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ANTI-Ia-REACTIVE CELLS IN MYCOSIS FUNGOIDES: A STUDY OF SKIN BIOPSIES, SINGLE EPIDERMAL CELLS AND CIRCULATING T LYMPHOCYTES

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Abstract. Skin sections, epidermal cells in suspension and circulating T lymphocytes were investigated for the presence of Ia-like antigens in a group of patients with the cutaneous T-cell lymphoma mycosis fungoides (MF) and in healthy controls. Highly purified anti-Ia antibodies were used in indirect immunofluorescence, indirect peroxidase and peroxidase–antiperoxidase techniques. Allogeneic and autologous mixed leukocyte reaction tests with epidermal cells from MF lesions as stimulator cells were also performed. The majority of the dermal lymphohistiocytes and mononuclear cells in the epidermal Pautrier microabscesses expressed Ia-like antigens. So also did a greater proportion of circulating T lymphocytes than in the controls. In some MF patients a high percentage of single epidermal cells of non-lymphoid character also reacted with the antibodies. The possible significance of the present findings for the pathogenesis of MF is discussed.

Key words: Mycosis fungoides; Ia(HLA-DR)-like antigens; T lymphocytes; Mixed leukocyte culture reaction

Mycosis fungoides (MF) is a rare disease which is considered to be a T-cell lymphoma with its first symptoms in the skin. The clinical course varies widely and shows no appreciable correlation to distinctive histological features or other measurable parameters. The most current staging classification divides MF from the clinical point of view into three successive cutaneous stages: premycotic erythema (stage I), infiltrated plaques (stage II), and tumours (stage III). Later stages of the disease are characterized by the involvement of lymph nodes (stage IV) and blood and viscera (stage V) (30). The disease can also be tumorous from the beginning (*d'emblée* form) or be manifested as erythroderma.

The most typical histological feature (12) is the mainly lymphohistiocytic band-like dermal infiltrate comprising atypical mononuclear cells named mycosis (MF, Lutzner) cells (13). These cells may penetrate into the epidermis and gather in sharply demarcated cavities, so-called Pautrier microabscesses. Mycosis cells have morphological similarities to the abnormal circulating cells in the small cell variant of Sézary's syndrome (SS), a disease often considered to be the leukemic form of MF (4).

In situ studies have revealed that the majority of the infiltrating mononuclear cells, both dermal and epidermal, have T-lymphocyte characteristics (1, 3, 18).

It has been suggested by some authors that MF skin lesions are the secondary signs of a primarily malignant clone of T cells homing in the skin (5). Others believe that MF is a disease that starts in the skin as a reaction to a persistent antigen (25). In order to study these and other possibilities, it seemed pertinent both to further characterize the cells in the skin of MF lesions and to look for systemic abnormalities in MF.

We have previously shown that in MF the number of epidermal cells expressing Ia-like antigens is increased as compared with normal skin (26). In normal skin, epidermal Langerhans cells are Ia-expressive but the kerotinocytes and melanocytes are not (9, 16, 23). Ia antigens seem to be the critical membrane constituents of the macrophages for their capacity to present antigens to T-helper cells

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The gene product(s), coded for by a locus (or several loci) closely associated or identical with the HLA-D locus of the major histocompatibility complex in man, are called HLA-DR antigens or, in analogy with the terminology used in other species, Ia-antigens. Antigens reacting with immunosorbent-purified hetero-anti-Ia (HLA-DR)-antibodies are for brevity called Ia-like antigens in this paper.

Table I. *Clinical data*

Pat no.	Sex	Stage or type of MF	Age at first skin symptoms (years)	Duration (years)	Clinical picture at time of test
1	♀	II	64	3	Poikiloderma, plaques
2	♂	II	59	3	Few plaques
3	♀	II	71	10 (†)	Extensive plaques
4	♂	III	28	8	Plaques, tumours
5	♂	III	39	25	Plaques, noduli, tumours
6	♀	III	57	5	Extensive tumorous plaques
7	♀	III	58	9	Plaques, tumours, extensive erythema
8	♀	III	53	23	Extensive plaques, few tumours
9	♀	III	68	15	Plaques, tumours
10	♂	IV	73	2 (†)	Extensive tumours (*d'emblée*). MF histology in lymphnodes
11	♀	Erythroderma	66	3	Erythroderma. MF histology in skin
12	♂	Sézary's syndrome?	63	4	Erythroderma. Circulating Sézary cells. Dermatopathic lymphadenopathy

(15). The fact that Langerhans cells can replace macrophages in the antigen presentation to T cells has demonstrated the close association between functional characteristics of Langerhans' cells and their expression of Ia antigens (24). Morphological studies suggest that Langerhans cells are the focus of cell damage by mononuclear cells in contact dermatitis (20) and in MF (17). Recent data further indicate that antigen-activated T lymphocytes synthesize and express Ia-like antigens (6). The earlier finding of Ia-expressing lymphocyte-like cells among other epidermal anti-Ia-reactive cells in lesions of MF (26) therefore aroused our interest to further study of these different kinds of cells.

The present investigation comprised a morphological and functional study of Ia-expressing epidermal cells of lesions and an estimation of circulating T lymphocytes expressing Ia-like antigens in patients with MF.

MATERIAL AND METHODS

Patients

Skin specimens were obtained from 8 healthy persons and 11 patients with a clinical and histopathological diagnosis of MF. Patient No. 12 had erythroderma without conclusive histological evidence of MF, and had circulating Sézary cells and dermatopathic lymphadenopathy (Table I). Care was taken to obtain the biopsies from new, previously untreated lesions. Infiltrated plaques were chosen and tumours were avoided so that the material should be as homogeneous as possible.

