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Intraclonal diversity in a Sezary syndrome with a differential response to 2-deoxycoformycin of the two lymphoma cell populations

Elisa Granjo,[1] Margarida Lima,[2] José Manuel Lopes,[3] Nória Cunha,[4] Maria dos Anjos Teixeira,[2] Filipe Santos,[5] Jorge Candeias,[5] Carlos Resende,[4] Ana Helena Santos,[2] Ana Balanzategui,[6] Alberto Orfão[7] and Estella Matutes[8] [1]*Department of Clinical Haematology, Hospital Geral de São João,* [2]*Service of Clinical Haematology, Unit of Flow Cytometry, Hospital Geral de Santo António,* [3]*IPATIMUP and Porto Medical Faculty,* [4]*Department of Dermatology and Venereology,* [5]*Department of Immunology, Hospital Geral de São João, Porto, Portugal,* [6]*Unit of Molecular Biology, Service of Haematology, Hospital Universitário de Salamanca,* [7]*Service of Cytometry, Hospital Universitário de Salamanca and Centro de Investigacion del Cancer, Salamanca, Spain, and* [8]*Academic Department of Haematology and Cytogenetics, the Royal Marsden NHS Trust, London, UK*

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Summary. We report a case of Sezary syndrome with two abnormal $CD4^+$ T-cell populations detected in the peripheral blood by flow cytometry immunophenotyping and DNA cell content, suggesting a biclonal T-cell lymphoproliferative disorder. Despite these findings, molecular analysis of the T-cell receptor genes was consistent with a monoclonal T-cell proliferation, supporting the existence of intraclonal diversity rather than a true biclonal disease. The patient achieved a transient response with 2-deoxycoformycin, with a selective decrease of the larger/hyperploid T-cell population; later on, an increased representation of this T-cell population was observed concomitantly with clinical relapse.

Keywords: Sezary syndrome, biclonal, intraclonal diversity, 2-deoxycoformycin, flow cytometry.

Sezary syndrome (SS) is a cutaneous $CD4^+$ T-cell non-Hodgkin's lymphoma that manifests with generalized erythroderma and blood circulating cerebriform cells (Siegel *et al*, 2000). Treatment includes photopheresis, total skin electron beam therapy, single-agent and combined chemotherapy and immunotherapy. Recent studies suggest that pentostatin (2-deoxycoformycin, 2-DCF), a potent inhibitor of the adenosine deaminase with activity in a range of lymphoid malignancies, is an effective single-agent therapy for SS (Dearden *et al*, 2000).

In contrast to B-cell disorders, in which clonality can be demonstrated through the finding of immunoglobulin light chain restriction, assessment of T-cell clonality by immunophenotyping still remains complex and currently depends on either the demonstration of an aberrant immunophenotype and/or the study of the T-cell receptor (TCR) β-chain variable region (Vβ) repertoire using a large panel of monoclonal antibodies; in such cases, T-cell clonality is considered when a single TCR-Vβ domain is expressed (Lima *et al*, 2001a). However, demonstration of T-cell clonality formally needs molecular analysis of the configuration of the TCR genes.

Here we report a patient with SS with a monoclonal proliferation of $CD4^+$ T cells in the peripheral blood in whom intraclonal heterogeneity was demonstrated by flow cytometry immunophenotyping and DNA cell content; these two distinct $CD4^+$ T-cell populations responded differently to therapy with 2-DCF.

To the best of our knowledge this is the first report describing a cutaneous T-cell lymphoma in which intraclonal diversity correlated with clinical manifestations and response to therapy.

CASE REPORT

A 79-year-old man was diagnosed with SS in 1998 and subsequently treated with Interferon α2b (3×10^6 U, subcutaneously, three times per week) plus photochemotherapy

Correspondence: Elisa Granjo, Serviço de Hematologia Clínica, Piso 2, Hospital S. João, Alameda Prof. Hernani Monteiro, 4202-451 Porto, Portugal. E-mail: npp46740@mail.telepac.pt

