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INCREASED MIGRATION INHIBITION FACTOR PRODUCTION BY LEUKOCYTES OF PATIENTS WITH HERPES ZOSTER GIVEN THE LEUKOCYTE ULTRAFILTRATE

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Summary. — Using the direct leukocyte migration inhibition assay, the cell-mediated immune response was followed in together 47 patients suffering from herpes zoster. Of these, 19 persons were treated with partially purified and concentrated lysed leukocyte ultrafiltrate. Enhancement and/or earlier production of the leukocyte migration inhibition factor — in the presence of varicella-zoster virus antigen — was observed by leukocytes from patients given the ultrafiltrate on days 2—3 since the onset of the vesicular stage of the disease. Highly significant ($\alpha = 1\ \%$, $P < 0.01$) differences in the migration inhibition values were observed between non-treated patients and patients given one dose of the ultrafiltrate during the days 3—12 after appearance of the first herpes zoster vesicles; on days 13—30 these values were not significant.

Key words: *herpes zoster*; *lysed leukocyte ultrafiltrate*; *cell-mediated immunity*

Introduction

The host cell-mediated immune (CMI) response is considered as decisive for the recovery from primary as well as from reactivated infection with varicella-zoster virus (VZV) (Gershon *et al.*, 1979; Gershon *et al.*, 1984). The efficiency of the CMI defence mechanism was shown generally lowered in elderly and in immunocompromised persons suffering from malignant diseases (Gershon *et al.*, 1979). Severe and protracted herpes zoster (HZ) was frequently observed in patients with early and advanced stages of acquired immune deficiency syndrome.

It was repeatedly confirmed that dialysed or ultrafiltered extracts of lysed leukocytes (LLU) transfer not only specific skin test reactivity to previously non-reactive or anergic recipients (delayed hypersensitivity) (Borkowski and Lawrence, 1979; Lawrence and Borkowski, 1983) but also induce and/or enhance the production of various lymphokines. The production of the

migration inhibition factor (MIF) is also enhanced as a part of LLU's effect on CMI (Wilson *et al.*, 1979).

The clinical effectivity of LLU was repeatedly proven in the treatment of various infectious complications occuring mainly in patients suffering from diseases causing or caused by impairment of CMI functions (Fudenberg, 1987; Mayer *et al.*, 1987; Moulias *et al.*, 1979; Pekárek *et al.*, 1987). The human leukocyte migration inhibition assay (LMI) is recommended as a suitable method for the study of the CMI in humans, especially when investigating the effect of LLU either *in vitro* or *in vivo* (Fudenberg *et al.*, 1983; Wilson *et al.*, 1983; Tsang and Fudenberg, 1986).

The present study was undertaken in order to analyse the production of the MIF — a soluble mediator important for CMI effector mechanisms — by leukocytes obtained from blood samples taken at intervals during the clinical course of HZ in patients administered LLU or placebo.

Patients, Materials and Methods

Patients and treatment. Two groups of patients with acute, localized HZ were studied. The total patient population included 47 persons. Group 1 consisted of 19 persons who were given LLU. It was administered in a single dose subcutaneously within three days after the first vesicle formation. One dose was equivalent to 3×10^8 lymphocytes.

Group 2 consisted of 28 subjects receiving one ml of phosphate-buffered saline (pH 7.2) subcutaneously.

The average age of HZ patients in groups 1 and 2 was 46.3 years (range 10—73) and 56.1 years (range 15—82), respectively.

Blood samples from the patients were collected in three intervals expressed in days. We considered for day 0 the appearance of first HZ vesicles on the involved dermatome. As interval 1 we considered the time period encompassing days 3—12, as interval 2 the period from days 13—30, and as interval 3 the period from days 35—60.

Lysed leukocyte ultrafiltrate. LLU was prepared from pooled "buffy coats" of blood of 150 random healthy donors. The ultrafiltrate of lysed leukocytes, in order to remove inhibitory substances, was precipitated with ethanol and subjected to gel permeation chromatography (Mayer *et al.*, 1983). The second chromatographic fraction, designed as P2/II, prepared in bulk quantity as described by Borvák *et al.* (1987, 1988) was used. The P2/II fraction exhibited a pronounced therapeutic effect in HZ patients (Mayer *et al.*, 1987). Patients in this study received LLU originating from one batch of the P2/II material.

