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Study of T-cell Antigen Receptor Gene Rearrangement: A Useful Tool for Early Diagnosis of Mycosis Fungoides

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A 50-year-old man was admitted to the hospital with a diagnosis of eczema. Using study of T-cell receptor gene rearrangement on skin biopsies, a diagnosis of mycosis fungoides was made, then confirmed by evolution. This observation provides the opportunity to discuss the usefulness of molecular biology for the early diagnosis of mycosis fungoides. ***Key word: Cutaneous T-cell lymphoma.***

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The early diagnosis of cutaneous T-cell lymphomas (CTCL) remains difficult, even using sophisticated methods such as DNA cytometry, cytogenetic analysis, quantitative electron microscopy and monoclonal antibodies (1). The recent cloning of genes encoding the T-cell receptor has provided useful markers of clonal T-cell proliferation (3). Since December 1986, we have tested this new technique on blood, lymph-node and skin specimens from all the patients in whom CTCL was confirmed or suspected. In one of these patients, the T-cell gene rearrangement study was of particular interest, allowing a diagnosis of mycosis fungoides (MF) at the eczematous stage.

CASE REPORT

A 50-year-old man was admitted to the hospital for evaluation of chronic pruriginous dermatitis. The first cutaneous lesions had been noted 7 months prior to the admission; initially they involved palms and quickly enlarged, spreading to elbows, knees and scalp. The patient had been evaluated 3 months before, at another institution, and a diagnosis of eczema had been made, confirmed by skin biopsy. There was no personal or familial history of atopic disease or psoriasis. A number of topical steroid preparations had been prescribed without persistent improvement.

In May 1987, the general condition of the patient was good; generalized skin xerosis was observed as well as irregular erythematous scaling and slightly infiltrated plaques on the chest and in proximal areas. Hyperkeratosis and painful fissuring were noted on palms and soles (Fig. 1), with pachyonychia and distal onycholysis.

Other clinical findings were normal, particularly lymph-node areas, liver and spleen palpation. Cutaneous tests with standard allergens proved non-reactive. The laboratory findings were as follows: white cell count: 8 300/mm^3 with 59% neutrophils, 13% lymphocytes, 11% eosinophils (910/mm^3), 16% monocytes, 1% basophils; platelets: 365 000/mm^3, haemoglobin: 16.7 g/dl, E.S.R.: 4 mm per hour, fibrine: 5.5. g/l, uric acid 416 µmoles/l ($n < 360$), creatinine 92 µmoles/l ($n < 120$), gammaglobulins 7.5 g/l, gamma glutamyl transpeptidase: 43.6 Ul/l ($n < 38$); ALAT and ASAT were within normal ranges. The serum IgE was 110 kUl/l ($n < 150$). The tests for syphilis were negative. There were no Sézary cells on blood smears. Ultrastructural study of peripheral blood showed cerebriform mononuclear cells. Skin biopsy was compatible with the diagnosis of eczema (see Discussion). Immunophenotypic studies revealed that the infiltrate was: CD1+ (more than 75% cells) (Fig. 2), CD2+, Ia+ (more than 50%), CD4+ (less than 50%) and CD3+ (less than 25%). The CD1+ cells seemed to be either reactive cells or Langerhans' cells. However, clinical doubts remained and DNA analysis was performed: DNA was extracted from peripheral blood leucocytes by routine methods, and digested with endonucleases EcoRI, Hind III. Placental DNA was used as control. DNA from nitrogen liquid frozen skin biopsy specimens was extracted as follows: the skin specimen was reduced to powder by French's press, then incubated for 1 hour at 37°C in 100 mM tris HCl, 150 mM EDTA pH 8, 0.02% collagenase, then mixed for 1 hour at room temperature on a rotary wheel with 6M guanidine hydrochloride (from BRL, Bethesda) and 0.1 M sodium acetate pH 5.5. The suspension was incubated at 60°C for a minimum of 1 hour with 1% sarkosyl and 100 µg/ml proteinase K (Sigma, St Louis), before being layered under 2.5 vol. of absolute ethanol. The DNA was rinsed in 2×5 ml of 70% ethanol and resuspended in 10 mM tris HCl, 1 mM EDTA pH 7.5 on a rotary wheel overnight. Then DNA was digested with endonucleases EcoRI and Hind III. The DNA fragments were subjected to electrophoresis in 0.7% agarose gels and transferred to nylon filters by Southern blotting. Filters were hybridized with 32 P-labeled probes specific for Ig and TCR genes, washed under appropriate conditions and subjected to autoradiography. The two probes used in this study were an

