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Interdigitating reticulum cells in the dermal infiltrate of mycosis fungoides

An ultrastructural and immunohistochemical study

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Summary. In order to provide insight into the role of accessory cells in lymphoproliferative neoplasms, 7 cases of mycosis fungoides at various clinical stages – patches, plaques and nodules – were studied ultrastructurally and immunohistochemically. The aim was to establish whether interdigitating reticulum cells are a constant finding in the dermal infiltrate. Their possible relationships with mycosic cells were also investigated. This study revealed that interdigitating reticulum cells were present in all the skin lesions examined, were present in considerable number in the patches and plaques and became sparse in the nodules. Furthermore, in the lesions at various clinical stages these cells showed varying ultrastructural features, probably related to different developmental stages. The close contacts between interdigitating reticulum cells and mycosic cells, the expression of antigenic markers of activation by mycosic cells and the morphological and immunohistochemical signs of progressive de-differentiation of mycosic cells in the more advanced stages suggest that interdigitating reticulum cells are involved in stimulating proliferation and – possibly – neoplastic progression of mycosic cells. A role for the T-cell microenvironment created in the dermis by lymphoid infiltrate in inducing the differentiation of interdigitating reticulum cells from their precursors is proposed.

Key words: Interdigitating cells – Mycosis fungoides

Introduction

Previous ultrastructural studies on mycosis fungoides (MF) have demonstrated the presence of interdigitating reticulum cells (IRCs) – accessory cells typical of T-cell areas of lymph nodes (Veldman 1970; Kaiserling and Lennert 1974; Friess 1976; Groscurth 1980) and spleen (Heusermann et al. 1974), and of thymic medulla (Kaiserling et al. 1974) – in the dermal infiltrate (Goos et al. 1976; Kaiserling 1978). Based upon these findings, these authors have suggested that IRCs might play a role both in creating a microenvironment in which mycosic cells (MCs) selectively home and in stimulating their proliferation (Goos et al. 1976; Goos 1976). In contrast, other ultrastructural studies have failed to reveal any IRC in the dermis of MF skin lesions (Braun Falco et al. 1977), thus raising doubt about their possible influence on MCs.

In addition, immunohistochemical studies have demonstrated that IRCs contain S-100 protein and are able to express T6 antigen (Takahashi et al. 1981; Ralfkiaer et al. 1984; Goordyal and Isaacson 1985). Several reports indicate that in the dermal infiltrate of MF dendritic cells are commonly present which show a positive staining with anti S-100 antibodies (Igisu et al. 1983) or with anti T6 antibodies (Chu et al. 1982; Holden et al. 1982; McMillan et al. 1982; MacKie and Turbitt 1982; Smolle et al. 1985). However, since an ultrastructural examination was not performed in these studies, it remains unclear whether they are IRCs or Langerhans cells, which are also known to contain S-100 protein and express T6 antigen (Cocchia et al. 1981; Fithian et al. 1981; Goordyal and Isaacson 1985).

Because of these conflicting reports we performed an ultrastructural and immunohistochemical study on 10 skin lesions clinically defined as patches, plaques and nodules from 7 patients with MF, with the aim of establishing whether IRCs are present in the dermal infiltrate. A further aim of this study was to determine what relationship,

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if any, exists between IRCs and MCs, in order to provide additional insight into the still unsolved problem of the role of accessory cells in MF.

Case reports

Seven Caucasian patients were studied. Clinical evaluation included history and physical examination, hemogram, chest X-ray and computerized abdominal tomography, skin and bone marrow biopsies. Clinical diagnosis of MF was confirmed by histopathology. The patients were staged according to Broder and Bunn (1980). It should be stressed that in the patients who underwent therapy, treatment had been interrupted at least one month before biopsy.

Case 1. PM, a 52 year-old woman, had erythematous scaling patches localized on face, arms, palms and soles. First lesions appeared 20 years before our observation. The patient received topical steroid therapy. Clinical stage was assessed as IA. A first biopsy was taken from the left arm and a second one, three months later, from the left hand.

