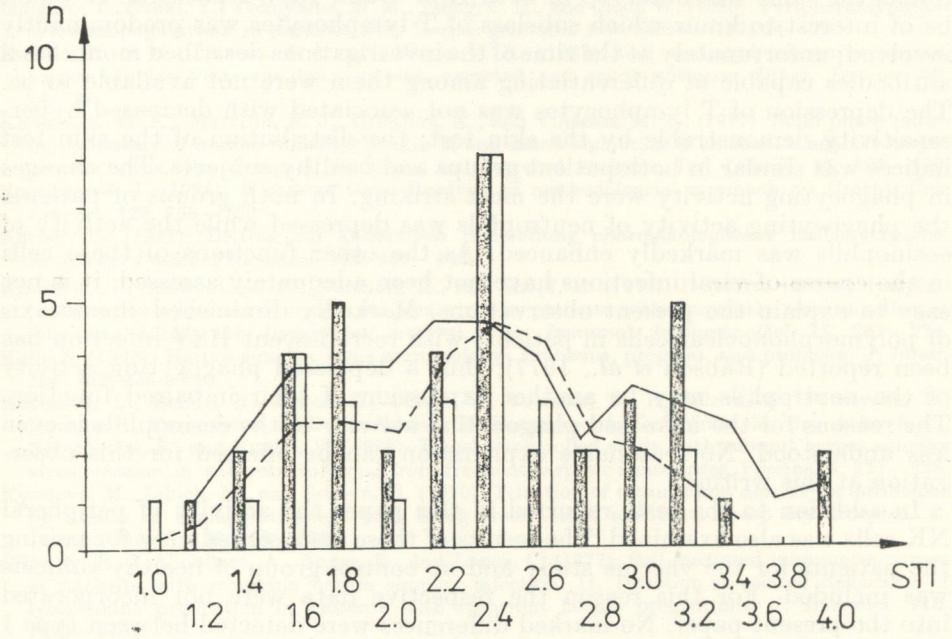

Fig. 1.
Distribution of skin test index (STI) in HSV patients and healthy individuals
Black columns HSV patients; open columns control subjects.

monstrated not only by the neutralization tests but also — and more definitely — by SPRIA with type specific gC-1 and gG-2 antigens. On the other hand, there was no significant difference in the EBV VCA and EA antibodies between patients and controls. This indicates that whatever may be the putative immunity defect involved in the pathogenesis of the recurrent disease it does not result in activation of latent EBV infection. Also the other findings concerning non-specific humoral immunity of the patients did not exhibit any marked or consistent deviation from the norm. The only exception was IgM; its elevation in the patients most probably reflects a strong and almost continuous stimulation by the virus antigens.

More abnormalities concerned cellular immunity. The finding of T lymphocytes depression in the patients was probably most important. Both active and total T lymphocytes were suppressed. This is at variance with the earlier observation by Sawanabori *et al.* (1977) but in agreement with the more

recent report by Ishiguro *et al.* (1980). Since the first group worked with type 2 patients while the latter with type 1 patients, it has been suggested that the discrepancy may be associated with the infecting virus type (Ishiguro *et al.*, 1980). However, this does not seem to be the case as in our materials the same was observed in both HSV-1 and HSV-2 patients. It would be of interest to know which subclass of T lymphocytes was predominantly involved; unfortunately at the time of the investigations described monoclonal antibodies capable of differentiating among them were not available to us. The depression of T lymphocytes was not associated with decreased hypersensitivity demonstrable by the skin test; the distribution of the skin test indices was similar in both patient groups and healthy subjects. The changes in phagocyting activity were the most striking. In both groups of patients the phagocyting activity of neutrophils was depressed while the activity of eosinophils was markedly enhanced. As the other functions of these cells in the course of viral infections have not been adequately assessed, it is not easy to explain the present observations. Markedly diminished chemotaxis of polymorphonuclear cells in patients with recrudescent HSV infection has been reported (Rabson *et al.*, 1977); thus a depressed phagocyting activity of the neutrophils may be another expression of their impaired function. The reasons for the increased phagocyting activity of the eosinophils is even less understood. No reasonable explanation can be offerred for this observation at this writing.

