

RESULTS

Ten basal cell carcinoma lesions were studied from patients aged 50 to 82 years and from 5 of them smears were prepared as well as sections. Using fluorescein-labelled anti-IgG, scattered foci of staining were found in the connective tissue of all the sections. In 3 cases of very faint fluorescence was visible in the cytoplasm of the carcinoma cells but was present to the same degree in the control sections too. The carcinoma cells proved easy to identify in the smears, all five of which showed a weak cytoplasmic fluorescence in about 70% of the tumour cells, the remainder being unstained. A similar proportion of cells in the control sections showed the same weak staining. Dilution of the conjugate to 1:64 quenched the fluorescence. There was no difference in staining between preparations treated with autologous serum and those treated with homologous serum. Staining with fluorescein-labelled anti-IgM was negative in all preparations. No surface membrane fluorescence was seen.

The faint cytoplasmic fluorescence was regarded as non-specific staining (4) because it was present in the control groups, was readily removed by diluting the conjugate, and was present to the same extent in preparations treated with homologous sera.

DISCUSSION

We have failed, using immunofluorescence techniques, to find evidence of anti-tumour IgG or IgM antibody in the sera of patients with basal cell carcinoma. Other explanations for this tumour's low metastatic potential should be sought. The high rate of individual cell death in basal cell carcinoma has been proposed (1) as an explanation of its slow growth rate, but the relationship of this finding to metastatic potential is unknown. Although non-immunological factors may well be important in the behaviour of basal cell carcinoma, a search for other immune responses, particularly cell-mediated, would be justified, as this tumour could represent one of the few situations in which host responses are relatively successful in limiting tumour spread.

REFERENCES

1. Kerr, J. F. R. & Searle, J.: A suggested explanation for the paradoxically slow growth rate of basal cell carcinomas that contain numerous mitotic figures. *J Pathol* 107: 41, 1971.
2. Lewis, M. G., Ikonopisov, R. I., Nairn, R. C., Phillips, T. M., Hamilton Fairley, G., Bodenham, D. C. & Alexander, P.: Tumour-specific antibodies in human malignant melanoma and their relationship to the extent of the disease. *Br Med J* 3: 547, 1969.
3. Lewis, M. G.: The role of circulating antibody in the control of metastases. *J Clin Pathol Suppl* 7: 83, 1974.
4. Nairn, R. C.: *Fluorescent Protein Tracing*. E. and S. Livingston, Edinburgh and London, 1969.

Rapid Diagnostic Tests for Cutaneous Eruptions of Herpes Simplex

N. K. Veien and B. F. Vestergaard

*Departments of Dermatology, Municipal Hospital,
and University Hospital, and Institute
of Medical Microbiology, University of Copenhagen,
Copenhagen, Denmark*

Received April 28, 1977

Abstract. Comparison was made of the results of virus isolation, indirect immunofluorescent staining (IF-test) and Tzanck smears from 32 patients with cutaneous eruptions clinically diagnosed as herpes simplex. Herpesvirus hominis was isolated from 21 patients; IF-test was positive in 22 patients; Tzanck smears were positive in 20 patients. 23 to 25 of the 25 patients in whom one of the diagnostic tests was positive could be identified by a combination of any two of the diagnostic methods employed. No false-positive reactions were observed for IF-test or Tzanck smear.

Key words: Herpesvirus hominis; Tzanck smear; Immunofluorescence; Herpes simplex

In 1948 Tzanck described a microscopy test for the identification of cutaneous disorders, using scrapings from diseased skin (Tzanck smear). The main area of employment for this test is in the diagnosis of herpes simplex, zoster and varicella (4). Immunofluorescent staining of smears (IF-test) from cutaneous eruptions and eruptions on the mucous membranes has also proven useful for the diagnosis of herpes simplex (1, 3, 7).