Case 2. MM, a 56 year-old woman, showed erythematous scaling patches on trunk and limbs. First lesions arose 16 years before our observation. The patient underwent P-UVA and topical steroid therapy. Clinical stage was assessed as IA. A first biopsy was taken from the left thigh and a second one, four months later, again from the same limb.

Case 3. BR, a 63 year-old woman, had erythematous scaling patches on trunk and limbs. First lesions arose 2 years before our observation. No therapy was given. Clinical stage was assessed as IA. Biopsy was taken from the left thigh.

Case 4. PMF, a 76 year-old woman, showed erythematous and oedematous plaques all over the body. First lesions arose 2 years before our observation. The patient was given topical steroid therapy. Clinical stage was assessed as IIA. Biopsy was taken from the abdomen.

Case 5. TR, a 53 year-old man, showed several erythematous plaques and a few nodules on trunk and limbs. First lesions arose 1 year before our observation. The patient was given topical steroid therapy. Clinical stage was assessed as IIB. Two biopsies were taken from two different plaques located on the left arm and the left gluteus respectively.

Case 6. VL, a 45 year-old woman, showed numerous erythematous plaques all over the body. First lesions arose 10 years before our observation. The patient underwent P-UVA therapy. Clinical stage was assessed as IIA. Biopsy was taken from the left forearm.

Case 7. MD, a 72 year-old man, had erythematous nodules all over the body. First lesions arose 8 years before our observation. The patient had received P-UVA, topical steroid and antiblastic chemotherapy. Clinical stage was assessed as IIB. Biopsy was taken from the left forearm. Diffusion of the disease led to the death of the patient 1 year after our observation.

Materials and methods

For electron microscopy, tissue fragments were fixed in 4% glutaraldehyde in 0.1 cacodylate buffer, pH 7.4, at 5° C for 3 h followed by postfixation in 1% OsO4 in phosphate buffer, pH 7.4, at room temperature for 2 h. The specimens were dehydrated in acetone series, passed through propylene oxide and embedded in Epon 812. Ultrathin sections were stained with uranyl-acetate and alkaline bismuth-subnitrate (Riva 1974) and examined with a Siemens Elmiskop 102 electron microscope at 80 kV.

For immunohistochemistry, tissue specimens were quickly frozen and cut at cryostat in 6μm thin sections, which were air-dried, fixed in a chloroform-acetone mixture for 5 min and incubated with primary monoclonal antibodies for 30 min (for their specificity see Gatenby et al. 1982; Foon and Todd 1986). The sections were subsequently incubated with sheep biotinylated anti-mouse serum (Amersham UK) for 30 min and then with streptavidin-biotin-peroxidase complex (Amersham UK) for 20 min. Peroxidase activity was demonstrated with aminoethyl-carbazole and hydrogen peroxide. The sections were finally counterstained with Mayer's haematoxylin. Normal human lymph nodes were used as positive controls. Sections incubated without the primary antibodies or without any antibody were used as negative controls for the second polyclonal antibodies and for the peroxidase enzymatic reaction respectively. A quantitative evaluation was performed by counting 100 cells per field in 5 consecutive microscopic fields at a magnification of $\times 400$. The percentage of labelled cells in respect to the non-labelled ones was then evaluated. Only cells whose nucleus was in the plane of the section were considered.

Results

In electron microscopy IRCs were present in the dermal infiltrate in large numbers in both the patches and the plaques but were sparse in the nodule. In the earlier lesions (cases 1, 2), judging by both their clinical and histopathological features – flattened patches with a scarce, mostly perivascular infiltrate – IRCs showed long cytoplasmic processes which gave to the cell a dendritic shape and tended to join with similar processes (Fig. 1) and cell bodies (Fig. 2) of neighbouring IRCs, thus forming a rudimentary cellular network. The nucleus was ovoid or irregularly shaped, but was always characterized by the presence of small chromatin clumps preferentially located peripherally. One or two nucleoli were present and sometimes a fibrous lamina apposed to the inner face of the nuclear envelope was also evident. Usually, IRCs showed rather numerous vesicular profiles of RER and a large Golgi apparatus.