Most patients were treated daily for 2–4 weeks with psoralen baths followed by UV-A irradiation (PUVA) (7). The treatments were generally given at 3-month intervals. All biopsies for this study were taken just before a new PUVA treatment period was started. Patients 6 and 11 had had no treatment before the biopsies. Five years or more prior to this study one patient (no. 9) had had short periods on cytostatics (cyclophosphamide, methotrexate, bleomycin) and 2 patients (Nos. 3 and 8) had had topical HN_2 treatment. Patient 12 received an intermittent low dose of methotrexate (15 mg/week) one year prior to this study, with no effects on the skin lesions. Patient 10 had had cytostatics (cyclophosphamide, vincristine, prednisone) 5 weeks prior to this investigation. Most patients in stage III had had local radiotherapy for single tumours.

Specimens of skin and blood

Biopsies were taken with a punch 3–4 mm in diameter from normal skin and lesions. They were quick-frozen in isopentane at −70°C and stored at the same temperature unless sectioned immediately. All these specimens were cut 6 μm thick on a cryostat.

Oval shave biopsies about 15 mm in the greater diameter were taken with a razor blade for immediate epidermal cell separation. Heparinized blood samples were drawn from the MF patients, from 6 patients with psoriasis (age: 22–76, mean: 60) and from 10 healthy controls. The patients had no signs of either local or systemic infection at the time of the blood sampling, which was performed on the same occasion as the biopsies were taken.

Antisera

The IgG fractions of previously described rabbit anti-Ia and anti-beta-2-microglobulin were isolated (10, 14). The IgG fractions of the anti-Ia antibodies were further purified by immunosorbent chromatography, either on Sepharose-coupled highly purified, detergent-solubilized Ia antigens isolated from chronic lymphatic leukemia cells (11), or on Sepharose-coupled highly purified papain-solubilized Ia (HLA-DR) antigens from pooled cadaveric spleens (K. Wiman et al., in preparation).

The Fab fragments of the IgG fractions of the anti-Ia

antibodies and of the normal rabbit IgG were prepared as described earlier (11).

Single-cell suspension of epidermal cells

Single-cell suspensions were prepared mainly by the technique described by Steinmuller & Wunderlich (21) with some modifications: The skin was cleaned with 70% ethanol. Shave biopsies including epidermis and superficial dermis were immediately placed in phosphate-buffered saline (PBS) and thoroughly washed. After blotting on dry filter paper the specimens were spread dermal side up in a Petri dish and soaked with 0.5% trypsin (type III, Sigma) in PBS for 20 min at 37°C. The dermis was easily removed with fine forceps and the exposed epidermis was covered with medium (RPMI 1640, 5% FCS, 20 mM HEPES and rubbed with the forceps. The resulting epidermal suspension was repeatedly pipetted, collected and spun down in the medium for 10 min at 500 *g*. Cornified flakes were left in the Petri dish. The pellet of epidermal cells was resuspended in 1 ml of 0.025% DNase (DN-25 Deoxyribonuclease I, Sigma) in PBS for 1–3 min at 37°C. Following a triple wash in the medium the suspended cells were counted. Viability was estimated by trypan blue exclusion and ranged in normal skin 65–95 (mean 83) % and in lesional skin 55–95 (mean 72) %.

Separation of T lymphocytes from peripheral blood

Mononuclear cells were isolated from heparinized peripheral blood by centrifugation on Ficoll-Hypaque as described by Bøyum (2). The cells were washed three times in PBS and then depleted of adherent cells by incubating $5–8\times10^6$ cells/ml in RPMI 1640 containing 10% FCS in Petri dishes for 45 min at 37°C. The non-adherent cells were washed twice with the medium, and are referred to in the following as peripheral blood lymphocytes (PBL). These were then fractioned in T and non-T lymphocytes by separating cells forming spontaneous rosettes with sheep red blood cells (SRBC) (8): Equal volumes of PBL (3×10^6 cells/ml) and neuraminidase-treated SRBC (1% suspension in RPMI) were mixed and incubated for 15 min at room temperature. Following centrifugation for 5 min at 80 *g*, the cells were kept at 4°C for 30 min. The pellet was resuspended, and rosette-forming cells were separated from non-rosetting cells by centrifugation on a Ficoll-Hypaque gradient (400 *g*, 20 min) in the presence of 10% FCS. Non-T cells were recovered from the interface and were washed three times. The pellet was carefully resuspended in RPMI with 20% FCS, and layered on top of a second Ficoll-Hypaque gradient. T cells were then recovered from the pellet after lysis of the SRBC by a Tris-buffered NH_4Cl solution. The purity of the T-cell populations, as determined by rerosetting with SRBC, was 97–98%.

Indirect immunofluorescence method (IIF) on sections

Air-dried 6 μm sections were initially washed in PBS for 10 min and then incubated with 50 μl portions of the different rabbit IgG fraction, diluted in PBS containing 4% bovine serum albumin (PBS–BSA). The IgG protein concentration of the anti-Ia antibodies was 0.01 mg ml^{-1}. The antibodies were allowed to react with the tissue sections for 30 min at room temperature in a humid atmosphere, were extensively washed in PBS and then allowed to react in a second step for 30 min with fluorescein-isothiocyanate (FTC)-conjugated porcine anti-rabbit IgG (Daco, Denmark, lot 107) at a working protein concentration of 0.1 mg ml^{-1}. After several washes in PBS the sections were mounted in glycerol containing 10% PBS and examined in a Leitz Orthoplan fluorescence microscope with incident light and blue narrow-band activation illuminated with a Philips Xenon XBO 75 lamp.