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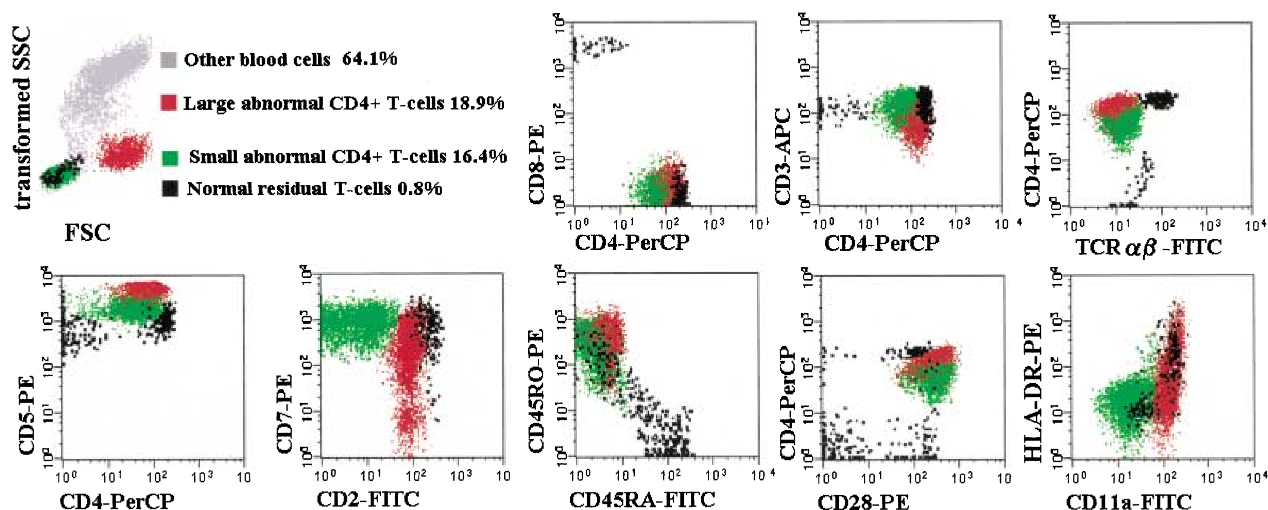

Fig 1. Dot plots illustrating the main phenotypic features of both small (green dots) and large (red dots) aberrant CD4$^+$/CD3$^+$/TCRαβ$^+$ T cells, in comparison to normal residual T lymphocytes (black dots).

(Psoralen and ultra-violet A, PUVA) without an objective response. He was first observed in the Haematology Department of the Hospital S. João (Porto, Portugal) in March 1999 with generalized erythroderma and pruritus. Blood counts were as follows: haemoglobin 12·3 g/dl, platelets 163×10^9/l and white blood cell count (WBC) $11{\cdot}8 \times 10^9$/l (lymphocytes $4{\cdot}3 \times 10^9$/l). The peripheral blood film showed a mixture of small- and large-sized lymphocytes with a cerebriform nucleus and the skin biopsy revealed dermal infiltration by atypical lymphoid cells with epidermotropism. Flow cytometry analysis of peripheral blood leucocytes performed in whole blood by a stain-and-lyse direct immunofluorescence technique with a four-colour panel of monoclonal antibodies revealed two abnormal CD4$^+$ T-cell populations co-existing with a small population of normal residual CD4$^+$ T cells (Fig 1). The two abnormal CD4$^+$ T-cell populations had different light scatter properties, one with a forward scatter (FSC) and a side scatter (SSC) similar to normal CD4$^+$ T cells (small Sezary cells) and the other being larger and more complex (large Sezary cells). In addition, they also differed one from each other on the pattern of expression of CD2, CD3/TCRαβ, CD4, CD5, CD7, CD11a and HLA-DR. Small Sezary cells were CD7$^{+bright}$ and expressed virtually no CD2, whereas large Sezary cells were CD2$^{+bright}$ and showed dim and heterogeneous reactivity for CD7. Although with different intensities, both cell populations expressed CD5 brightly and the TCRαβ, CD3 and CD4 antigens dimly in comparison to normal CD4$^+$ T cells. In addition, small abnormal CD4$^+$ T cells were CD11a^{+dim}, CD25$^-$ and HLA-DR$^-$ and large abnormal CD4$^+$ T-cells were CD11a$^{+bright}$, HLA-DR$^{-/+dim}$ and CD25$^{-/+dim}$. Both T-cell populations were CD28 and CD45RO positive while negative for other T-/natural killer (NK)-cell markers tested (CD8, CD11b, CD11c, CD16, CD38, CD45RA, CD56, CD62L, CD57, CD94, CD122, CD158a, CD161, NKB1). TCR-Vβ repertoire studies using a panel of monoclonal antibodies that identify 23 different TCR-Vβ families and covers around 60 ± 4% of the TCR-Vβ repertoire of CD4$^+$ T cells present in the normal adult peripheral blood (Lima *et al*, 2001a) showed a dilution pattern, with 93% of CD4$^+$ T cells remaining unidentified with the panel of anti-TCR-Vβ monoclonal antibodies used in the study. Flow cytometry DNA cell content studies showed that small abnormal CD4$^+$ Sezary cells were diploid (DNA index, 0·98) whereas large aberrant ones were hyperploid (DNA index, 1·90); in addition, the small Sezary cells had a higher percentage of cells in S phase (1·9% *vs* 0·7%) whereas large Sezary cells had a higher mitotic index (% G2/M phase cells of 5·3% versus 0·6%) (Table I). Southern blot (SB) analysis of the DNA extracted from blood mononuclear cells treated with the *Eco*R1 and *Hin*dIII restriction enzymes and hybridized with 32P-labelled probes for the constant region of the TCRβ gene (cβ) showed a TCR rearrangement pattern compatible with a monoclonal T-cell proliferation. At diagnosis, the bone marrow aspirate had 18% lymphoid cells, of which 97% were CD4$^+$ T cells (54% large abnormal; 45% small abnormal and 1% small normal residual CD4$^+$ T cells).