Intermingled with these cells a few monocytes were found (Fig. 3), showing all the well-known ultrastructural features, and numerous cells with transtional features between monocytes and dendritic IRCs: compared with monocytes, the latter cells displayed a similarly dispersed chromatin and clear cytoplasm, but more abundant RER, more developed Golgi apparatus and more numerous cytofilaments. In some cases, a nuclear fibrous lamina could be observed. Furthermore, these transitional cells had a dendritic shape (Fig. 4).

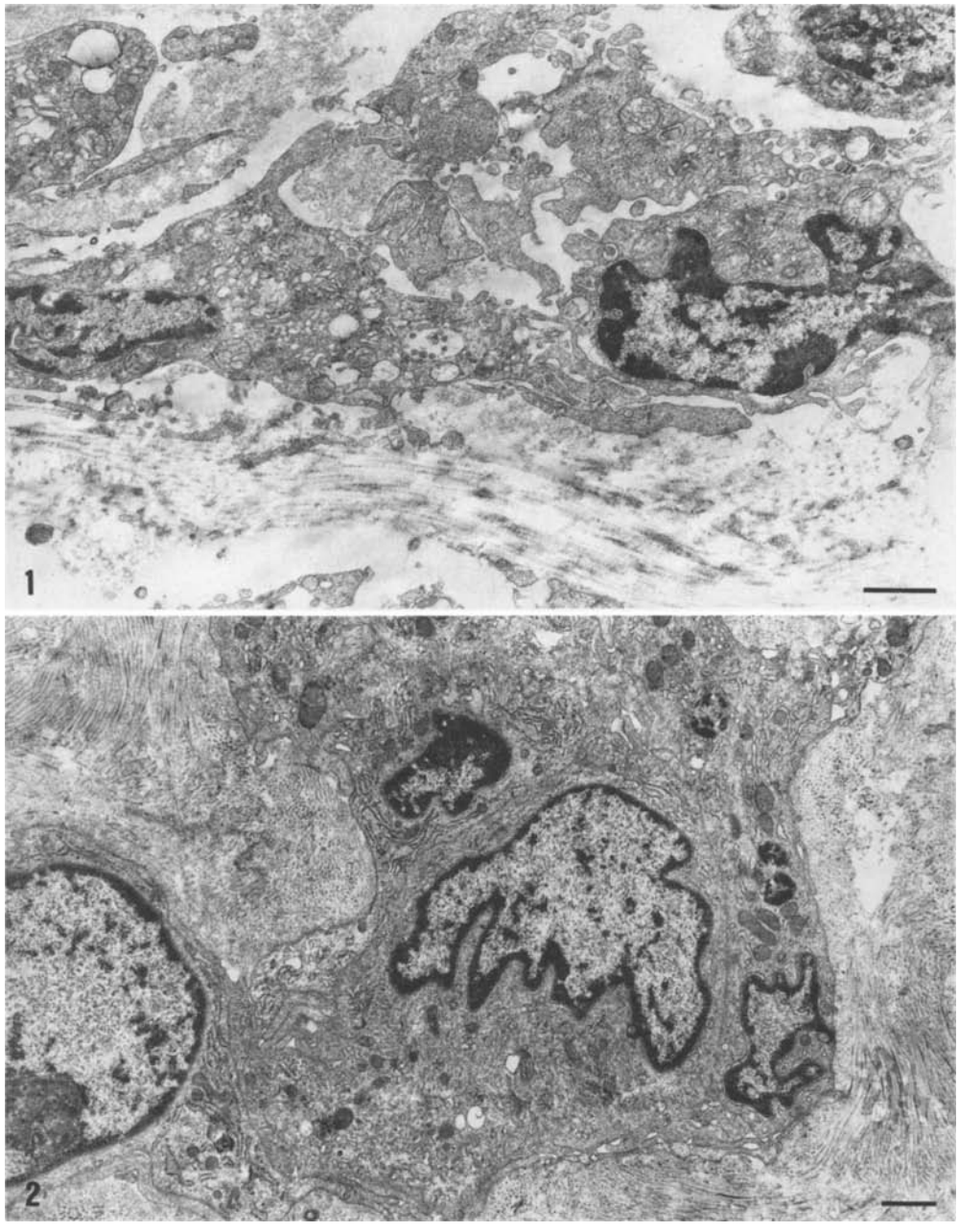

Fig. 1. Dermal infiltrate of mycosis fungoides. Patch stage. An interdigitating reticulum cell (*left*), containing moderate amounts of endoplasmic reticulum and showing cytoplasmic processes adherent to similar formations of interdigitating reticulum cells nearby, is seen near to a mycosic cell (*right*). EM, ×9600. *Bar* = 1 μm

Fig. 2. Dermal infiltrate of mycosis fungoides. Patch stage. Interdigitating reticulum cells are seen showing inconspicuous RER and closely apposed to each other. EM, ×7600. *Bar* = 1 μm

All the above cells established close contacts between each other, which may be considered as a marker for IRC lineage. Furthermore, IRCs were frequently encountered in contact with MCs (Fig. 1) but tight appositions, as seen in Fig. 4, were only occasional in the early lesions.

In the more advanced patches (case 3) and in the plaques (cases 4, 5, 6) – thickened lesions characterized by a denser dermal infiltrate – IRCs were more numerous and most of them showed morphological signs of enhanced functional activity (Fig. 5) in an abundant cytoplasm with large amounts of mitochondria, multiple Golgi stacks and numerous vesicles of rough and smooth endo-