The specificity of the binding of the anti-Ia antibodies was assessed by replacing these by specific rabbit anti-human-beta-2-microglobulin and normal rabbit IgG at protein concentrations of 0.02 and 0.01 mg ml^{-1} respectively, and also by omitting the first incubation step.

Further specificity tests included preincubation of the specific anti-Ia antibodies with Raji and M●LT cells as previously described (27) before they were used as above in the IIF.

IIF on epidermal cell suspensions

$2–4\times10^5$ packed epidermal cells were incubated for 30 min in tubes at 4°C with 25 μl portions of the specific antibodies diluted in RPMI 1640, 5% FCS and 20 mM HEPES to a concentration of 0.01 mg ml^{-1}. After three washes in the medium the cells were incubated for a further 30 min at 4°C with the FTC-labelled porcine anti-rabbit IgG (working concentration 0.05 mg ml^{-1}) and then washed again three times in the medium. 25 μl of the cell suspension was examined on slides in the fluorescence microscope. Controls included normal rabbit IgG (0.01 mg ml^{-1}) in the first incubation step.

IIF on peripheral blood T lymphocytes

The highly purified peripheral blood T lymphocytes (5×10^6/ml) were incubated with the anti-Ia antibodies for 30 min and the following steps were carried out, essentially as described above.

The peroxidase–antiperoxidase method (PAP)

Principles according to Sternberger (22) were followed, by and large. After blockage of endogenous peroxidase with 0.3% H_2O_2 in PBS the 6 μm sections were allowed to react with 10% normal porcine serum for 10 min. The washed sections were then incubated for 30 min at room temperature in a humid atmosphere with the specific antibodies diluted in PBS to 0.01 mg ml^{-1}. After extensive washing in PBS the sections were exposed to porcine anti-rabbit IgG (Daco, lot 129A, conc. 0.8 mg ml^{-1}) for another 30 min and after several washings they were incubated in a third step with horseradish peroxidase–rabbit anti-horseradish peroxidase (horseradish peroxidase conc. 0.02 mg ml^{-1}, Daco, log 019). After extensive washing the enzymatic activity of horseradish peroxidase was developed with 3-amino-9-ethylcarbazol (Sigma) with H_2O_2 in acetate buffer, pH 5.5. The sections were counterstained in hematoxylin (Meyer) and mounted in gelatin–glycerin. Normal rabbit IgG in the first step (0.01 mg ml^{-1}), and omission of the first step served as controls.

Indirect peroxidase method (IIP)

To take advantage of the IgG-Fab fragments of the specific

anti-Ia antibodies, an indirect peroxidase method was used on some sections. After initial H_2O_2 treatment as above, the sections were allowed to react with 10% normal sheep serum and incubated with the rabbit anti-Ia IgG-Fab (0.04 mg ml^{-1}). After washings, the sections were exposed to horseradish-peroxidase-conjugated sheep anti-rabbit IgG-$F(ab')_2$ (Cappel, US, 11980). Development and counterstaining were performed as described above.

PAP and IIP on epidermal single-cell suspensions

All incubation steps and development were carried out in tubes at 4°C. The counterstaining was performed on slides. The same concentrations of the antibodies as mentioned above were used except for anti-Ia IgG-Fab, which were used at 0.02 mg ml^{-1}. Controls included normal rabbit IgG and IgG-Fab.

Autologous and allogeneic mixed leukocyte culture reactions (MLR)

MLR tests were performed in round-bottomed microtitre plates (Greiner, Nuertingen, FRG), using 5×10^4 responder cells (T cells) from patients or PBL from normal donors and 5×10^4 irradiated (3000 rad) stimulator cells (epidermal cells from patients or non-T cells from normal donors) in a total volume of 0.2 ml. The culture medium was RPMI 1640 supplemented with antibiotics/antimycotics, 2 mM L-glutamine, 10 mM HEPES and 10% heat-inactivated human AB serum obtained from male donors or heparinized plasma from the examined patient. The microcultures were incubated for 6 days in a humidified atmosphere containing 5% CO_2, and 1 μCi of [^{3}H]thymidine was added to each well for the last 4 hours. The cultures were then harvested with a Skatron multiharvester and processed for scintillation counting. Triplicate cultures were set up for each determination, and results are expressed as Δcpm, which is the mean cpm of stimulated cultures minus the mean cpm of control cultures.

RESULTS

Sections (IIF, PAP, IIP)

Skin from healthy controls and apparently normal skin from patients showed epidermal dendritic cells in a mainly suprabasal position staining with the anti-Ia antibodies. Scattered cells in the dermis as well as endothelial cells of blood vessels also reacted with the antibodies (Fig. 1*a, b*, 2*a*).

In the dermis of the MF lesions the majority of lympho-histiocytic cells in the more or less dense infiltrate of all patients in this study stained with the anti-Ia antibodies (Fig. 1*c, d, f, i*). The intensity of staining varied between different cells. In some patients, clusters of deeply located mononuclear cells did not stain, but in others, cells with this position were also brightly stained. With a less dense infiltrate some stained dendritic cells could be distinguished in the dermis. In sections consisting of epidermal cavities with mononuclear cells, most of these cells also reacted with the antibodies (Fig. 1*j*, 2*b*).

