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Human T-cell Leukemia Virus in Cutaneous T-cell Lymphoma in Denmark

A Possible Association of HTLV and Aneuploidy

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Cutaneous T-cell lymphomas (CTCL) are neoplasias of mature T-cells and comprise Sezary syndrome, mycosis fungoides and some cases of lymphomatoid papulosis. Clinically this group of disorders differ from the more aggressive neoplasias of mature T-cells known as adult T-cell leukemia/lymphoma and T-cell lymphosarcoma leukemia which are associated with human T-cell leukemia virus (HTLV). We have found that of 68 patients from Denmark with CTCL ten were positive for HTLV antibodies and that the neoplastic T-cells from skin specimens in seven of eight HTLV-antibody positive patients studied by DNA flow cytometry exhibit DNA aneuploidy. Either one or two hyperdiploid cell clones were present. Aneuploidy was found in two patients with histologically verified mycosis fungoides, in four patients with histological non-diagnostic mycosis fungoides, and in one patient with lymphomatoid papulosis. The present data indicate that further seroepidemiologic survey studies of cutaneous T-cell lymphomas should include the early histological non-diagnostic stages, especially when aneuploidy is present. (Received January 14, 1984)

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Cutaneous T-cell lymphomas comprise the Sezary syndrome, mycosis fungoides and some cases of lymphomatoid papulosis (1). These diseases have cutaneous lesions that histologically are characterized by a dermal cell infiltrate with OKT 4, Leu 2 A positive T-cells as defined by monoclonal antibodies, although OKT 8, Leu 3 A positive cell infiltrates have also been reported (2, 3). Single cell DNA measurements by flow cytometry have demonstrated aneuploidy in the early, histological non-diagnostic stages of mycosis fungoides and have thus shown to be helpful in establishing an early malignancy diagnosis. The pathogenesis and etiology of this group of disorders is unknown. Recently however, Gallo and coworkers have isolated and characterized the human T-cell leukemia virus (HTLV), a human type-C retrovirus (4–6) from patients with adult T-cell leukemia/lymphoma and acute lymphosarcoma cell leukemia of T-cells (7–9) but also from a few patients with cutaneous T-cell lymphoma (4). The adult T-cell leukemia/lymphoma is characterized by an aggressive course, poor prognosis, cutaneous and visceral involvement, hypercalcemia and in some cases unusual lytic bone lesions (7–9). Except for the cutaneous manifestations cutaneous T-cell lymphoma has none of these features. During the last year we have developed an indirect ELISA microtest for the determination of HTLV antibodies (10) and by this method we have studied 68 patients with cutaneous T-cell lymphoma, mainly in the early stages. We found that 10 of these cases were HTLV antibody positive, and we have now examined these 10 HTLV-antibody patients with single cell DNA measurements by flow cytometry on skin specimens.

Table I. *Number of HTLV-antibody positive sera from 68 patients with different subtypes of cutaneous T-cell lymphoma*

Diagnosis	HTLV antibody positive
SS	0/5
MF III	2/4
MF II	0/15
MF I	7/40
LP	1/4
Total	10/68

MATERIAL AND METHODS

Patients

Sixty-eight patients with cutaneous T-cell lymphoma were examined. There were five with Sezary syndrome, four with mycosis fungoides stage II (plaque stage, diagnostic histology), 40 with mycosis fungoides stage I (plaque stage, non-diagnostic histology), and four with lymphomatoid paulosis. Control patients have previously been published (11, 12). Single cell DNA measurements were performed on skin specimens from eight of the ten HTLV positive antibody cases.

Indirect ELISA microtest

An ELISA assay for detection of HTLV antibodies in human sera has been developed and presented in detail earlier (11). An additional confirmatory neutralization test was used, also described earlier in detail (11). A suppression of the ELISA value by 50% in the sample exposed to the unlabelled anti-HTLV, relative to a standard normal human serum, was considered *positive*, indicated by +: confirmatory result for the presence of anti-HTLV. In some cases, no specificity could be detected, however, due to extremely low titer complete reduction of the serum to the level of normal human serum background could be achieved with less than a 50% reduction. These cases were termed *suspect positive* and indicated by (+).

Single cell DNA measurements

Single cell DNA measurements by flow cytometry have previously been published in detail (12, 13). Dermal biopsies are placed in 2 ml calcium and magnesium free phosphate buffered saline with 1% fetal calf serum and cut into pieces smaller than 1 mm and shaken vigorously for 20 sec. The samples are then filtered through a 100 μm filter, centrifuged and the sediment carefully resuspended with a hypotonic buffer solution 10 mM tris-HCl; pH 7.4, containing 1 mM EGTA detergent (Nonidet P40, BCH-Chemicals, 1% v/v), propidium iodide (Sigma, 50 mg/l) and ribonuclease (Sigma type I A, 100 mg/l). The resulting nuclear suspensions were allowed to stain for 15 min and filtered through a 30 μm filter. Internal standards were added. At least 50000 nuclei were measured without and with internal standards in a Becton Dickinson FACS IV flow cytometer. DNA index values were calculated as follows: the patient DNA sample peak was divided by the simultaneously measured DNA trout peak, and this result was divided by the mean value of normal male or female lymphocyte DNA relative to trout.

