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DIAGNOSIS OF *HERPESVIRUS HOMINIS* INFECTIONS WITH THE USE OF EHRLICH MOUSE ASCITES TUMOUR CELLS

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Summary. — A new technique for the detection of *Herpesvirus hominis* (HVH) in materials from patients, based on mixed haemadsorption with Ehrlich mouse ascites tumour cells, was proposed. Investigations on 100 pustule punctates and smears from 75 patients with typical clinical signs of various primary and recurrent infections yielded positive results in 80% of cases. This was in good accordance with HVH isolations in various cell cultures. The new technique can, therefore, be recommended for routine diagnosis. Its main advantage is the rapid virus diagnosis.

Key words: *Herpesvirus hominis*; *diagnosis*; *mixed haemadsorption*

Introduction

Laboratory diagnosis of *Herpesvirus hominis* (HVH) is required especially in perilous conditions and for the exclusion of clinically similar symptoms. A rapid virological diagnosis is becoming particularly important in herpetic meningoencephalitis, herpetic sepsis of new-borns and in HVH-induced generalised atypical skin diseases as a prerequisite for an efficient treatment with antiviral chemotherapeutics (Kaplan, 1973; Eichmann, 1977; Wilken, 1978).

Routine HVH diagnosis by inoculation of cell cultures and subsequent typing in the neutralisation test takes under optimal conditions at least one week, but usually 2 — 3 weeks. The clinical diagnosis can thus be confirmed or corrected only with considerable delay.

The aim of the present investigations was to test whether mixed haemadsorption (Fagraeus and Espmark, 1961) with Ehrlich mouse ascites tumour cells (EATC) could be utilized in rapid and reliable diagnosis of HVH infections. The study was stimulated by the successful use of EATC in rapid

diagnosis of influenza (Adamczyk and Adamczyk, 1975; Adamczyk *et al.*, 1975). Virus isolation in cell cultures and the direct HVH assay by immunofluorescence were carried out for comparison.

Materials and Methods

Test materials. A total of 100 pustule punctates and smears from 75 patients with various primary and recurrent HVH infections were examined by inoculation in cell cultures and by mixed haemadsorption. The immunofluorescence test was carried out on 35 smears from skin efflorescences taken from 25 patients. The patients were selected based solely on clinical symptoms, mainly typical efflorescences: herpes labialis — 26 patients; herpes facialis (except herpes labialis) — 20 patients; herpes integumentalis (extremities) — 11 patients; herpes integumentalis (trunk) — 7 patients, eczema herpeticatum (generalized form), herpes genitalis and keratitis herpetica — 3 patients each; and stomatitis aphthosa — 2 patients.

Fifty smears from the skin and oral and pharyngeal mucosa from patients with various pustular dermatoses of no HVH aetiology and from healthy persons served as controls.

The materials destined for mixed haemadsorption and inoculation of cell cultures were immediately placed into gelatin solution (Schweizer, 1968) and kept at −20 °C until examined. For immunofluorescence assay, the materials from patients were smeared directly on microscope slides and fixed with acetone.

Virus assay in cell cultures. Three days old monolayer tube cultures of human struma cells and lung fibroblasts were inoculated with the test materials and examined daily under the microscope. If there were no cytopathic changes within 8 days, two further cell culture pasages were carried out, Any cytopathic agents were typed by appropriate immune sera in neutralisation tests.

Ehrlich mouse ascites tumour cells. The EATC used were continuously passed in SPF mice (AB albino mice of the ZIMET, Jena, breed). The mice were inoculated intraperitoneally each with about 10^7 EATC. Ascites, harvested under sterile conditions 6—8 days after inoculation, was used in the experiments.

Mixed haemadsorption. The method of Espmark (1965) proposed for virus diagnosis, was employed. We found in pilot tests that four prototype strains of HVH types 1 and 2 are adsorbed on to EATC. In the first step of the procedure, the materials from patients were incubated for 48 hr (this period of time proved to be optimal) in a suspension of EATC (3×10^6) cells per ml) at 34 °C. Then followed two washings with phosphate buffered saline (PBS), pH 7.3. The second step consisted in an incubation for 1 hr with specific HVH antibody. The respective antisera were obtained following an immunization schedule according to Plummer *et al.* (1970) as modified by Wutzler *et al.* (1976). The third step of the reaction included incubation with goat anti-rabbit globulin (produced by Staatliches Institut für Immunpräparate, Berlin). Unbound antibody was