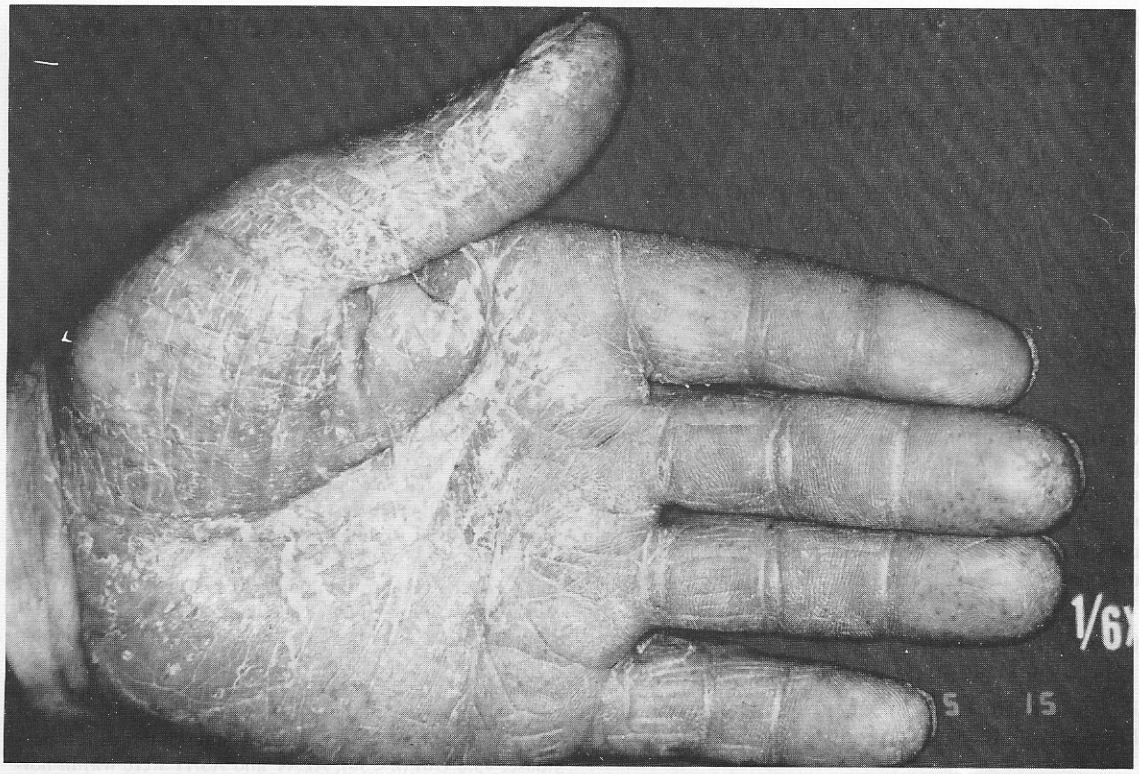

Fig. 1. Hyperkeratosis and fissuring (05/15/87).

