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Assessment of a tissue transport-medium in preservation of tissue-fixed immunoglobulins and complement demonstrated by direct immunofluorescence

A pilot study with skin from SLE patients

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SUMMARY The present work was undertaken in order to test the value of a tissue transport-medium (Histocon) for direct immunofluorescence studies. For this purpose one skin biopsy was performed on each forearm of 26 patients with systemic lupus erythematosus. One of the specimens was left in ice-cold Histocon solution for 4, 8, or 20 hours, and the other was immediately quick-frozen. The results of the immunofluorescence tests with the two methods yielded similar results. It is concluded that the solution allows the preservation of tissue-fixed immunoglobulins and complement during short periods of transport.

Direct immunofluorescence tests on skin and mucosal biopsy specimens are valuable adjuncts in the diagnosis of vesicobullous dermatoses and systemic lupus erythematosus (SLE) (Jablonska *et al.*, 1975; Nisengard *et al.*, 1975). Currently, for the preservation of tissue-fixed immunoglobulins and complement, the method of choice is to quick-freeze the specimen and to keep it in a frozen state until it reaches the laboratory. This procedure has, however, hampered the wide use of immunofluorescence tests in clinical practice since facilities for quick-freezing are not always readily available.

Since 1971 biopsy specimens for histopathological diagnosis have been sent to our laboratory in a transport solution (Histocon) (Heyden *et al.*, 1972), which allows the specimen to be frozen after arrival. Cold microtome sections are routinely prepared for diagnostic reporting. This transport fluid and intra-laboratory freezing have been shown to allow combined histological, enzyme histochemical, and sometimes electron microscopical studies (Morgan *et al.*, 1978) to be made. The present investigation was designed to determine whether tissue transported in Histocon could also be used for immunofluorescent studies.

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Material and methods

Twenty-six patients (3 men and 23 women aged 21-73, mean 46, years) fulfilling the criteria for diagnosis of SLE as established by the American Rheumatism Association (Cohen *et al.*, 1971) were selected for study. Details regarding age, sex, and therapy are given in Table 1.

Two punch biopsy specimens (5 mm diam) from clinically normal skin were obtained from each patient. Under local anaesthesia (1% lidocain) the skin was taken from the dorsal side of each forearm approximately 10 cm below the elbow.

From each patient one of the two specimens was immediately frozen in isopentane, prechilled in liquid nitrogen, mounted on cryostat chucks, and kept on dry ice until it reached the laboratory. The other specimen was placed in ice-cold (0-4°C) Histocon (Histo-Lab, Göteborg, Sweden) and transported in an unfrozen but cold state to the laboratory. After intervals of 4, 8, and 20 hours this

Table 1 *Results of direct immunofluorescent studies of clinically normal skin in 26 patients with SLE*

Patient	*Age (yr)*	*Sex*	*Therapy*	*D/T*	*IgG*	*IgM*	*IgA*	*C_3*
1	73	F	CS+AT	D/T4	+/+	+/+	−/−	−/−
2	37	F	CS	D/T4	−/−	+/+	−/−	+/+
3	47	F	CS	D/T4	−/−	+/+	−/−	−/+
4	21	F	CS+AT	D/T4	−/−	+/+	−/−	+/+
5	40	F	CS	D/T4	−/−	+/+	−/−	+/+
6	34	F	CS+AT	D/T8	−/−	+/+	−/−	−/−
7	34	F	CS+AT	D/T8	−/−	+/+	−/−	−/−
8	40	F	CS	D/T8	−/−	+/+	−/−	−/+
9	33	F	CS+AT+AM	D/T8	−/−	+/+	−/−	+/+
10	41	F	CS+AT	D/T8	−/−	−/−	−/−	+/+
11	27	F	No	D/T8	−/−	+/+	−/−	−/−
12	27	F	No	D/T20	−/−	+/+	−/−	−/−
13	68	F	CS+AT	D/T20	−/−	+/+	−/−	−/−
14	27	M	CS	D/T20	−/−	+/+	−/−	−/−
15	46	F	CS	D/T4	−/−	−/−	−/−	−/−
16	63	M	CS	D/T4	−/−	−/−	−/−	−/−
17	69	F	CS+AM	D/T4	−/−	−/−	−/−	−/−
18	59	F	CS+AT	D/T4	−/−	−/−	−/−	−/−
19	35	F	CS+AM	D/T4	−/−	−/−	−/−	−/−
20	55	F	CS+AM	D/T4	−/−	−/−	−/−	−/−
21	47	F	CS	D/T8	−/−	−/−	−/−	−/−
22	46	F	CS+AT	D/T8	−/−	−/−	−/−	−/−
23	38	M	AM	D/T8	−/−	−/−	−/−	−/−
24	70	F	CS	D/T8	−/−	−/−	−/−	−/−
25	65	F	CS+AT	D/T8	−/−	−/−	−/−	−/−
26	48	F	CS	D/T20	−/−	−/−	−/−	−/−

CS = corticosteroids; AM = antimalarial drugs; AT = azathioprin; D = direct freezing; T = time (hours) in Histocon before freezing.

Table 2 *Fluorescein/protein ratios, antibody concentrations, and protein concentrations of antisera used*

	IgG	*IgM*	*IgA*	*C_3*
Fluorescein/protein ratio (molar)	2·4	2·4	2·6	2·5
Antibody concentration (μg/ml)	200	100	100	100
Protein concentration (mg/ml)	3·4	1·7	11·3	2·3

specimen was also frozen as above and stored together with the first specimen in a freezer at −70°C. Within one week all biopsy specimens were sectioned in a cryostat (−25°C) to a thickness of about 6 μm.