Preparation of leukocytes. Whole heparinized blood (30 ml) obtained from HZ patients was supplemented with 6 % dextran T-500 (Pharmacia, Uppsala). The unclotted blood was layered over a Ficoll-Verografin cushion. 13 parts of 12 % Ficoll (Pharmacia, Uppsala) were mixed with 2 parts of 76 % Verografin (diatrizoate) (Léčiva, Praha). The blood was allowed to sediment at 37 °C for 45—60 min. The leukocyte-enriched supernatant (with minimum contaminating erythrocytes) was carefully aspirated from the cushion, washed in Minimal Eagle's medium by centrifugation at 390 g and then, repeatedly, at 200 g for 10 min. The leukocyte-containing pellet was finally resuspended in RPMI-1640 (Flow Laboratories) to a concentration of 250×10^6 cells/ml.

Preparation of antigen. The washed pooled suspensions of human embryo fibroblasts infected with VZV (the 12th passage of a strain isolated from the vesicular fluid of a 14-year-old boy suffering from chickenpox) were disrupted by sonification at 20 kHz (Raytheon sonic oscilator) for 3×30 sec in an ice bath. The cell debris was removed by centrifugation at 800 g for 15 min. The VZV antigen residual infectivity was inactivated at 56 °C in a water bath for 30 min. The preparation contained 2.6 mg of protein/ml as determined by the method of Lowry *et al.* (1951).

The control antigen was prepared in a similar way from uninfected cell cultures.

The titres of the virus material estimated in the complement-fixation reaction were 16—32. The antigen was stored in aliquots at — 20 °C until used.

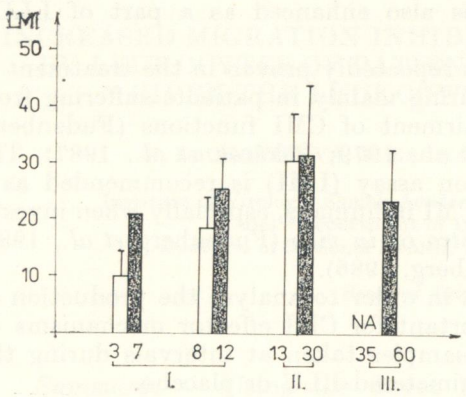

Fig. 1.
Leukocyte migration inhibition values in patients suffering from herpes zoster and treated with lysed leukocyte ultrafiltrate
LMI: leukocyte migration inhibition activity expressed in per cent according to the comparison of migration areas of leukocyte in the presence and in the absence of the varicella-zoster virus
□ Patients non-treated with lysed leukocyte ultrafiltrate (LLU) — empty columns
■ Patients treated with LLU — dark columns
NA: samples not available
Bars represent the standard deviation ($\bar{x} \pm$ SE)
Digits indicate days after first vesicle formation; Roman digits indicate intervals of the disease.

Preparation of agarose plates and the leukocyte migration inhibition assay. The plates were prepared according to Spitler and Müller (1976) with minor modifications. To prepare two plates, always 65.2 mg agarose (Pharmacia, Uppsala) was dissolved in 4.2 ml of sterile distilled water. Agarose was melted in a boiling-water bath for 30—40 min. Horse serum (0.94 ml) and RPMI-1640 medium (4.2 ml) were added, and the final mixture was poured on plastic Petri dishes (⌀ 60 mm, KOH-I-NOOR) the surface of which was treated with 0.05 % PDDA (poly-dimethyl-diallyl-ammoniumchlorid) in order to facilitate a strong adherence of leukocytes in consequence of electrostatic interactions (Kupper *et al.*, 1983). A 2 mm puncher was used to cut wells in the hardened agarose plates.

For LMI assay the leukocytes obtained from individual patients (collected not later than 2—3 hr before use) were preincubated at 37 °C for 90 min with four different VZV antigen concentrations obtained at dilutions 25.5, 76.5, 229.5, 688.5 of the stock material in RPMI-1640 supplemented with 10 % horse serum. Increasing antigen concentrations were used in order to detect the MIF response (Zachar — unpublished observation). The respective antigen dilution (22.5 μl) was added to 5.25 × 10^6 cells. Aliquots of the cell suspension (7 μl) were added into the wells in triplicate. The plates, containing cell suspension without antigen (control) and with antigen, were placed into a 37 °C incubator with 5 % CO_2 atmosphere and examined for 16—18 hr later on a Documator DL II (Carl Zeiss, Jena) apparatus. Cell migration areas traced on a sheet of transparent paper were measured using a planimeter.

Migration inhibition activity was calculated according to comparison of migration areas of leukocytes in the presence and in absence of VZV antigen and expressed in per cents according to the following formula:

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

LMI value extending 20 % was considered to be significant in the system used, e.g. witnessing of an increased MIF production.

Statistics. Statistical evaluation was carried out by Wilcoxon's rank test.