The anti-Ia reactivity of epidermal cells displayed a more varied picture in different patients (Fig. 1*c–i*). In some patients no obvious difference from normal skin was found. In others an increased number of dendritic cells, often arranged in groups, or lying in an unusually high position within the epidermis, and also stained round, non-dendritic cells were observed (Fig. 1*d–f*). In other patients not only dendritic cells but also units or islands of more or less flat cells were stained (Fig. 1*g*). In some patients all epidermal cells in the sections reacted with the antibodies (Fig. 1*h, i*). In Table II these four patterns are referred to respectively as normal, increased, units, and intercellular. The pattern of cells reactive with the anti-Ia antibodies in consecutive sections was the same when examined with IIF, PAP and IIP.

Normal rabbit IgG, normal rabbit IgG-Fab, and the Raji-preincubated anti-Ia antibodies gave no staining. The anti-beta-2-microglobulin stained all epidermal and dermal cells and the MOLT-preincubated anti-Ia antibodies stained as the specific antibodies but with a dilution effect of 3–4 dilution steps (IIF).

Epidermal single-cells (IIF, PAP, IIP)

The percentage of epidermal cells expressing Ia-like antigens was estimated by counting the cells reactive with the antibodies in IIF and PAP (in two cases, instead of PAP, IIP was used with IgG-Fab fragments of the primary antibodies). Cells with a brilliant rim fluorescence and reddish brown rim staining, respectively, were judged as positive, and about 300 cells were counted in each case.

The proportion of stained cells in normal skin from patients varied between 1 and 2% (mean 1.6%) and in 8 healthy controls between 2 and 3% (mean 2.2%) (Table II). In lesions the proportion of anti-Ia-reactive epidermal cells varied between 1 and 24% (Table II, IIF results). The values for IIF and PAP/IIP corresponded very well. Fig. 2*c–j* shows different cells reactive with the anti-Ia antibodies (PAP). Stained small epidermal lymphocyte-like cells were seen (Fig. 2*c–d*). Another type of stained cell consisted of round cells larger than lymphocytes, with a lower nucleus–cytoplasm ratio and a more dense nuclear appearance than would have been expected from the lymphoid cell

Table II. *Expression of Ia-like antigens (IIF) in sections and among individual epidermal cells and circulating T lymphocytes in patients with mycosis fungoides, and in control subjects*

Pat no.	Expression of Ia-like antigens					
	Epidermis				Circulating T cells	
	Sections		Separated cells %			
	Normal	MF lesion[a]	Normal	MF lesion	%	Total ($\times 10^9$/l)
1	N	N	nd	1	2	1.7
2	N	Increased	2	5	12	9.5
3	N	Units	nd	14	9	6.0
4	N	N	1	1	15	14.1
5	N	Increased	2	6	7	5.8
6	N	Units	nd	10	nd	–
7	N	Units	nd	12	25	35.5
8	N	Units	2	13	18	29.4
9	N	Intercellular	nd	24	15[b]	32.7
10	N	N	1	3	8	8.1
11	N	Intercellular	nd	24	nd	–
12	N	Intercellular	nd	23	26	81.3
Controls						
Psoriasis (n=6)	–	–	–	–	mean=2.7 range=0–6	–
Healthy controls (n=8)	N	–	mean=2.2 range=2–3	–	–	–
Healthy controls (n=7)	–	–	–	–	mean=2.8 range=0–5	–

[a] See Fig. 1 and Results for key to symbols.
[b] 2 months after skin biopsies, N = normal, nd = not done, n = number.

population (Fig. 2*f*). Fairly large stained irregular cells with small nuclei were also observed (Fig. 2*h–i*). The controls were negative.

Peripheral T lymphocytes reactive with the anti-Ia antibodies

Table III shows the percentage of circulating T lymphocytes in patients with MF and psoriasis and in healthy controls. The values for both categories of patients lay within the normal range for the method. The values for peripheral T cells expressing Ia-like antigens in healthy controls (range 0–5%) and in psoriasis patients (range 0–6%) are shown in Table II. From this table it is also seen that many of the MF patients had elevated levels of anti-Ia-reactive circulating T lymphocytes (range 2–26%).

Table III. *Percentage of circulating T lymphocytes in mycosis fungoides and in controls*

	T-cells (%)	
	Mean	Range
MF-patients (n=10)	70.6	66–79
Controls: Psoriasis patients (n=6)	70.3	68–72
Healthy controls (n=10)	72.8	67–78

Stimulation by the epidermal cells in allogeneic and autologous MLR

The stimulatory capacity of epidermal cells in suspension was tested in allogeneic and autologous MLR. Epidermal cell suspensions were prepared from 3 patients with MF who exhibited a markedly increased number of Ia-expressing cells (patients 7, 9 and 12) and one patient with a slight increase in these cells (No. 2).