The rapid establishment of the diagnosis of a cutaneous eruption of herpes simplex is important when eczema herpeticum is suspected. It is, likewise, important that the diagnosis of genital

eruptions caused by Herpesvirus hominis (HVH) be made without delay if these occur near the time of delivery or when the differential diagnosis may be a contagious venereal disease (5, 6).

The purpose of this study was to show whether the IF-test and Tzanck smears are as valuable as virus isolation in the diagnosis of cutaneous eruptions of herpes simplex and whether the diagnostic sensitivity is enhanced by the use of more than one type of test.

MATERIALS AND METHODS

Thirty-two consecutive patients with a clinical diagnosis of herpes simplex participated in this investigation. Each patient had a history of recurrent vesicular eruptions in the same area, and physical examination showed grouped vesicular or crusted lesions on inflamed skin. The duration of the eruptions at the time of examination was 1–21 days (mean 6 days). Three patients with vesicular hand eczema and 2 with herpes zoster served as controls.

The vesicles were opened using a No. 15 scalpel blade, and both the contents of the vesicles and scrapings from the base were smeared on four slides. Additional vesicle contents and scrapings were placed in 1 ml transport medium (Eagle's minimal essential medium supplemented with 5% calf serum and antibiotics) and within 2 hours

taken to the virus laboratory where aliquots of 0.1 ml were added to four tissue culture tubes (2 with human diploid fibroblasts and 2 with a rabbit cornea cell line) essentially as previously described (7). From crusted lesions similar scrapings were taken after removal of the crust. No distinction was made between HVH types 1 and 2. For the Tzanck smears the material on two of the slides was fixed with Sprayfix® and stained with freshly prepared Giemsa for 10 min. Microscopy was performed before the results of virus isolation or IF-test were known. The criteria for a positive identification included the presence of multinuclear giant cells, while intranuclear inclusion bodies were not considered necessary (8).

The two remaining smears, used for the IF-test, were fixed in acetone at 4°C for 10 min, and a 1:100 dilution in phosphate-buffered saline (PBS) of a purified rabbit anti-HVH type 1 and 2 IgG preparation was applied for 30 min at 37°C. A similar antibody preparation is available from DAKO, Copenhagen (7). After rinsing twice in PBS for 10 min each time and briefly in distilled water, the slides were dried. Fluorescein-isothiocyanate (FITC) conjugated swine anti-rabbit IgG (DAKO F21090) diluted 1:20 in PBS was then applied for 30 min at 37°C. The slides were rinsed as described above and mounted with 12% Elvanol in PBS adjusted to pH 8.

These slides were examined using a Leitz fluorescence microscope equipped with an HBO 200W lamp, a KP 490 exciter filter and a K510 barrier filter. A sample was considered positive when there was obvious fluorescence of at least five intact cells. The microscopy was performed without previous knowledge of the results of virus isolation or Tzanck smears.

RESULTS

HVH was isolated from the lesions of 21 patients. The Tzanck smears were positive in 20 patients. There was agreement in the reading of the duplicate smears for 28 of the 32 patients. All patients in whom at least one slide was positive are categorized as positive. The number of epithelial multinuclear giant cells varied greatly from patient to patient, but not between the duplicate slides from the same patient. Intranuclear inclusion bodies were seen in approximately two-thirds of the positive slides and in none of the negative slides.

There were no positive smears from the 3 patients with eczema, and no intranuclear inclusion bodies were present in these smears. The slides from both patients with herpes zoster contained multinuclear giant cells as well as intranuclear inclusion bodies. HVH was not isolated from any of the control patients.

The IF-test was positive in 22 patients. Identical readings of the duplicate IF-test slides were made for all 32 patients. In the positive slides, intense yellow-green fluorescence was seen both in cells



Fig. 1. Fluorescent and non-fluorescent epithelial cells ($\times 260$).