The patient was treated with 2-DCF (4 mg/m^2, intravenously, once a month, for 5 months) with significant improvement of the skin lesions and marked reduction in the blood lymphocytosis to $0{\cdot}779 \times 10^9$/l in April 2000 (Fig 2). Large CD4$^+$ Sezary cells almost disappeared whereas small CD4$^+$ Sezary cells persisted in the blood (Fig 2). He remained stable until July 2000, when the disease recurred with erythroderma, lymphadenopathy, increasing lymphocytosis (WBC $9{\cdot}9 \times 10^9$/l; lymphocytes $4{\cdot}6 \times 10^9$/l) and reappearance of the large CD4$^+$ Sezary cells in blood (Fig 2). He was restarted on 2-DCF (4 mg/m^2, intravenous, once a month) plus prednisolone (100 mg, *per os*, 5 d, once a month) with clinical and haematological response but died from lung infection in January 2001.

Table I. DNA content and cell cycle distribution of peripheral blood T cells at diagnosis.

	Ploidy	DNA index	% G0/G1	% S	% G2M
Small abnormal $CD4^+$ T cells	Diploid	0·98	97·5	1·9	0·6
Large abnormal $CD4^+$ T cells	Hyperploid	1·90	94·0	0·7	5·3
Normal residual T cells	Diploid	1·00	98·2	1·0	0·8