Results

Altogether 19 patients were treated with LLU and 28 patients were administered placebo. Significant difference ($P < 0.01$) between the LMI values observed in the LLU treated (26.8 ± 5.4 %) and placebo-given per-

Table 1. Positive outcome of the leukocyte migration inhibition test in patients suffering from herpes zoster whom was administered LLU or placebo

Interval studied	Days after first appearance of vesicles	LMI positivity in patients given	
		LLU	Placebo
I.	3—12 (3—7, (8—12)	7/7 (2/2) (5/5)	5/20 (0/5) (5/15)
II.	13—30	5/7	8/8
III.	35—60	4/5	samples not available

LLU: dialysed or ultrafiltered extracts of lysed leukocytes
LMI: for explanation see Fig. 1
Reduction of the migration area size extending 20 per cent comparatively to the control, was considered as positive result.
nominator: number of samples fielding a positive result in LMI
denominator: number of samples studied

sons (14.08 ± 6.15 %) was found in samples taken during days 3—12 (i.e. during the 1st interval) since the appearance of vesicular eruptions (Fig. 1). During days 3—7 the LMI values seen in HZ patients treated with LLU were in average 2.5-times higher than those seen in placebo-administered patients (n=5), each of them being positive (Table 1). The highest production of MIF, as measured in the described system was reached in both groups of patients, in average in the 2nd interval, i.e. during days 13—30. Considering the individual values, the decline of MIF response started beginning from day 20 after appearance of HZ blisters, i.e. in the stage when the clinical healing of dermal lesions was already completed at least for a week. Nevertheless, during interval 2 no significant differences between the LLU-treated and placebo-given groups were found (Fig. 1).

LMI values in LLU treated HZ patients (n=5) investigated during the interval 3, i.e. at days 35—60, fulfilled the criteria of a positive reaction, although, as MIF production is considered, a generally weaker response of leukocytes was seen in the presence of VZV antigen.

LMI values found in placebo-administered patients (n=28) suggest patterns of the natural dynamics at least of early and advanced stages of the MIF response in a matched group of adult patients. The average values seen in samples from the days 3—12 (14.08 ± 6.15 %) differed significantly ($P < 0.01$) from the values (30.5 ± 8.5 %) seen in samples taken at interval 2 (days 13—30), thus witnessing the specific CMI response (as paralelled by the MIF production) with a steep rise in the period between intervals 1 and 2.

Discussion

Higher activation rate of latent viral infections under conditions where a decline in the host defences (mainly T cell functions) is presumed (in elderly persons) or where an impairment of CMI may be present (in advanced stages of cancer) seems a frequent observation.

The pathogenesis of HZ — a disease with a wide age span — occurring mostly in otherwise "healthy" persons has not been definitely established as yet. Especially the triggering mechanisms leading to virus activation in the spinal ganglia are obscure. The general belief is that a certain still not clearly defined and covert CMI deficiency is needed for the clinical exacerbation of VZV latency.

The LLU, prepared from leukocytes of immunocompetent donors contains at least 200 different low-molecular moieties (Fudenberg, 1987). Substances with M_r of 3.5 kD antigenically nonspecific, amplify and accelerate the CMI activities in man, promoting the natural ability of the T4 cells (CD4) to produce important biological regulators such as MIF, IFN-γ and others. Substances with M_r of 2—5 kD are antigen-specific and have unique ability to transfer the specific information for the T cell-mediated immune responses to previously non-responsive host (transfer factor, TF). According to Fudenberg (1987), TF binds to immunologically uncommitted T lymphocytes rendering them antigen-sensitive and specifically responsive.

Our preparation of LLU exerted unexpectedly rapid and favourable effect upon parenteral administration in acute HZ, if applied at an early developmental phase of the disease. Acceleration of healing was associated with a significant cessation of virus shedding in the skin lesions of patients given LLU, unlike in the placebo group (Mayer *et al.*, 1987).

In the context of the present study it is of particular interest to note that rapid interruption of VZV replication in the HZ skin lesions coincides well with the time when increased LMI values were observed in the patients. This finding is consistent with the findings of Dörfling and Schröder (1987) concerning the presence of activated macrophage components in LLU preparations.

It seems that in some individuals with advanced age a still not defined defect(s) of T cells may develop facilitating the activation of VZV latency. The LLU administered in the critical phase of the mounting HZ may then stimulate apparently the CMI effector mechanisms, as witnessed also by our LMI data, targeted on VZV replication, what in turn reflects in beneficial clinical effects. LLU does not stimulate B cells neither in HZ nor during primary VZV infection (Zachar *et al.*, 1988). We cannot discriminate which component of LLU in our preparation was more beneficial, that augmenting the CMI response or that inducing the specific TF. Probably both components were instrumental, because substances with M_r 10 kD were present in the preparation from the "buffy-coats" of healthy adult donors who had experienced varicella by 80—90 per cent. The relative contribution of the discussed two main components warrant exploration using more purified and separated materials.

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