Table 1. *The combined results of virus isolation, Tzanck smear and IF-test*

Virus isolation	Tzanck smear	IF-test	No. of patients
+	+	+	16
—	—	—	7
+	—	+	3
—	+	+	3
+	—	—	2
—	+	—	1
Total			32

slightly larger than leukocytes and in some up to five times this size. The latter were judged to be multinuclear giant cells. The most intense fluorescence was usually seen just below the cell membrane and in the cytoplasm, whereas nuclear fluorescence was weaker. In some slides, cells with a morphology similar to normal epithelial cells showed weak fluorescence. In no instance was this sufficiently intense as to be confused with specific fluorescence (Fig. 1). No fluorescence was seen in any of the slides from the control patients.

Table I shows the correlation between the results of virus isolation, IF-test and Tzanck smear. For 23 of 32 patients the results of the three techniques were identical.

The average duration of the eruption among patients with homogeneously positive tests was found to be 5 days, among those with homogeneously negative tests, 9 days.

DISCUSSION

The diagnostic tests employed in this investigation proved to be almost equally sensitive (Table I). However, it proved necessary to prepare two Tzanck smears from each patient in order to achieve this result.

20 of the 25 patients with positive diagnostic test results were identified using Tzanck smear as the sole diagnostic tool. The addition of virus isolation identified the remaining 5 patients. Combination of any two diagnostic tests used in this study will produce similar results. Although a combination of IF-test and Tzanck smear gave the poorest result, this combination identified 23 of the 25 patients in whom positive diagnostic tests were made.

IF-test was seen to be the single most sensitive diagnostic tool, and previous studies have shown

that distinction can be made between herpes simplex type 1 and 2 infections using this test (2, 7).

All three of the techniques employed gave consistently negative results for 7 patients. This might have been due to the fact that the average disease duration for this group of patients was 9 days, whereas it was 5 days in the group for whom homogeneously positive test results were found. The results of Tzanck smears and virus isolation are frequently negative in herpes simplex infections of more than 8 days' duration (8). It is also possible that not all of these patients had eruptions caused by HVH.

REFERENCES

1. Black, M. M., McQuillin, J. & Gardner, P. S.: A rapid diagnostic aid for Herpesvirus hominis infections in dermatology. *Dermatologica* 144: 45, 1972.
2. Fraser, C. E. O., Melendez, L. V. & Simeone, T.: Specificity differentiation of herpes simplex virus types 1 and 2 by indirect immunofluorescence. *J Infect Dis* 130: 63, 1974.
3. Gardner, P. S., McQuillin, J., Black, M. M. & Richardson, J.: Rapid diagnosis of Herpesvirus hominis infections in superficial lesions by immunofluorescent antibody techniques. *Br Med J* 4: 89, 1968.
4. Jones, E. W.: Cytodiagnosis. In *Textbook of Dermatology*, 2nd ed. ed. (ed. A. Rook, D. S. Wilkinson & F. J. G. Ebling), vol. 1, pp. 73–76. Blackwell, Oxford, 1972.
5. Nahmias, A. J., Josey, W. E., Naib, Z. M., Freeman, M. G., Fernandez, R. J. & Wheeler, J. H.: Perinatal risk associated with maternal genital herpes simplex virus infection. *Amer J Obstet Gynec* 110: 825, 1971.
6. Rubin, S. J., Wende, R. D. & Rawls, W. E.: Direct immunofluorescent test for the diagnosis of genital herpes virus infections. *Appl Microbiol* 26: 373, 1973.
7. Vestergaard, B. F. & Thybo, H.: Rapid diagnosis and typing of Herpesvirus hominis by the fluorescent antibody method. *Scand J Infect Dis* 6: 113, 1974.
8. Veien, N. K.: Cytology and viral and bacterial culture in herpes simplex, zoster and varicella. *Cutis*, unpubl. data.

Effect of Cyproterone acetate on Skin Surface Lipids

D. Fanta and M. M. Müller

2nd Department of Dermatology, and Institute of Medical Chemistry, University of Vienna, Vienna, Austria

Received July 12, 1977

Abstract. Skin surface lipids in female patients with acne were examined before and after treatment with the oral