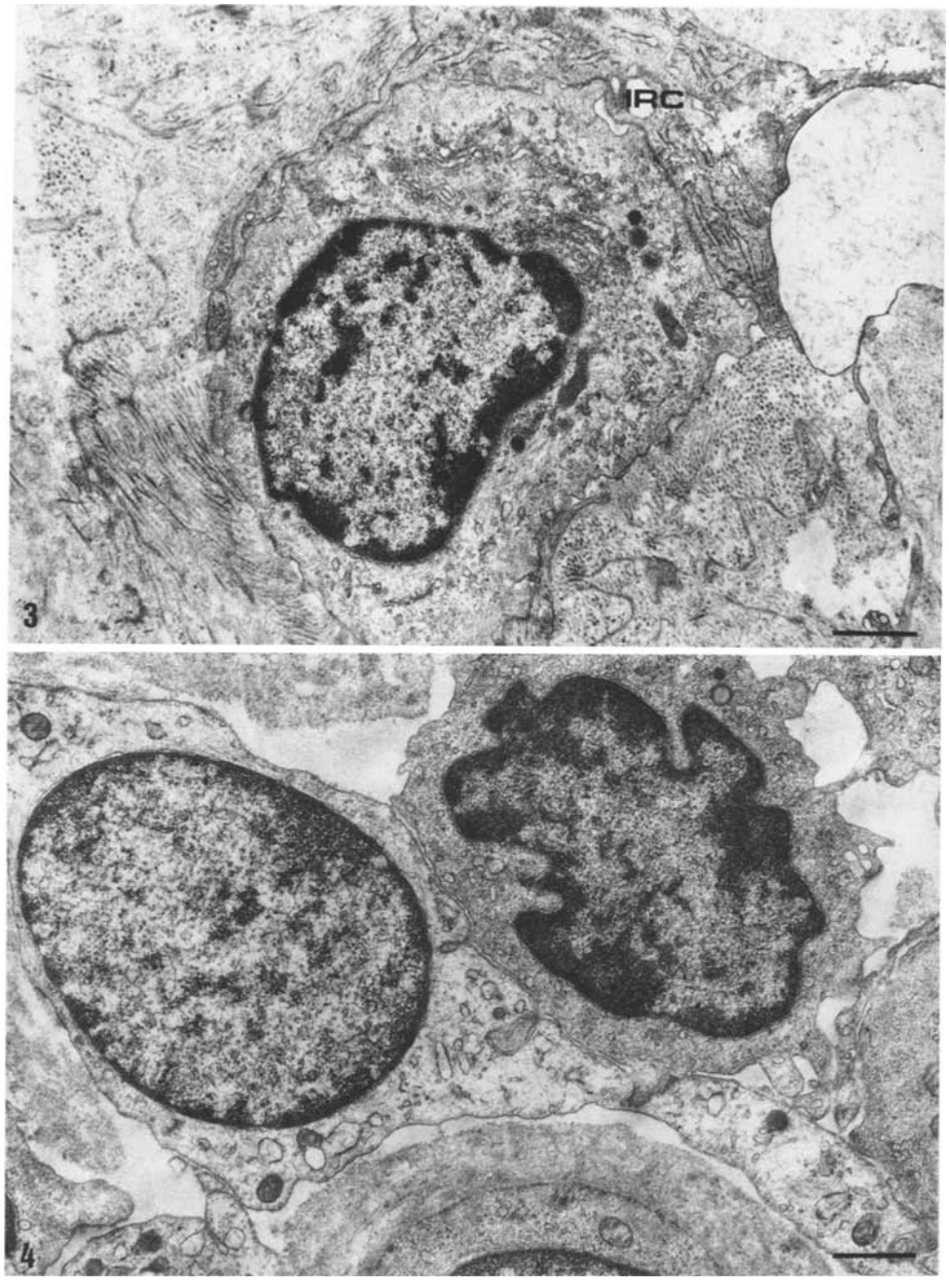

Fig. 3. Dermal infiltrate of mycosis fungoides. Patch stage. A monocyte is seen in close contact with a cytoplasmic process of an interdigitating reticulum cell (IRC). EM, ×11600. *Bar*=1 μm

Fig. 4. Dermal infiltrate of mycosis fungoides. Patch stage. A transitional interdigitating reticulum cell, dendritic in shape and showing some RER cisternae and nuclear fibrous lamina, tightly adherent to a mycosic cell (*upper right*). EM ×11600. *Bar*=1 μm

plasmic reticulum. Their nuclei generally had an irregular outline. The IRCs were provided with long and branched cytoplasmic processes (Fig. 6), formed an extended cellular network, and were always closely apposed to MCs, which frequently featured blastic and anaplastic cells – abundant cytoplasm filled with ribosomes and clear nucleus with large nucleoli (Figs. 5, 6).

In the nodular lesions (case 7) IRCs were sparse and less rich in organelles, and the contacts with MCs, most of which had blastic and pleomorphic features, became occasional (Fig. 7), despite the