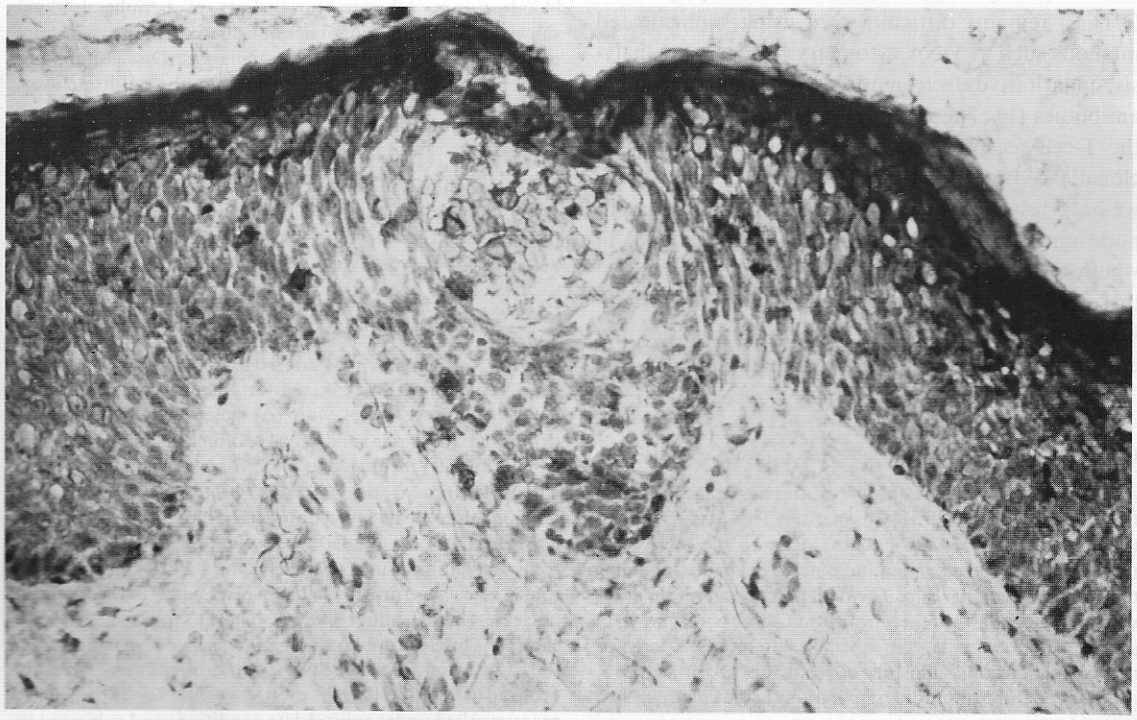

Fig. 2. CD1 staining of the infiltrate cells (×225).

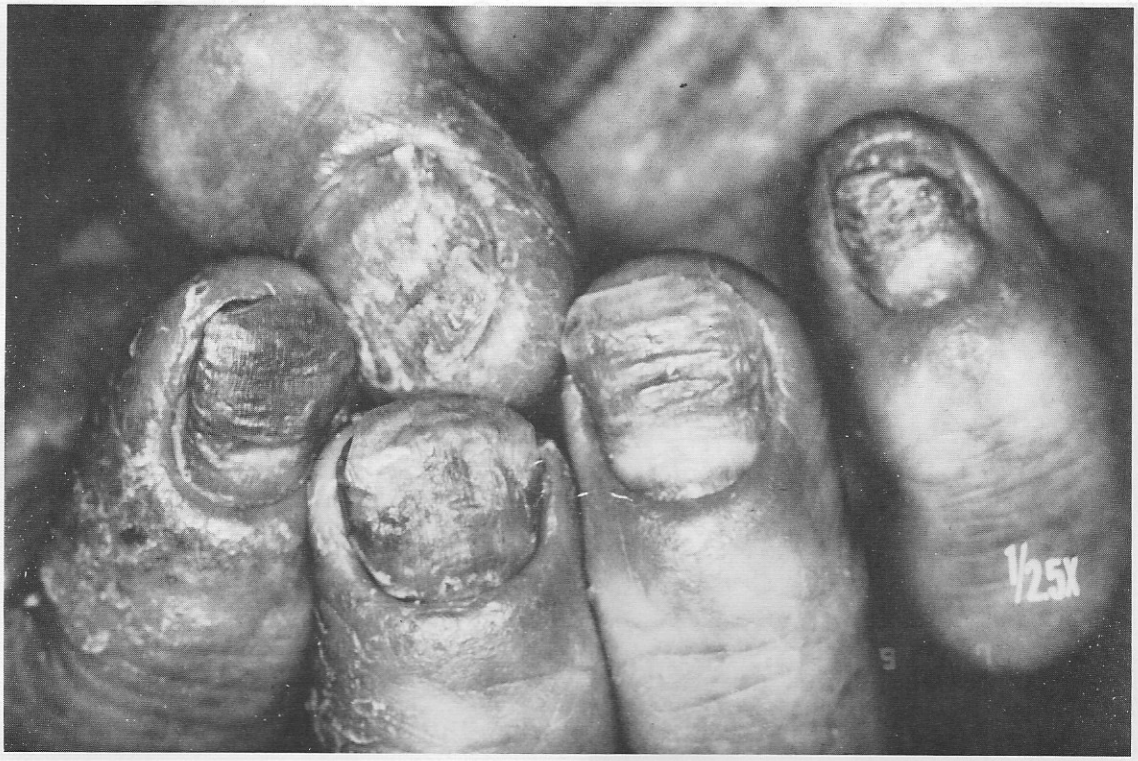

Fig. 3. Perionyxis and nail alterations (09/07/87).