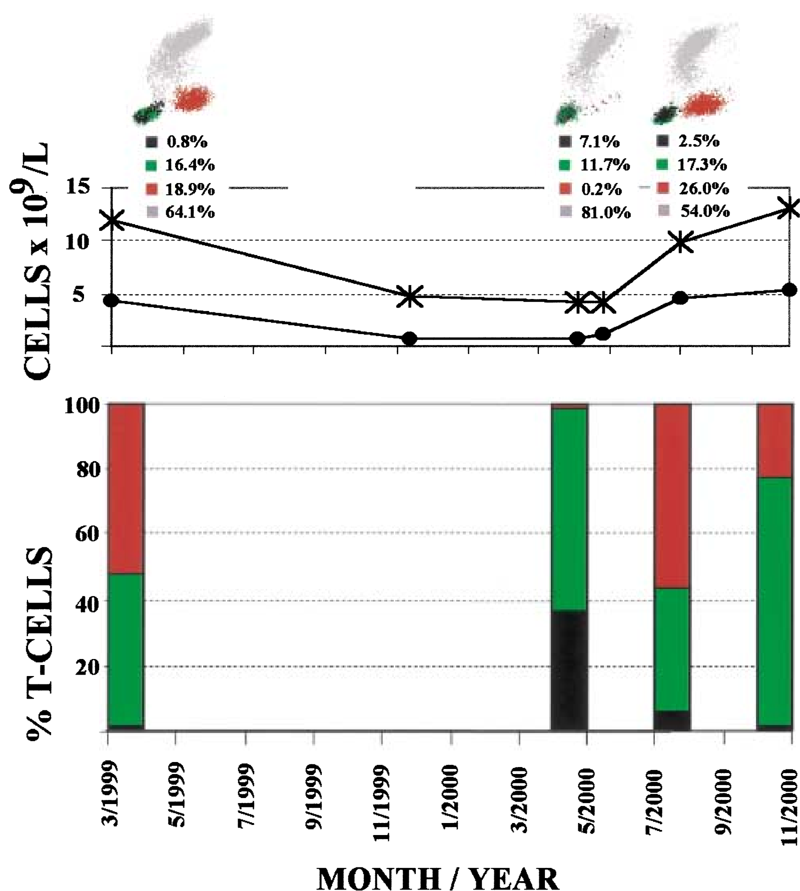

Fig 2. Blood cell counts and immunophenotypic enumeration of peripheral blood lymphocytes at diagnosis and at different times during follow-up after starting treatment with 2-DCF. The WBC counts (–⚹–) and the lymphocyte counts (–●–) counts are shown in the upper panel. The lower panel shows the relative representation of large abnormal $CD4^+$ Sezary T cells (red bars), small abnormal $CD4^+$ Sezary T cells (green bars) and small normal residual $CD4^+$ plus $CD8^+$ T lymphocytes (black bars), as the percentage of total peripheral blood T cells. Dot plots in the top of the upper panel represent the SSC/FSC distribution of peripheral blood cells as well as the percentage of each cell population among total white blood cells from the sample (for key, see Fig 1).

DISCUSSION

In recent years an increasing number of biclonal lymphoproliferative disorders (LPD) have been described due to a more accurate approach to the diagnosis. Most of the documented cases were referred to as biclonal B-cell LPD and a few as concomitant B- and T-cell LPD (Moss & Gillespie, 1997; Lima *et al*, 2001b), the latter including patients with cutaneous T-cell lymphoma co-existing with a B-cell leukaemia (Alves *et al*, 1998; Paolini *et al*, 2000). Cases of biclonal T-cell LPD are quite rare, only three well-documented reports have been described in the literature during the last decade (UltraMed, including MedLine®, 1990–2001) (Richards *et al*, 1994; Kondo *et al*, 1995; Shibata *et al*, 1995). Two of these cases were biclonal human T-cell leukaemia virus type I (HTLV1)-positive adult T-cell leukaemia/lymphoma, in which two different T-cell clones were identified by immunophenotyping, TCR gene rearrangement studies and analysis of the integration pattern of HTLV-I proviral DNA (Kondo *et al*, 1995; Shibata *et al*, 1995); the remaining case was a patient with large granular lymphocytic leukaemia that proved to be biclonal by immunophenotyping and demonstration of the simultaneous presence of distinct TCR gene rearrangements (Richards *et al*, 1994). A case of a patient with SS in whom biclonality was considered highly probable based on the identification of two different abnormal T-cell populations by immunophenotyping has also been reported, although the different clonal origin was not demonstrated (Dereure *et al*, 1994).

In the present case, two abnormal $CD4^+$ T-cell populations were detected by flow cytometry immunophenotyping and DNA cell content studies, raising the question of whether these two T-cell populations were the result of

intraclonal diversity associated with chromosome instability or rather represent a true biclonal disease. Unfortunately, flow cytometry TCR-Vβ studies were of limited value in clarifying this point as the panel of anti-TCR-Vβ monoclonal antibodies used did not identify the TCR-Vβ family expressed in either of the two phenotypically aberrant T-cell populations. Molecular studies revealed a monoclonal TCRβ chain gene rearrangement pattern, strongly favouring the hypothesis of intraclonal heterogeneity rather than the existence of a biclonal T-cell LPD. Although it could be argued that SB has a relatively low sensitivity and that a more sensitive polymerase chain reaction (PCR)-based technique should be used to clarify this point, the possibility of missing one of the clones in SB analysis is very unlikely as each of the two abnormal $CD4^+$ T-cell populations represented nearly half of the total circulating mononuclear cells at the time when the molecular studies were performed, a value which is clearly above the cut-off level of 5–10%, usually considered as the sensitivity limit for this technique.

Intraclonal diversity has been demonstrated in both B- and T-cell LPD. Histological transformation of B-cell lymphomas is frequently associated with clonal heterogeneity due to ongoing somatic mutations and acquisition of new chromosomal abnormalities (Whang-Peng *et al*, 1995; Matolcsy *et al*, 1999; Szereday *et al,* 2000). The occurrence of large cell transformation has been documented in patients with mycosis fungoides/SS (Diamandidou *et al*, 1998; Li *et al*, 1998; So *et al,* 2000) and, as in this patient, at least one case has been previously reported in which large cells proved to be hyperploid by conventional karyotyping and DNA cell content staining (So *et al,* 2000).

In the case described here, flow cytometry proved to be of great help in detecting intraclonal heterogeneity, and in quantifying and characterizing the two abnormal T-cell populations as well as in monitoring during and after therapy. Interestingly 2-DCF seemed to act preferentially in one of the lymphoma T-cell populations, as large DNA hyperploid $CD4^+$ Sezary T cells selectively decreased after treatment. Moreover, the clinical manifestations correlated to the presence of such a clone, as reappearance of erythroderma occurred simultaneously with an increase in the large DNA hyperploid $CD4^+$ Sezary cells in the peripheral blood.

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