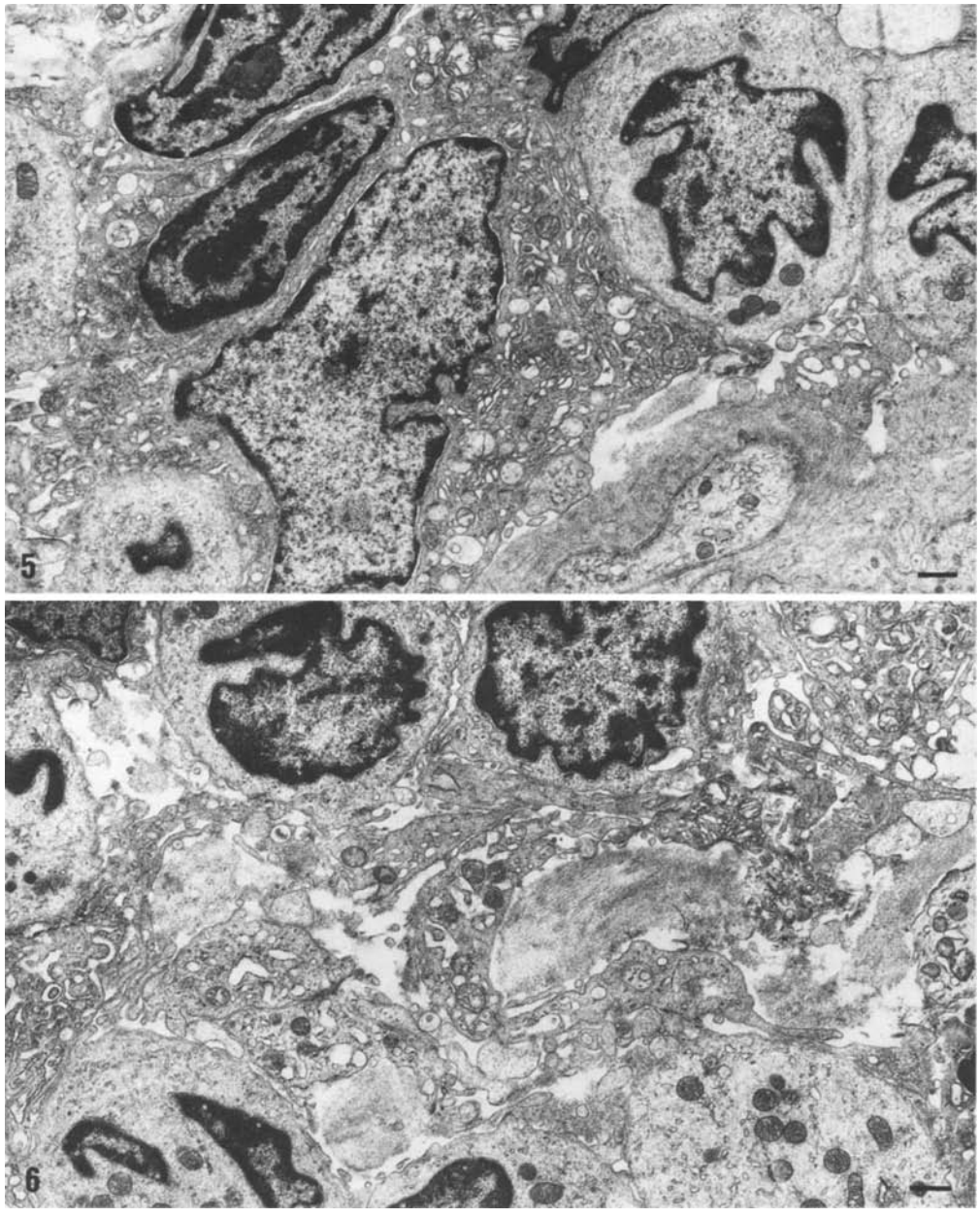

Fig. 5. Dermal infiltrate of mycosis fungoides. Plaque stage. An interdigitating reticulum cell, containing multiple Golgi stacks and several RER cysternae, is closely apposed to a mycosic cell with blastic features (*upper right*). EM, × 5600. *Bar* = 1 μm

Fig. 6. Dermal infiltrate of mycosis fungoides. Plaque stage. Several interlocking cytoplasmic processes of interdigitating reticulum cells forming an extended network and establishing intimate contacts with blast-like mycosic cells. EM, × 5600. *Bar* = 1 μm

large numbers and great density of the MCs infiltrating the dermis.

It should be stressed that, in all the numerous sections examined, only one Langerhans cell, recognized by the presence of Birbeck granules, was detected in the dermis and no MCs were found in the vicinity of this cell.

The results of the immunohistochemical study are summarized in Table 1. In the patches (cases 1, 2, 3) the majority of lymphoid cells expressed T11, T3 and T4 antigens, which are generally associated with T-cells, mature T-cells and helper/inducer subsets respectively. In the plaques (cases 4, 5, 6) the number of T3- and T4-positive cells decreased. In the nodule (case 7) only few and sparse lympoid cells were still T11-, T3- and T4-positive, the majority of them expressing none of these antigens.