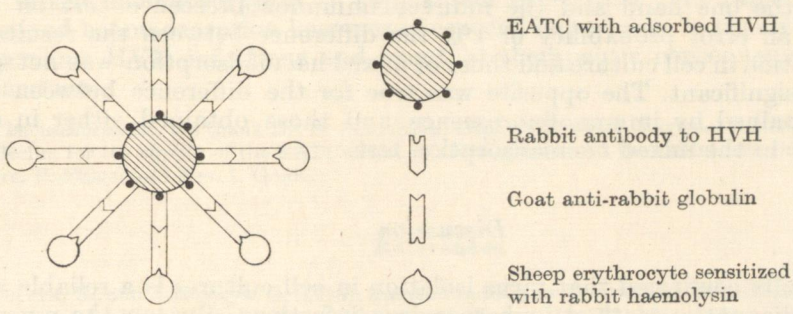

Fig. 1.
Arrangement of mixed haemadsorption for the detection of HVH

removed by two washings with PBS. To visualize microscopically the viruses adsorbed on to EATC, the cell suspensions were finally incubated with sheep erythrocytes sensitized with rabbit haemolysin (Hämolysin gegen Schaferythrozyten DAB 7; VEB Sächsisches Serumwerk, Dresden). In case of positive reactions, the adsorption of sensitized erythrocytes on to EATC led to the formation of the so-called "mulberry forms" which could be easily recognized in the light microscope (Fig. 1; and Fig. 2 — see Plate XI). HVH type 1-infected and uninfected EATC were included as a positive and negative control, respectively, in each experiment.

Direct virus assay by immunofluorescence. Acetone-fixed smear preparations of 35 test materials were incubated for 1 hr at 37 °C with rabbit anti-HVH immune serum, then washed for 10 min with PBS and stained with fluorescein isothiocyanate-labelled anti-rabbit globulin. The controls included a preparation treated with rabbit serum containing no HVH antibody and acetone-fixed HVH-infected struma cells. The preparations were examined in a fluorescence microscope in incident light, using a HBO 200 lamp and BG 12 and OG 1 filters. Bright fluorescence of the nuclei or cytoplasm of intact cells was evaluated as positive.

For *statistical evaluation* of the results, the 2I-test (Sachs, 1974) was employed.

Results

HVH was isolated in human struma cell and lung fibroblast cultures from 65 of 100 pustule punctates and smears taken from 75 patients with various HVH infections. Cytopathic changes were observed in 57, 7 and 1 case in the lst, 2nd and 3rd passage, respectively. On the average, the cytopathic changes appeared in either cell culture system after four days. Isolation attempts from smears of the control group were invariably negative.

HVH diagnosis by mixed haemadsorption was positive in 80 of the 100 materials examined. All materials of the control group were negative.

As distinct from the high positive detection rate in cell cultures and the mixed haemadsorption test, immunofluorescence revealed specific fluorescence of HVH-infected cells only in 13 out of the 35 materials tested. The majority of the smear preparations could not be evaluated in spite of the use of hyperimmune serum because the dead cells and leukocytes showed a rather strong nonspecific fluorescence.

The results of virus isolation in cell cultures showed an 81% coincidence with the results of mixed haemadsorption. The coincidence was only 43% between the results of virus isolation in cell cultures or of mixed haemadsorption on the one hand and the indirect immunofluorescence test on the other. At an error probability of 1%, the difference between the results of virus isolation in cell culture and those of mixed haemadsorption was not statistically significant. The opposite was true for the difference between the results obtained by immunofluorescence and those obtained either in cell cultures or in the mixed haemadsorption test.

Discussion

Our results confirmed that virus isolation in cell cultures is a reliable and sensitive diagnostic method in herpesvirus infections. But on the average 1-2 weeks were required for a definite answer including the typing of the cytopathic agent. Negative conclusions were possible with adequate security

at least after three weeks from the onset of the first pasage. The results obtained with 8 test materials which yielded HVH only in the 2nd or 3rd passage showed that several passages may be necessary. Because of the long time interval from acceptance of the material to the transmission of the required information, the detection of virus in cell cultures frequently has only little diagnostic value for the clinician. It must be stressed, however, that this classical mode of virus detection has a great advantage in its high reliability and sensitivity. Moreover it offers the possibility of isolating the virus for further studies like subtype identification in HVH. Finally, also other viruses can simultaneously be isolated in cell cultures, which might be helpful in the diagnostic elucidation of a given picture of disease.

The comparative tests with mixed haemadsorption yielded at least the same HVH detection rate as cell cultures. Moreover, HVH was detected in two smears from patients with typical skin efflorescences, in which cases the isolation experiments had to be discontinued due to bacterial contamination. As compared with isolation in cell culture, another advantage of the mixed haemadsorption is that, under optimal conditions, a diagnosis is possible within 2-3 days. An important prerequisite is the availability of fresh EATC. Preservation of EATC at −70 °C in dry ice or at −190 °C in liquid nitrogen, as recommended by Adamczyk and Adamczyk (1977) in influenza diagnosis, would probably be adequate for providing a steady supply of EATC for immediate use.

As compared with the relatively time and material consuming propagation, cultivation and passaging of cells, the easier technique of EATC passaging is another advantage. A disadvantage of mixed haemadsorption is the fact that in this way the herpesviruses are not isolated for subsequent typing. Moreover the use of a HVH specific antiserum excludes the possibility of detection by mixed haemadsorption of any other concomitant virus.

The relatively few positive findings by immunofluorescence are in contrast to the results of virus isolation in cell cultures and those of mixed haemadsorption. The nonspecific fluorescence of damaged or dead cells, tissue fragments and leukocytes was the most important disturbing factor.

Under optimal conditions, the two methods — isolation in cell cultures and mixed haemadsorption — supplement each other. The rapid diagnosis by mixed haemadsorption becomes of especial importance in timely diagnosis of perilous HVH infections and eventual therapeutic consequences in this connection.

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