Ig heavy-chain joining region JH probe (M 13 C76 R51A) and a TCR beta-chain gene probe (derived from YT 35). All rearrangements were shown using at least two different restriction enzymes in order to exclude the possibility of abnormal patterns being caused by DNA polymorphism. With the TCR β chain probe, 3 rearranged bands beside germinal configuration were found in the skin, but not in the peripheral blood, with a biclonal pattern of lymphoproliferation.

Evolution: three months later, by August 1987, in spite of intensive care with dermocorticoids, the dermatologic condition became worse and the patient was reevaluated. New cutaneous lesions were present on the flanks and axillar areas: they appeared as large round plaques with slight infiltration; hyperkeratosis of the palms and soles and nail alterations worsened (Fig. 3), and an axillary palpable lymphadenopathy, two centimeters in size, was found. There were no Sézary cells in the peripheral blood. The CD4/CD8 ratio was 2. A new cutaneous biopsy was interpreted as chronic eczema but dermal infiltrate was more important than on previous biopsies. Axillary lymphadenectomy examination showed partial lymph-node involvement, with persistent architecture; the infiltrate was pleomorphic, with large and small lymphocytes, plasmocytes and immunoblasts; these features were of the mixed type of lymph-node involvement (2). Unfortunately no DNA analysis could be performed. A treatment with methotrexate was begun (25 mg intramuscular weekly). Four months later, MTX was stopped, in spite of a partial improvement, because of liver test abnormalities. At that time, the diagnosis of MF was obvious clinically (Fig. 4) and histologically. A new skin biopsy was studied by molecular biology: a monoclonal pattern of lymphoproliferation was found, with only 1 rearranged band (TCR β probe) (Fig. 5); this band was identified as one of the rearranged bands found in the first skin specimen, on a different agarose gel because of the same length with both EcoRI and Hind III enzymatic digestion. Blood sample DNA remained in germinal configuration (Fig. 5).

DISCUSSION

Using the Southern blot hybridization technique, Weiss et al. (3) analysed the DNA of the β T-cell receptor genes in lymph-node specimens of patients with MF. Clonal rearrangements were not only found in all specimens showing definite histologic involvement but also in 7 of 9 lymph nodes that were histologically not diagnostic of MF. This study suggests that the DNA hybridization technique is more sensitive than histologic and cytologic examinations in detecting lymph-node and peripheral blood involvements in patients with MF. However, whether such studies will contribute to an early diagnosis of MF has

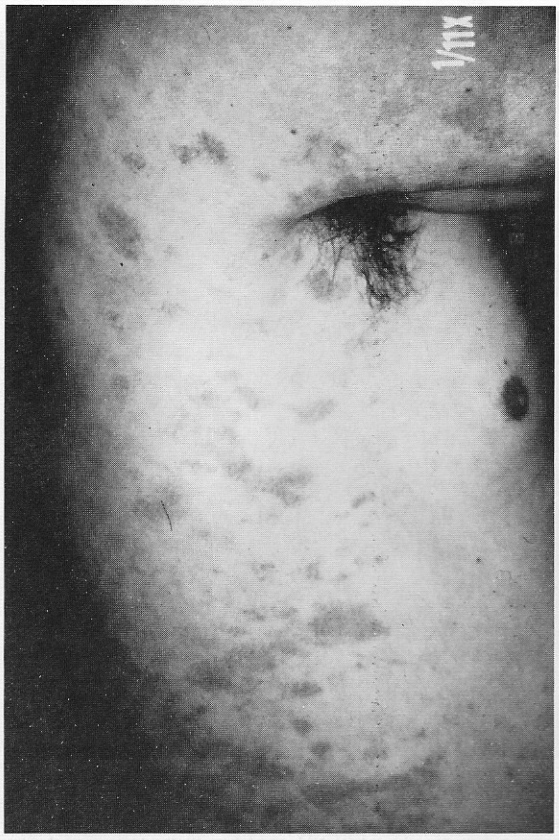

Fig. 4. Infiltrated plaques (12/07/87).