The epidermal cells were strong stimulators in

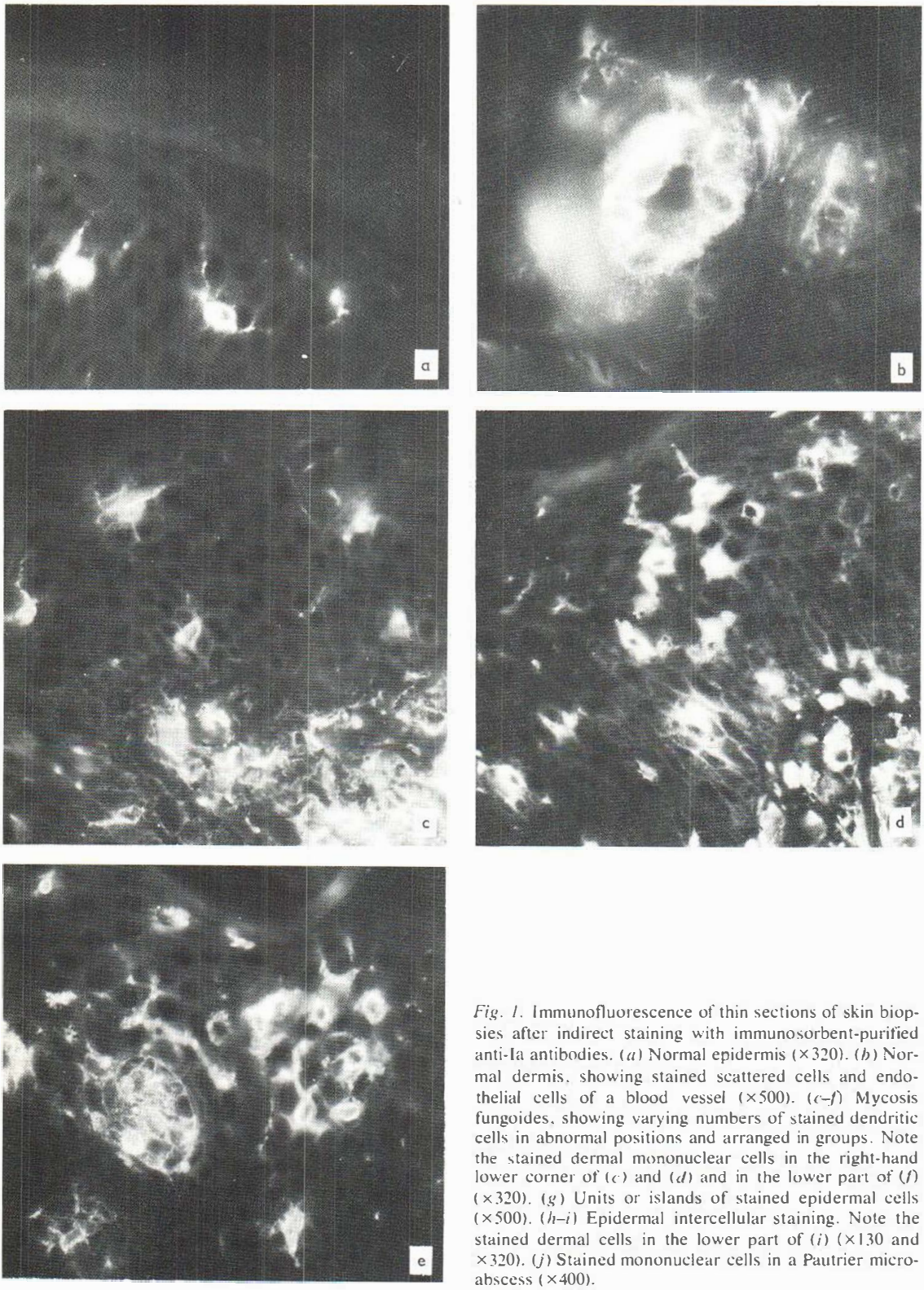
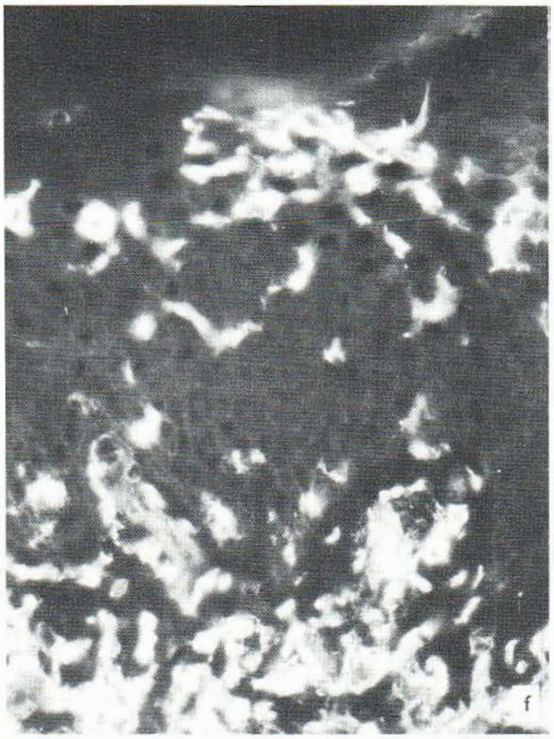