In addition to the tests reported in this paper the activity of peripheral NK cells was also examined. The results of these tests served only for pairing the patients for the vaccine study and no control group of healthy subjects was included. For this reason the respective data were not incorporated into the present paper. No marked differences were detected between type 1 and type 2 patients.

References

Böyum, A. (1968): A one-stage procedure for isolation of granulocytes and lymphocytes from human blood. *Scand. J. Clin. Lab. Invest.* **97**, suppl. 21, 51—76.

Boguszaková, L., Hirsch, I., Břicháček, B., and Vonka, V. (1983): Relationship between Epstein-Barr virus nuclear antigen and DNA genome number in superinfected and lymphoblastoid cell lines. *J. gen. Virol.* **64**, 887—894.

Czarnetzki, B. M., and Macher, E. (1980): *In vitro* studies on the human cellular immune response to commercially available herpes simplex antigens. *Arch. Dermatol. Res.* **268**, 247—255.

Corey, L., Reeves, W. C., and Holmes, K. K. (1978): Cellular immune response in genital herpes simplex virus infection. *N. Engl. J. Med.* **299**, 986—991.

Cunningham, A. L., and Merigan, T. C. (1983): Gamma-interferon production appears to predict time of recurrence of herpes labialis. *J. Immunol.* **130**, 2397—2400.

Douglas, R. G., and Couch, R. B. (1970): A prospective study of chronic herpes simplex virus infection and recurrent herpes labialis in humans. *J. Immunol.* **104**, 289—295.

Doutlík, S., Vacek, Z., Pekárek, J., and Vepřeková, A. (1984*a*): A new preparation for evaluation of cell immunity *in vivo* — Immunoskintest (in Czech). *Prakt. Lékař (Praha)* **64**, 758—760.

Doutlík, S., Vacek, Z., and Deutschmannová, J. (1984*b*): Evaluation of the state of cellular and humoral immunity against tetanus (in Czech). *Čas. Lék. čes.* **123**, 1182—1186.

El Araby, I. I., Chernesky, M. A., Rawls, W. E., and Dent, P. B. (1978): Depressed herpes simplex virus-induced lymphocyte blastogenesis in individuals with severe recurrent herpes infections. *Clin. Immunol. Immunopathol.* **9**, 253—263.

Gange, R. W., de Bats, A., Park, J. R., Bradstreet, C. M. P., and Rhodes, E. L. (1975): Cellular immunity and circulating antibody to herpes simplex virus in subjects with recurrent herpes simplex lesions and controls as measured by mixed leucocyte migration inhibition test and complement-fixation. *Br. J. Dermatol.* **97**, 539—544.

Hoffman, T., and Kunkel, H. C. (1976): E rosette test, pp. 71—81. In Bloom, B. R., David, J. R. — *In vitro methods in cell-mediated and tumor immunity*, New York, Acad. Press.

Ishiguro, T., Ozaki, Y., Yokoyama, M. M., Tseng, C. H., and Chao, W. (1980): Peripheral lymphocytes subpopulations in patients with Herpes genitalis. *Immunobiol.* **157**, 24—29.

Kalimo, K. O. K., Joronen, I, A., and Havu, V. K. (1983): Cell mediated immunity against herpes simplex virus envelope, capsid, excreted and crude antigens. *Infect. Immun.* **39**, 24—28.

Kalimo, K. O. K., Marttila, R. J., Granfors, K., and Viljanen, M. K. (1977): Solid phase radioimmunoassay of human immunoglobulin M and immunoglobulin G antibodies against herpes simplex virus type 1 capsid, envelope, and excreted antigens. *Inf. Immun.* **15**, 883—889.

Kamínková, J. (1981): E rosette tests. Analysis of methodological approach (in Czech). *Čas. Lék. čes.* **120**, 756—760.

Kärber, G. (1931): Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. *Arch. exp. Pathol. Pharmakol.* **162**, 480—483.

Kirchner, H., Schwenteck, M., Northoff, H., and Shöpf, E. (1978): Defective *in vitro* lymphoproliferative responses to herpes simplex virus in patients with frequently recurring Herpes infections during the disease-free interval. *Clin. Immunol. Immunopathol.* **11**, 267—274.