RESULTS

Of the 68 patients with cutaneous T-cell lymphoma 10 patients were found to be HTLV antibody positive (11), five males, five females, age range 40 to 88 with a median value of 62 years. The 58 HTLV-antibody negative patients, 29 males and 29 females with an age range of 26 to 90 with a median value of 68 years. The distribution of these HTLV-antibody positive patients among the different subgroups is given in Table I. The clinical data, DNA index values and HTLV-antibody results of the positive patients are given in Table II. All HTLV-antibody positive patients had dermal T-helper cell infiltrates determined by monoclonal antibodies by an immunoperoxidase technique (2). The two patients

Table II. *Clinical data, single cell DNA-measurements on skin biopsies and HTLV-antibody results*

DNA index assessed by flow cytometry of skin specimens where values differed from euploid DNA content ($p<0.01$ according to t-distribution). +, HTLV-antibody positive; (+), suspected HTLV-antibody positive (see Materials and Methods).
CR = remission; PR = partial remission. ND = not done

Patient	Age	Sex	Diagnosis	Histology	Duration of disease (yrs)	Clinical stage of disease	Skin DNA-index	HTLV-antibody
ISP	59	M	MF III	Epidermotropic pleomorphic dermal cell infiltrate with Pautrier abscesses	19	Relapse	1.036	+
JG	60	M	MF III	Same	–	Relapse	1.057 1.125	+
IM	76	F	MF I	Sparse infiltrate of mature lymphocytes	4	Relapse	1.043	+
GP	57	F	MF I	Same	15	PR	ND	+
CN	40	F	MF I	Same	1	Relapse	1.075 1.170	(+)
KL	72	M	MF I	Same	3	PR	1.020 1.133	(+)
PH	88	F	MF I	Same	50	Relapse	1.019	+
AP	62	M	MF I	Same	–	CR	1.083	(+)
ET	68	F	MF I	Same	33	PR	ND	+
LB	62	M	LP	Pleomorphic dense dermal cell infiltrate vasculitis	4	PR	1.031 1.122	+

with mycosis fungoides stage III had active disease when first studied. Patient ISP demonstrated one hyperdiploid cell clone, whereas JG demonstrated two hyperdiploid cell clones. Patient ISP had four sera taken during a period of one year, all HTLV-antibody positive. When the first sample was taken the patient was in relapse, but in remission at the time of the three subsequent samples. In four of five antibody positive patients with mycosis fungoides stage I (non diagnostic histology) aneuploidy was found with either one or two hyperdiploid cell clones (IM, CN, KL, AP). Finally the patient with lymphatoid papulosis (LB) had a histogram which revealed a hyperdiploid cell clone. DNA histograms from peripheral blood lymphocytes in all HTLV-antibody positive patients were normal (data not shown). The presence of HTLV-antibodies in the sera and aneuploidy in the dermal infiltrates could not be related to the activity or the stage of the disease.

DISCUSSION

Since the first isolation and characterization of HTLV by Gallo and coworkers (4, 5, 14, 15) seroepidemiologic studies have demonstrated that HTLV is associated with a particular form of mature T-cell malignancy in different parts of the world (16–20). This mature T-cell malignancy has been called adult T-cell leukemia/lymphoma in Japan and acute lymphosarcoma T-cell leukemia in England. These diseases are known to cluster in rural districts in Southwest Japan (18, 19) and in Caribbean blacks (17), while they occur sporadically elsewhere (4, 5). They are probably the same disease but exhibit divergent

clinical features (7–9). We have now found HTLV antibodies in the sera from patients with cutaneous T-cell lymphoma and these HTLV antibody positive patients have further been examined with single cell DNA measurements. Two patients with mycosis fungoides in the tumor stage had hyperdiploid cell clones from skin specimens but the activity or the stage of the disease did not seem to influence their HTLV-antibody titers (11). An important finding was that seven HTLV antibody positive patients had early mycosis fungoides stage I with non-diagnostic histology and in four of five of these patients hyperdiploid cell clones were determined. No alterations in single cell DNA or phenotype of the peripheral blood in these early stage patients have been found (12, 21). Thus, even patients with early histologically not yet diagnostic but clinically characteristic mycosis fungoides with aneuploidy of the skin lesions may be HTLV-antibody positive in highly specific HTLV antibody assays. Some of these patients have had their disease for several years (Table II) and it has not been possible to determine when the HTLV-serum-antibodies developed. Whereas it is now possible unequivocally to demonstrate HTLV in neoplastic cells and determine HTLV antibodies in sera from patients with the adult T-cell leukemia of the type which clusters in Japan and the Caribbean, the less aggressive, non-clustering CTCL cases require much more attention and work mainly because the virus antibody titer is much lower. The present data indicate that further seroepidermiologic survey studies of cutaneous T-cell lymphomas also should include the early stages, especially when aneuploidy is present in the dermal lymphocytes and further research into the molecular biology of HTLV in cutaneous T-cell lymphoma is clearly indicated.

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