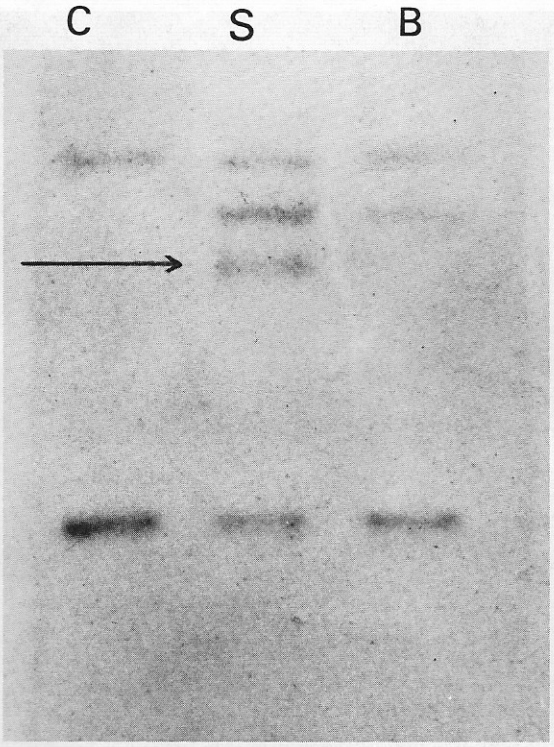

Fig. 5. Autoradiograph of DNA digested with EcoRI and then hybridized with the TCR-β chain probe. → = rearranged band in skin. C = control (placental DNA); S = second skin biopsy ; B = blood.

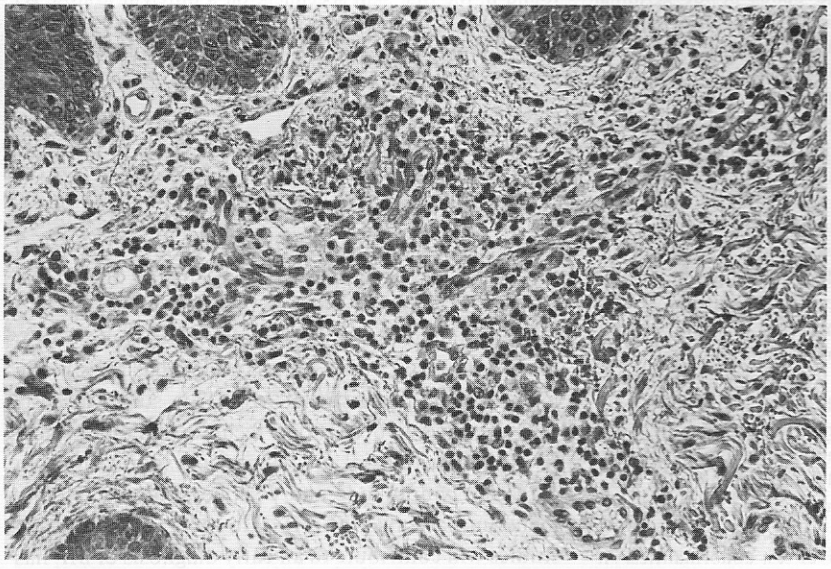

Fig. 6. Skin biopsy with more dense infiltrate (×200).

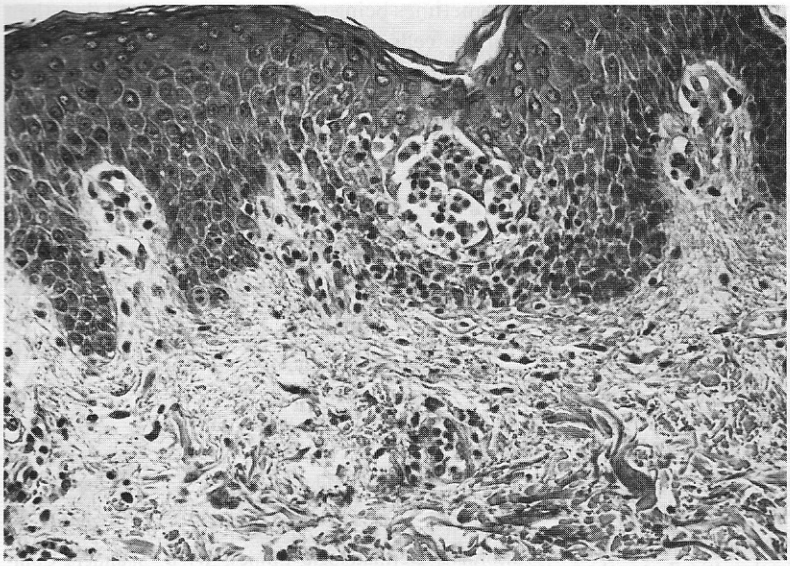

Fig. 7. Pautrier's microabscess (initial skin biopsy) (×200).