In most of the lesions examined variable

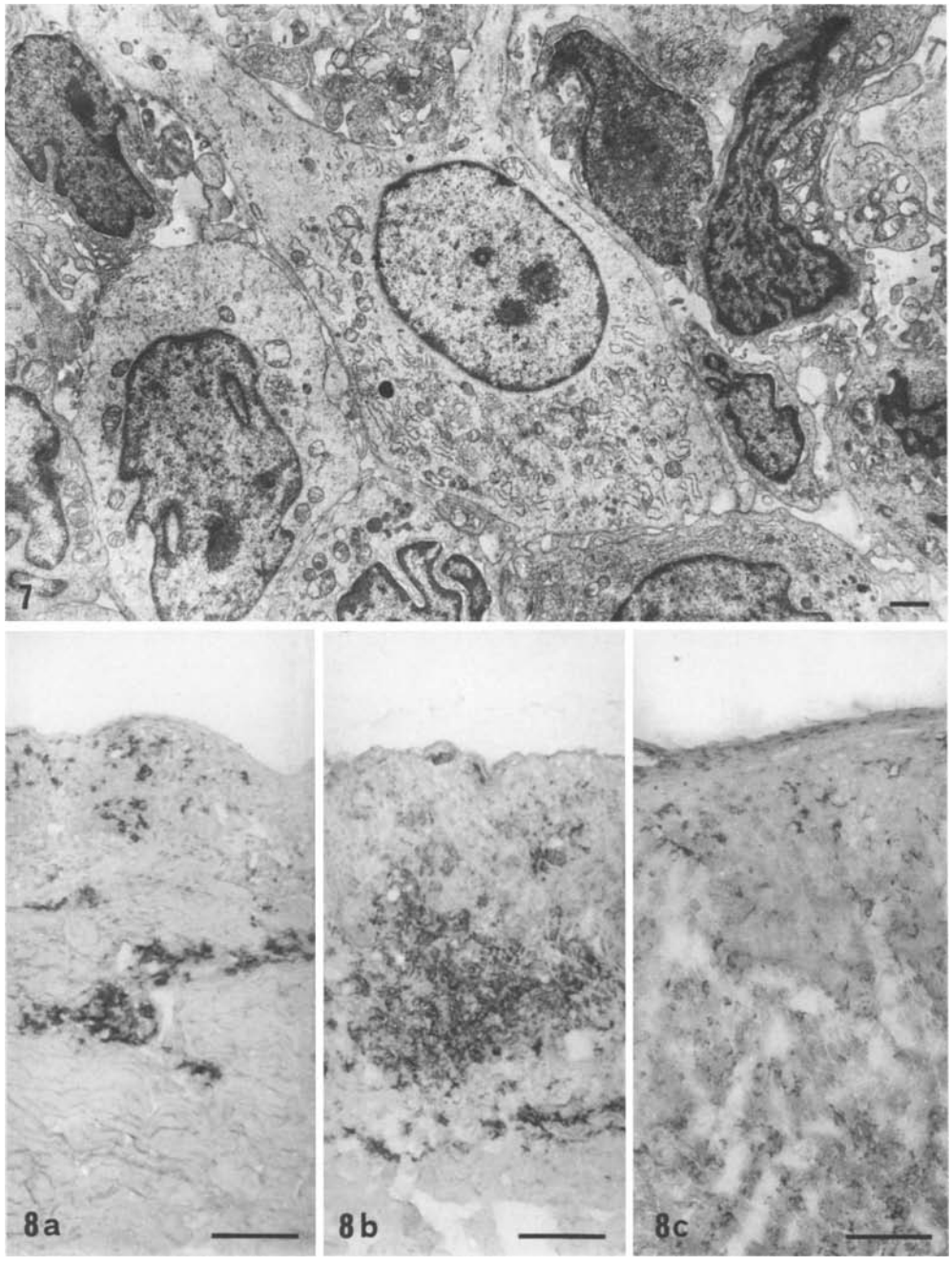

Fig. 7. Dermal infiltrate of mycosis fungoides. Nodular stage. An interdigitating reticulum cell with reduced organellular complement is seen touching some blastic mycosis cells, which show abundant cytoplasm, clear nucleus and prominent nucleolus. EM, × 5600. *Bar* = 1 μm

Fig. 8. Mycosis fungoides skin lesions. T6-positive dendritic cells are seen. **A** patch stage. **B** plaque stage. **C** nodular stage. Frozen sections, immunoperoxidase method, × 120. *Bar* = 100 μm

amounts of lympoid cells reacted with T9 and anti-IL2-receptor antibodies, which identify antigens usually associated with activated T-cells.