For staining, commercially available fluorescein-conjugated rabbit antihuman antisera (Dakopatts, A-S, Copenhagen, Denmark) to IgG, IgM, IgA, and C_3 were used. The properties of the antisera are listed in Table 2. Before staining, the sections were left to air-dry for 15 min. The incubation was performed in a moist chamber at room-temperature with a drop of conjugate for 30 min, the tissue was washed three times for 5 min in phosphate-buffered saline (PBS, pH 7·2), and mounted in 10% glycerol in PBS, pH 7·2. The working titre was found by serial dilution and subsequent staining to be 1:40 for IgG and 1:20 for IgM, IgA, and C_3. Whenever a positive reaction was detected, a control was used; the tissue sections were incubated with specific unlabelled antibody before the addition of conjugated antiserum.

The sections were examined with incident illumination. The microscope equipment consisted of a Leitz Orthoplan fluorescence microscope with a vertical illuminator. The light source was an Osram HBO 200 lamp. The objectives were Leitz oil immersion objectives. Filters used were: BG 12 and BG 38 for excitation, KP 490, dichroic mirror TK 495, and built-in barrier filter K 495 and K 510.

All sections were read blind, the investigator being unaware of the transport procedure used.

Results

Positive reactions appeared as homogenous, fibrillar, or granular patterns of bright green fluorescence in the dermal-epidermal junction of the skin.

Fresh frozen biopsy specimens from 14 out of the 26 SLE patients (nos 1-14) showed positive reactions for IgG, IgM, or C_3, or a combination of these findings. Almost identical results were obtained with the transport solution tested (Table 1). As shown in the Figure, no visible difference in intensity or pattern of immunofluorescence could be recorded with the two preservation techniques used. The only exceptions were the specimens from patients 3 and 8. These showed positive reactions for C_3 after Histocon

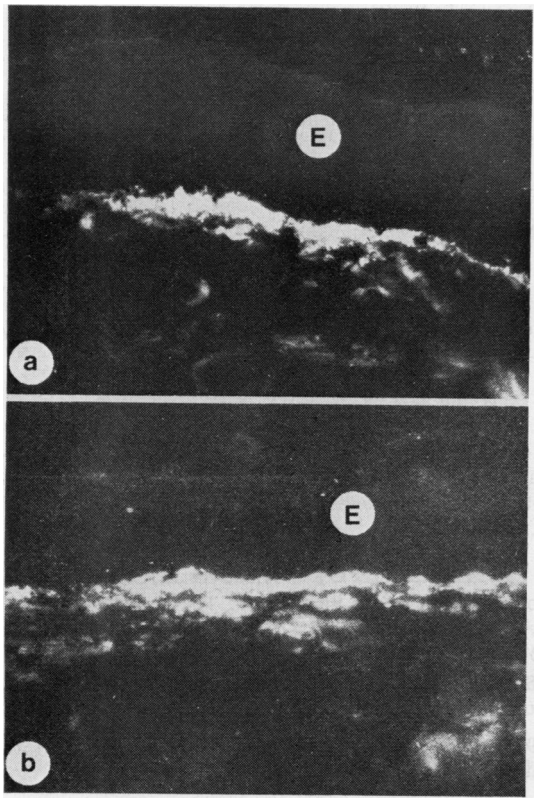

Figure *Comparison of IgM fluorescence in the dermal-epidermal junction (patient 1) in direct frozen (a) and transported specimen (b). E denotes epidermis.* (× *250*)

transport whereas the direct frozen biopsy specimens gave negative results. None of the subjects was positive for IgA. The remaining SLE patients (15-26) were negative for fluorescence in the dermal-epidermal junction with both techniques used (Table 1).

All the control tests, incubated with unlabelled antibody, were read negative.

Discussion

It is well recognised that the skin of patients with SLE frequently shows deposits of immunoglobulins and complement (Tuffanelli, 1972; Jablonska *et al.*, 1975; Monroe, 1977). Therefore we decided to carry out the present study on a group of patients with SLE.

The results have shown that Histocon is a useful tissue transport medium for immunofluorescent studies of tissue-fixed immunoglobulins and complement. Thus, with the use of cold Histocon and a transport time not exceeding 20 hours (Table 1) the results were almost identical with those obtained for fresh-frozen specimens. Unpublished data from our laboratory also suggest that the transport medium has equivalent preserving capacity on immunoglobulins, complement, and fibrinogen in other skin diseases, for example, discoid lupus erythematosus, pemphigoid, dermatitis herpetiformis, and lichen planus.

With the use of Histocon it was observed that two biopsy specimens were positive for C_3 whereas the direct-frozen specimens obtained from contralateral arms were negative. It is believed that this finding reflects differences in skin deposition of C_3 met with in the same patient.

In 1973 Michel *et al.* introduced a similar approach for the transport of biopsy specimens aimed at immunofluorescent studies. A liquid fixative (an N-ethyl malmeide buffer with ammonium sulphate) was used and excellent preservation of tissue-fixed immunoglobulins was reported. However, it was claimed that sections obtained for routine histology were not always as good as those after formalin fixation. Recently, in a comprehensive study, Skeete and Black (1977) investigated the value of the method described by Michel *et al.* (1973). It was concluded that the liquid fixative was useful but consistently reliable only if the specimens were received within one day of biopsy.

In the present series, all biopsies were performed on clinically normal skin. Nevertheless, tests for immunofluorescence were positive in 14 out of the 26 patients (54%) studied. This observation is, however, in accord with the experience of others (Kay and Tuffanelli, 1969; Burnham and Fine, 1971; Jablonska and Chorzelski, 1974), who reported positive reactions in 35-80% of uninvolved skin of SLE patients.

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