

liquid water between electrically charged plates, and we made a general statement that an electric field mechanism helps align the water molecules into ice-like clusters.

Thus, our analysis does not involve the difference in energy between "ice" and "water" but is rather a comparison of the interaction energy of the polar substrate crystal with an ice nucleus (partially) proton-ordered along the hexagonal axis as against a nonpolar substrate with (completely) disordered ice nucleus.

Model calculations (Table 1) imply that unless polar ice is introduced at the nucleation stage, there is no advantage of D,L-alanine over the L form. With polar ice we have in effect increased the dielectric constant of the intercalated ice medium.

With regard to the last point raised by Wilen, our overall aim was to demonstrate the ice nucleating ability of polar crystals by unraveling the riddle of the difference in ice nucleating behavior of the racemic and

chiral forms of α -amino acid crystals. In this way, we also hope to provide other approaches for understanding phenomena such as the ice nucleating ability of frost bacteria.

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HTLV-1 Provirus and Mycosis Fungoides

Human T cell lymphotropic virus type-1 (HTLV-1) is associated with a mature post-thymic T cell malignancy, adult T cell leukemia/lymphoma (ATL), which often affects cutaneous tissues. ATL is clinically distinct from mycosis fungoides (MF), although in both conditions the neoplastic CD4⁺ T cells are epidermotropic (1).

W. W. Hall *et al.* found a partially deleted HTLV-1 provirus in a cell line established from one patient with MF (2). Defective HTLV-1 provirus has also been found in some cases of ATL (3). The findings of Hall *et al.* and others (4) implicate HTLV-1 or a related retrovirus in the pathogenesis of cutaneous T cell lymphoma in some patients.

To assess the frequency of this association, we analyzed genomic DNA from freshly isolated peripheral blood mononu-

clear cells and from lesional skin biopsies of 40 patients with MF (5). Under low stringency conditions, Southern (DNA) blot hybridization with a full-length HTLV-1 probe (λ 23-3) revealed identical multiple bands in the 40 patients and in normal controls, which presumably indicates the presence of endogenous HTLV-1-like sequences within human genomic DNA (6). In contrast, at high stringency we found discrete bands in tissue DNA from only three (seronegative) Caucasian patients. The sizes of these bands were different from those characteristic of HTLV-1-associated ATL samples. Specifically, in one patient a truncated 8-kb Eco RI fragment was detected (Fig. 1), while the other two patients had an extra 3-kb Hind III fragment. We did not succeed in amplifying HTLV-1 sequences from these latter two patients, but

in the former patient amplification by polymerase chain reaction with primers flanking a conserved HTLV-1 *pol* sequence yielded a 119-base pair fragment. Subcloning into bacteriophage M13 and single-strand DNA sequence analysis revealed a 119-base pair *pol* sequence which, with the exception of a single base substitution, was identical to that of HTLV-1 (7).

These data indicate monoclonal integration of exogenous defective HTLV-1 sequences, which agrees with the findings of Hall *et al.* However, our results indicate that defective HTLV-1 sequences can be detected only in about 10% of patients with MF. Therefore, in our view, the role of these defective retroviruses in the pathogenesis of mycosis fungoides remains in question.

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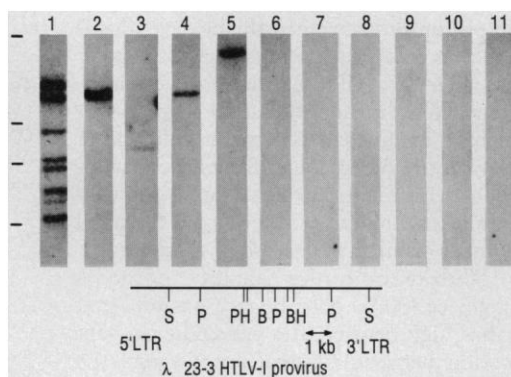
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Cutaneous T cell leukemias/lymphomas (CTCLs) are rare neoplasms that include mycosis fungoides (MF), its variant Sézary syndrome (SS) (considered to be the leukemic phase of MF), and adult T cell leukemia/lymphoma (ATL) (1). ATL, in its acute or chronic forms, is closely associated with HTLV-I infection (2) and consequently has a marked geographic predominance, with a high incidence in Japan, the Caribbean, and parts of eastern Europe. The role played by HTLV-I in MF and SS has been the subject of contradictory reports.