Fig. 1. Immunofluorescence of thin sections of skin biopsies after indirect staining with immunosorbent-purified anti-Ia antibodies. (*a*) Normal epidermis (×320). (*b*) Normal dermis, showing stained scattered cells and endothelial cells of a blood vessel (×500). (*c–f*) Mycosis fungoides, showing varying numbers of stained dendritic cells in abnormal positions and arranged in groups. Note the stained dermal mononuclear cells in the right-hand lower corner of (*c*) and (*d*) and in the lower part of (*f*) (×320). (*g*) Units or islands of stained epidermal cells (×500). (*h–i*) Epidermal intercellular staining. Note the stained dermal cells in the lower part of (*i*) (×130 and ×320). (*j*) Stained mononuclear cells in a Pautrier microabscess (×400).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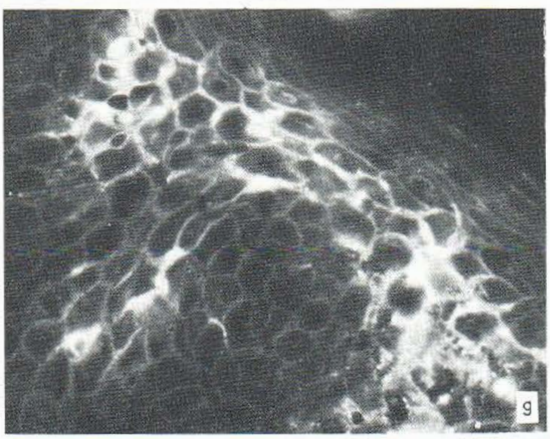
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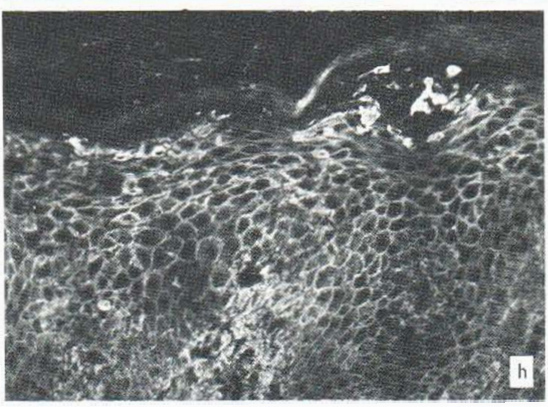
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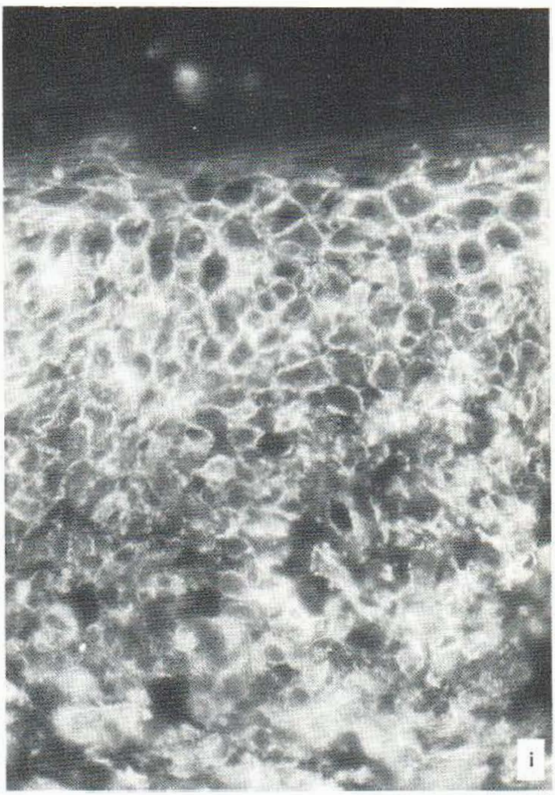
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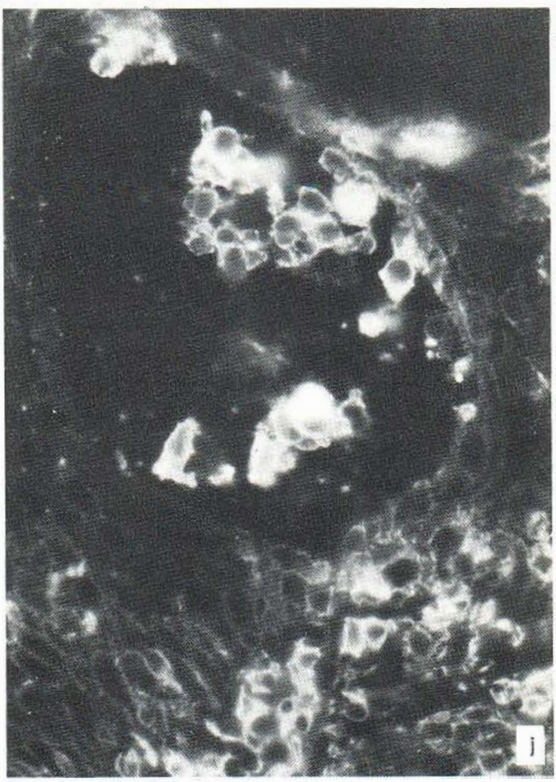
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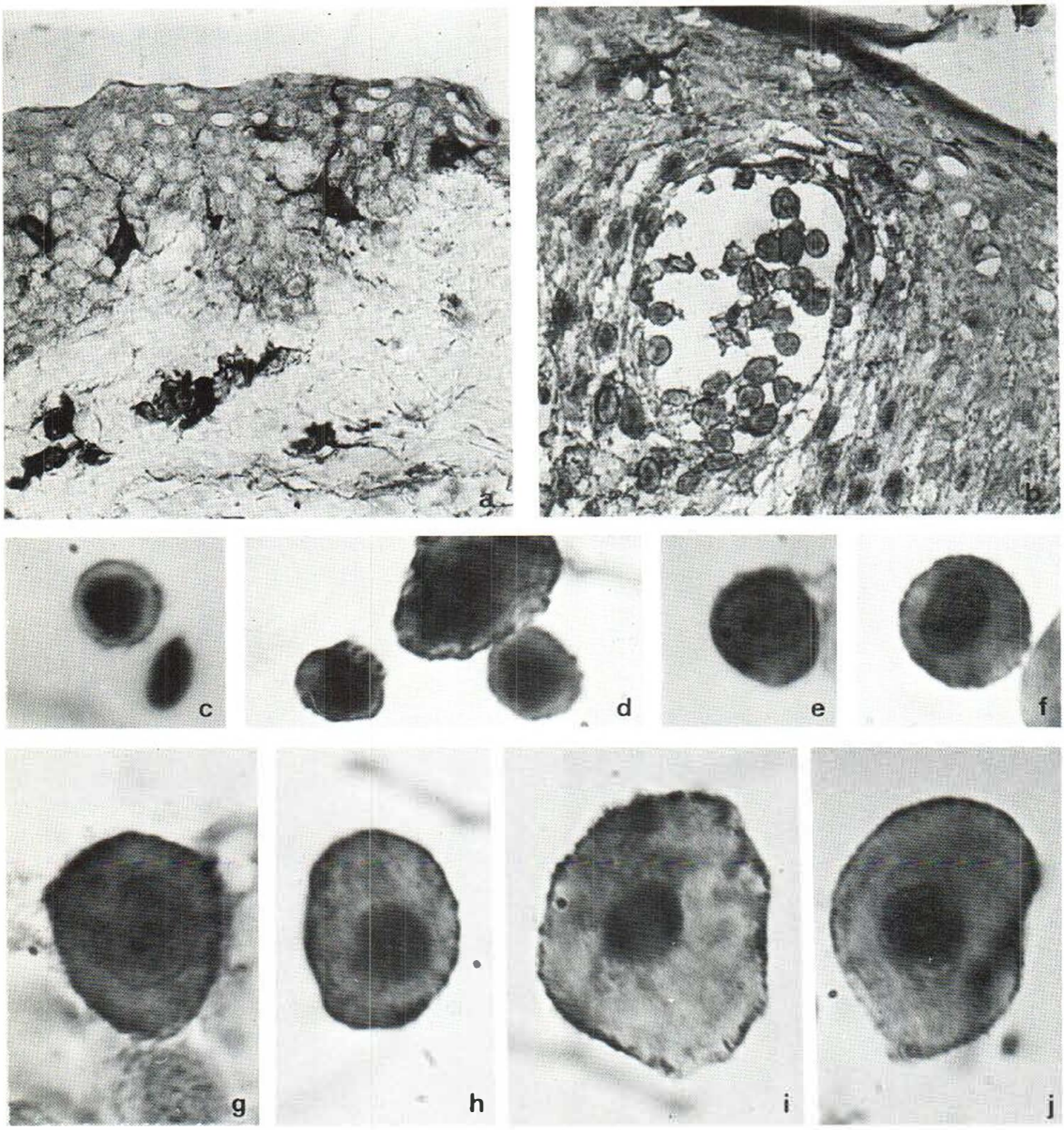