Kohl, S. (1985): Herpes simplex virus immunology: Problems, progress, and promises. *J. infect. Dis.* **152**, 435—440.

Kutinová, L., Benda, R., Kaloš, Z., Dbalý, V., Votruba, T., Kvíčalová, E., Petrovská, P., Doutlík, S., Kamínková, J., Domorázková, E., Bergmanová, V., Krčmář, M., Suchánková, A., Němečková, Š., and Vonka, V. (1988): Placebo-controlled study with subunit herpes simplex virus vaccine in subjects suffering from frequent herpetic recurrences. *Vaccine*, **6**, 223—228.

Kyselová, M., Libich, M., and Srbová, H. (1970): Titration of tetanus and diphtheria antibodies by means of hemagglutination test, using freeze-dried sensitized sheep erythrocytes. *Z. Immun.-Forsch.* **139**, 228—235.

O'Reilly, R. J., Chibbare, A., Anger, E., and Lopez, C. (1977): Cell-mediated immune responses in patients with recurring herpes simplex infection. II. Infection-associated deficiency of lymphokine production in patients with recurrent herpes progenitalis. *J. Immunol.* **118**, 1095—1102.

Rabson, A. R., Whiting, D. A., Anderson, R., Glover, A., and Koornhof, H. J. (1977): Depressed neutrophil motility in patients with recurrent Herpes simplex virus infections: *In vitro* resoration with levamisol. *J. infect. Dis.* **135**, 113—116.

Russell, A. S. (1974): Cell-mediated immunity to herpes simplex virus in man. *J. infect. Dis.* **129**, 142—146.

Sawanabori, S., Ashman, R. B., Nahmias, A. J., Beningo, B. B., and Lavia, M. F. (1977): Rosette formation and inhibition in cervical dysplasia and carcinoma *in situ*. *Cancer. Res.* **37**, 4332—4335.

Sheridan, J. F., Beck, M., Aurelian, L., and Radowsky, M. (1985): Immunity to herpes simplex, virus: virus reactivation modulates lymphokine activity. *J. infect. Dis* **152**, 449—456.

Shillitoe, E. J., Wilton, J. M. A., and Lehner, T. (1977): Sequential changes in cell mediated immune responses to Herpes simplex virus after recurrent herpetic infection in humans. *Infect. Immun.* **18**, 130—137.

Steinbuch, M., and Audran, R. (1969): The isolation of IgG from mammalian sera with the aid of caprylic acid. *Arch. Biochem. and Biophys.* **134**, 279—284.

Suchánková, A., Hirsch, I., Krčmář, M., and Vonka, V. (1984): Determination of Herpes simplex virus type-specific antibodies by solid-phase RIA on *Helix pomatia* lectin-purified antigens. *J. infect. Dis.* **149**, 964—972.

Thong, Y. H., Vincent, M. M., Hensen, S. A., Fucillo, D. A., Rola-Pleszcynski, M., and Bellanti, J. A. (1977): Depressed specific cell-mediated immunity to herpes simplex virus type 1 in patients with recurrent herpes labialis. *Infect. Immun.* **12**, 76—80.

Větvička, V., Fornůsek, L., Kopeček, J., Kamínková, J., Kašpárek, L., and Vránová, M. (1982): Phagocytosis of human blood leucocytes: a simple micromethod. *Immunol. Letters* **5**, 97—100.

Weinberg, M. A., Portnoy, J. D., Clecner, B., Hubschman, S., Lagacé-Simard, J., Rabinovitch,

N., Remer, Z., and Mendelson, J. (1985): Viral inhibition of lymphocyte proliferative responsiveness in patients suffering from recurrent lesions caused by herpes simplex virus. *J. infect. Dis.* **152**, 441—448.

Wilson, J. M. A., Ivanyi, L., and Lehner, T. (1976): Cell-mediated immunity in herpesvirus hominis infections. *Br. Med. J.* **i**, 723—726.

Wybran, J., and Fudenberg, H. H. (1971): Rosette formation, a test for cellular immunity. *Trans. Assoc. Am. Physicians* **84**, 239—247.

Yasukawa, M., and Zarling, J. M. (1983): Autologous herpes simplex virus-infected cells are lysed by human natural killer cells. *J. Immunol.* **131**, 2011—2016.