Hall *et al.* (3) report finding deleted HTLV-I provirus in cutaneous lesions of patients with MF. Polymerase chain reaction (PCR) analysis with HTLV-I *gag*, *pol*, *env*, pX, and long terminal repeat oligonucleotide primers, showed amplification of HTLV-I sequences in tissue samples from lesions of the five patients that they tested and in the blood of one of them. On this basis, they suggest that HTLV-I infection may be involved in at least certain cases of

Fig. 1. Southern DNA blot autoradiograph of Eco RI-digested DNA hybridized with a full-length HTLV-1 probe (λ 23-3) under high stringency conditions. A restriction enzyme map of the HTLV-1 provirus and the sizes of λ DNA fragments digested by Hind III (23.3, 9.4, 6.6, 4.4 kb) are shown. Positive controls are indicated by multiple bands in DNA from cell line MT2 (1) and a single 13 kb band in DNA from a patient with ATL (2). Single discrete Eco RI bands (3/8, 4/13.4, and 5/19.8 kb) are present in tissue DNA samples from three patients with MF, while negative results are seen in six other patients with MF (lanes 6 through 11). S, Sst I; P, Pst I; H, Hind III; B, Bam HI.



MF, thus unifying the etiology of CTCL.

We did not find HTLV-I infection markers in 24 cases of French and Portuguese patients with MF and SS, although we used immunological tests, electron microscopy of cultured lymphocytes, and PCR analysis of blood (4). To determine if complete or deleted HTLV-I proviral sequences were present in cutaneous lesions of MF and SS patients, we tested 18 DNA samples from previously analyzed patients (10 with SS and 8 with MF). The diagnosis of CTCL was based on clinical and histopathological findings; no patient had any clinical or pathological sign of acute or smoldering ATL. We used the same HTLV-I-specific oligonucleotide primers as Hall *et al.* (3) did, under identical technical conditions except that the number of cycles was increased from 30 to 35. The sensitivity of the PCR analyses was assessed by successive tenfold dilutions of a 1- μ g sample of control DNA that contained a single integrated HTLV-I provirus per genome. We observed HTLV-I amplification for all the primers even with a 10^5 dilution. When PCR was performed on DNA from cutaneous lesions, the results were negative for the 18 patients with all of the primers used, whereas the β gene that encodes for globin could be amplified in all of them.

We found no HTLV-I sequences in cutaneous lesions of our patients with MF and SS. The apparent contradiction between our results and those of Hall *et al.* (3) may derive from a mistaken diagnosis of MF in patients with chronic ATL, as occur in the case of the patient from whom the first isolate of HTLV-I was obtained (5). Another explanation could be that MF and SS have different etiologies that depend on the geographical origin of patients, as is the case for Burkitt lymphoma (6).

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Response: Whittaker and Luzzatto confirm our finding (1) that HTLV-1 provirus is present in the blood and in skin lesions of some patients with MF and SS. They also provide evidence, as we did (1), that the proviruses may be defective. They note, however, that HTLV-1 was found in only 10% of their European patients and, based on this observation, they question the role of the virus in the pathogenesis of MF and SS. In our report (1), we did not address the issue of the frequency of HTLV-1 infection in MF and SS patients. Our study (1) involved only six patients, which is not a large sample, and we did not intend to suggest that the frequency of infection was high. Another recent study found that 4 out of 27 American Caucasian patients with MF and SS were infected with HTLV-1 (2). These four patients were seropositive, and there was no evidence that the proviruses were defective.

These findings, (1, 2) from two distinct geographical areas, are significant; although infection would appear to occur in a minority of cases of MF and SS, the prevalence of infection is much higher than in the general population, which in the United States is less than 0.016% (3). The two studies (1, 2) suggest that the virus plays some role in the pathogenesis of at least certain cases of MF and SS, and they support the proposal that exogenous HTLV-1 infection has occurred in such cases. The HTLV-1 viral infection might be similar to that found in ATL, in that both complete (4) and deleted (5) forms of the provirus appear to exist in infected cells.

At present, it is unclear if patients with HTLV-1 represent a unique subset of MF

and SS patients that may, on further examination, be found to have distinct clinical, histological, or immunological features. Multidisciplinary studies on large patient populations should help clarify this and provide a better understanding of the role of the HTLV-1 virus in the pathogenesis of MF and SS. Such studies have recently suggested an association of HTLV-1 infection with the CDE30-positive, large cell, cutaneous lymphomas, and have also suggested that the proviruses may be defective (6). These studies could also establish criteria to distinguish between ATL and MF and SS associated with HTLV-1.

Bazarbachi *et al.*, in a PCR analysis with the same primers that were in our study, did not find provirus in cutaneous lesions of 14 patients. The reasons for the difference between their results and ours are unclear. Bazarbachi *et al.* suggest that our diagnosis of MF (1) may have been incorrect, and that the observed disease may actually have been chronic ATL. We agree that patients with MF and ATL can have similar clinical and histological features and that differentiation can be difficult, but we are confident that our diagnosis was correct.

We agree with Bazarbachi *et al.* that the geographical origin of the patients may also be important and that the association of HTLV-1 with certain cases of MF and SS may be analogous to Burkitt lymphoma, which may or may not be associated with Epstein-Barr virus infection. Alternatively, the differences could be accounted for if HTLV-1 is only associated with a certain subset of patients with MF and SS, and these may not have been included in their study.

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