Fig. 2. Light microscopy of anti-Ia antibody reactivity in skin sections and among individual epidermal cells with the peroxidase–anti-peroxidase technique. (*a*) Normal skin (×320). (*b*) Pautrier microabscess. Besides stained cells in the cavity, one stained dendritic cell is seen in a high position (left upper corner) and two round stained cells are seen at the lower left side of the cavity (×400). (*c–j*) Stained, individual epidermal cells from patients with mycosis fungoides. (2*c–f*: ×1 000 and 2*g–j*: ×1 600).

allogeneic MLR in all cases, but in no case was there any stimulation in atuologous MLR (Table IV). T cells from patients with MF were, however, able to proliferate normally after stimulation with allogeneic non-T cells. No difference was noted when the cultures were supplemented with 10% heparinized plasma from the patient instead of 10% normal pooled human serum.

Table IV. *Stimulatory capacity of individual epidermal cells from MF skin lesions in allogeneic and autologous MLR tests*

PBL_{allo} = allogeneic peripheral blood lymphocytes, Epid. = individual epidermal cells, $T_{Pat.}$ = autologous T cells, non-T_{allo} = allogeneic non-T lymphocytes

Patient no.	Responder	Stimulator	Δcpm×10^3
2	PBL_{allo}	Epid.	21.6
	$T_{Pat.}$	Epid.	0.1
	$T_{Pat.}$	non-T_{allo}	9.8
7	PBL_{allo}	Epid.	9.1
	$T_{Pat.}$	Epid.	0.3
	$T_{Pat.}$	non-T_{allo}	11.8
9	PBL_{allo}	Epid.	27.4
	$T_{Pat.}$	Epid.	0.2
	$T_{Pat.}$	non-T_{allo}	29.4
12	PBL_{allo}	Epid.	32.8
	$T_{Pat.}$	Epid.	0.0
	$T_{Pat.}$	non-T_{allo}	15.0

DISCUSSION

In an earlier study of 8 patients with MF, the number of epidermal cells expressing Ia-like antigens was found to increase with the severity of the disease (26). Among the patients in this study no clear-cut correlation was seen between the number of epidermal cells expressing Ia-like antigens and the staging classification used. Furthermore, another study of sections from several biopsies from 20 different patients with MF revealed 2 patients with MF stage IV without any intercellular staining pattern and 2 patients with MF stage II who, in some poikilodermic lesions, showed a staining of all epidermal cells (U. M. T., unpublished observation). In the present material of 12 patients, 3 exhibited epidermal intercellular staining.

This raises the question whether patients with cutaneous T cell lymphomas can show epidermal intercellular Ia-staining transitorily as a response to some unknown stimulus. Patients with advanced disease and without intercellular epidermal staining in several biopsies might on the other hand have a different type of MF.