yet to be established. Our observations in this case provide the opportunity to highlight the usefulness of molecular biology for the early diagnosis of MF. The differential diagnosis, in our patient, was between eczema and MF. Histologically, serial skin specimens sections were examined *a posteriori*. On all histologic sections there was a perivascular infiltrate in the upper dermis; moderate on the first biopsies, the infiltrate was dense on the more recent skin specimens (Fig. 6). This infiltrate consisted mainly of neutrophils, and lymphocytes with convoluted nuclei. Of particular interest was the finding of a single Pautrier's microabscess on one section from our initial biopsy (Fig. 7), and of an unusual immunophenotype of the infiltrate as the cells express CD1 and CD2 but lack CD4 and CD3. As the disease progressed the number of cellular atypias and mitosis increased and the cells became more characteristic with indented nuclei (Fig. 8).

In summary, diagnosis of MF was suspected, but no criteria was completely positive: neither immunophenotypic staining of the skin, nor the finding of

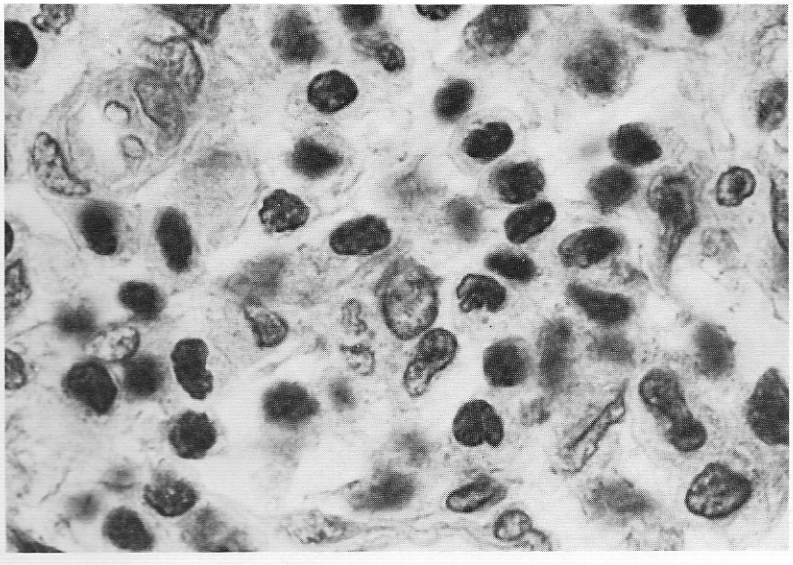

Fig. 8. Infiltrative cells with indented nuclei (×1 235).

cerebriform cells in the blood, were sufficient to establish an accurate diagnosis. The monoclonal T lymphoproliferation, proved by T-cell gene rearrangement study of the diseased skin, strongly supported the MF hypothesis, confirmed 6 months later by clinical and histological evolutions. At the time of the molecular study, the disease could be classified as stage I according to the staging system proposed by the Scandinavian MF Cooperative Group (4). Our results are in contradiction with those of Ralfkiaer (5) who could not detect any TCR β chain gene rearrangement, either in early suspected CTCL, or in the lesions of plaque stages of MF. Perhaps, in our patient, the MF was a particularly aggressive variant of the disease; indeed, lymph-node involvement was obvious only a few months after the first cutaneous manifestations. Finally, in our patient, the detection of 2 distinct monoclonal populations in the skin, only one of which could be identified in the skin 7 months later, leads to 2 hypotheses: firstly, it is possible that the 2 populations are present in the skin, one of them being undetectable because of insufficient sensitivity of the technique; secondly, that one of the monoclonal populations was selected in the evolution of the disease.

In conclusion, study of T-cell antigen receptor gene rearrangement is a very promising technique in the early evaluation of CTCL. However, it must be stressed that this method can only be used in conjunction with other diagnostic techniques. Moreover, finding a rearrangement is synonymous of monoclonality, but does not necessarily mean malignancy. Finally, gene rearrangement study diagnosis value is limited by the level of abnormal T cell clone infiltration sometimes being too low to be detected (less than 5% of the cells studied). Polymerase chain reaction (6), the sensitivity of which is 10^5-fold greater than Southern blotting, might be applied to the early diagnosis of CTCL but it remains an investigative method.

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