Dendritic S-100- T6-positive cells (Fig. 8) were already present in the early patches of case 1, were more numerous in the patches of cases 2 and 3 and in the plaque of case 4, and reached a maximum in the plaque of case 5. In the plaque of case 6, which could be considered a more advanced lesion by both its clinical and histopathological

Table 1

Antibodies	Case 1 patch %	Case 2 patch %	Case 3 patch %	Case 4 plaque %	Case 5 plaque %	Case 6 plaque %	Case 7 nodule %
OKT 11[a]	70	85	60	60	80	80	10
OKT 3[a]	60	80	50	30	35	10	5
OKT 4[a]	70	70	50	50	60	50	5
OKT 9[a]	10	10	20	15	10	less 5	less 5
Anti-IL2 rec.[b]	neg.	10	15	15	15	less 5	20
S-100[a]	10	25	25	30	30	15	less 5
OKT 6[a]	10	20	30	25	35	10	less 5

[a] Ortho, USA
[b] Becton & Dickinson, USA

features, S-100- T6-positive cells were less numerous and were present in very small amounts in the nodule of case 7.

Discussion

Our findings demonstrate that IRCs are usual components of the dermal infiltrate of MF. Our results are not in agreement with those of previous authors, who failed to reveal IRCs in the dermis of the MF skin lesions examined in their study (Braun Falco et al. 1977). This may be explained, in our opinion, by the fact that IRCs vary greatly in their cytological features in the lesions at different clinical stages. In fact, only in advanced patches and in plaques do IRCs show all the well-known ultrastructural characteristics and form an extended cellular network, similar to those of T-cell areas of lymphoid organs (Veldman 1970; Heusermann et al. 1974; Kaiserling et al. 1974a; Kaiserling et al. 1974b; Friess 1976; Veldman et al. 1978; Groscurth 1980) and hence can be easily identified. In fact, in earlier patches and in nodules IRCs do not show the typical features and therefore may escape identification. The variations in the cytological features of IRCs, especially evident in the earlier lesions, have been related to different developmental stages in a parallel study performed by us on the same cases examined here (Bani and Giannotti in preparation).

The close contacts between IRCs and MCs, the expression by MCs of antigenic markers of activated T-cells and the morphological signs of enhanced functional activity of IRCs in advanced patches and in plaques, strongly support the idea that IRCs are functionally related to MCs, probably playing a role in stimulating their proliferation. In the more advanced patches and in the plaques we observed activated IRCs in close apposition with blastic and anaplastic MCs. The latter progressively increase in number and loose their mature T-helper antigenic phenotype, according to previous reports (Holden et al. 1982; Willemze et al. 1983). These findings indicate that IRCs, by producing reiterated proliferative stimuli, may eventually induce the neoplastic progression of MCs. However, the decreased number of IRCs – also reported in previous immunohistochemical studies (Igisu et al. 1983; Willemze et al. 1983) – and the marked reduction of close contacts that we found in the nodule suggest the possibility that IRCs may only differentiate and persist under the influence of a mature T-cell microenvironment, such as that created in the dermis by the lymphoid infiltrate of MF (Goos 1976; Drijkoningen et al. 1987). This hypothesis is strengthened by the fact that T-cell lymphokines may be able to induce the differentiation of accessory cells (Schrader et al. 1984). Hence, it is conceivable that, when MCs loose their morphological, antigenic and – probably – functional characteristics of mature T-cells, the conditions which had previously induced the formation and functional activity of IRCs are no longer operating, and therefore these cells progressively diminish in number and reduce their contacts with anaplastic MCs.

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