To further evaluate the significance of our findings, epidermal single-cell suspensions were prepared from both normal and lesional skin of patients with MF in different stages. In spite of the lower viability of cells in our suspensions, the numbers of normal epidermal cells expressing Ia-like antigens were comparable to those found by others (23). We thus considered the observed difference in the number of anti-Ia reactive cells from certain MF patients to reflect a true increase compared to normal skin. There was a good relative correlation between the findings in sections and among cells in suspension with regard to this anti-Ia reactivity (Table II). The study on single cells thus strengthens the observation (26) that certain patients with MF have lesions containing an increased number of anti-Ia-reactive epidermal cells as compared with normal skin. The fact that never more than one-fourth of the epidermal cells in the suspensions were stained, whereas most of the epidermal cells in comparable sections appeared to be stained may well have been due to irregularities in cell distribution in the large shave biopsies used for single-cell preparations. It may, however, also be due to destruction of cell membrane proteins during the preparation process.

Among the anti-Ia-reactive epidermal cells derived from MF lesions some small lymphocyte-like cells and a number of other apparently non-lymphoid cells, some of which had very small nuclei could be distinguished. Their exact nature cannot be determined, however, until immuno-electron microscopy is available for these cells. Thus cells related to classical Langerhans cells might be present in all preparations, although at least some anti-Ia-reactive cells from the lesions designated "intercellular staining" show features suggestive of a keratinocyte nature. Whether the antibody-binding structures are synthesized or passively absorbed by the respective cells cannot yet be stated.

In an immuno-electron microscopic study of anti-Ia-reactive epidermal cells in sections of 8 MF patients in stages II–III, Rowden et al. (17) did not find small epidermal Ia-expressing lymphocytes in any of their patients, nor an epidermal intercellular Ia distribution as described in this paper. Besides the possibility of a true disparity between the examined MF patients in the two studies, the discrepancy between the results may also be explained by technical differences. Apart from using a different Ia antiserum, Rowden et al. (17) looked at tumour specimens from their stage III MF patients, with a possible risk of degenerative and secondary alterations in such a material. Furthermore, they used a fixative for their biopsies which in our hands significantly reduced the immuno-reactivity of Ia-like antigens. The need to study repeated biopsies

from untreated patients was also illustrated by our studies.

In addition to the present finding of aberrant epidermal anti-Ia staining in MF lesions, a majority of the dermal mononuclear cells in all patients in our study also reacted with the anti-Ia antibodies. *In situ* studies have shown that most of these cells are T lymphocyte (1, 3, 18). If such dermal T lymphocytes do express Ia antigens, this would be of special interest regarding the recent findings that activated T lymphocytes, in contrast to most normal T cells, express Ia-like antigens (6). In order to further substantiate the possibility of an increased number of Ia-antigen-expressing T lymphocytes in MF, peripheral blood T cells from a number of MF patients were also investigated for anti-Ia reactivity. The increased number of anti-Ia-reactive T cells thus recorded suggests an altered state of a significant part of the T-lymphocyte population also in some patients with early stages of MF (Table II).

Could these findings—together with the increased percentage and aberrant pattern of anti-Ia-reactive cells found in the epidermis of MF lesions—somehow help to explain the pathogenesis of MF? The basic problem, with deep implications for the treatment of MF, is whether this disease has its origin in the skin as a reaction to a persistent antigen (25) for instance, or whether the skin lesions are only the secondary signs of a primarily malignant T-cell clone homing in the skin (5).

On the basis of the present results, it might easily be envisaged that some chronic immunological stimulus, for example of viral origin, on the cell surface of Langerhans cells (29) will give rise to continuous T-lymphocyte stimulation, followed by a T-cell attack on such antigen-modified cells. Rowden et al. have previously suggested such a target role of Langerhans cells in MF (17) similar to the proposed model for contact dermatitis (20). Such an attack might lead secondarily to the numerical and morphological aberration of Ia-expressing cells observed in the epidermis. A continuous flow of T lymphocytes interacting with primarily or secondarily aberrant epidermal Ia-expressing cells might eventually cause a malignant T cell transformation. The likelihood that these aberrant Ia-expressing cells from MF lesions might account for the stimulation in primary MLR (Table IV) and could consequently probably present antigens to T lymphocytes would seem to make this alternative possible.

The present finding of an increased number of probably activated peripheral T lymphocytes could also reflect a persistent antigen stimulation. On the other hand, these cells might also belong to a primarily aberrant T-cell population. Furthermore, at least hapten antigenic stimulation does not seem to cause an epidermal intercellular Ia pattern (27, 28) as has been described here and previously (26) for some MF patients. Other claim from ultrastructural studies that keratinocytes—not Langerhans cells—are the target cells for the immunological process in MF (19). Our initial attempt to test the antigen persistence hypothesis by investigating the stimulatory capacity of individual epidermal cells from MF skin lesions in autologous MLR tests (Table IV), has also so far failed to support this idea. The possibility that an autologous epidermal antigen is destroyed in the preparation of single cells, however, is one among other risks that this *in vitro* test might poorly reflect the *in vivo* situation.

In conclusion, the results of the present investigation do not definitely favour either of the two proposed pathogenetic mechanisms for MF (5, 25). The clinical impression of heterogeneity among different MF patients is probably reflected in the involved cells at a membrane level. This will complicate an interpretation and comparison of results in different large series of patients with this disease. Further functional characterization of cells from the cutaneous MF lesions, the lymph nodes and blood in well defined groups of MF patients should further elucidate these questions.

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