HEMATOLOGICAL ONCOLOGY
Hematol Oncol 2002; **20**: 87–93.
Published online 22 March 2002 in Wiley InterScience (www.interscience.wiley.com). **DOI:** 10.1002/hon.695

REVIEW ARTICLE

$CD8^+/V\beta5.1^+$ LARGE GRANULAR LYMPHOCYTE LEUKEMIA ASSOCIATED WITH AUTOIMMUNE CYTOPENIAS, RHEUMATOID ARTHRITIS AND VASCULAR MAMMARY SKIN LESIONS: SUCCESSFUL RESPONSE TO 2-DEOXYCOFORMYCIN.

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SUMMARY

We report a case of $CD8^+/V\beta5.1^+$ T-cell large granular lymphocyte leukemia (T-LGL leukemia) presenting with mild lymphocytosis, severe autoimmune neutropenia, thrombocytopenia, polyarthritis and recurrent infections with a chronic disease course. Immunophenotyping showed an expansion of $CD3^+/TCR\alpha\beta^+/CD8^{+bright}/CD11c^+/CD57^-/CD56^-$ large granular lymphocytes with expression of the TCR-Vβ5.1 family. Southern blot analysis revealed a clonal rearrangement of the TCR β-chain gene. Hematopoietic growth factors, high dose intravenous immunoglobulin and corticosteroids were of limited therapeutic benefit to correct the cytopenias. During the disease course, the patient developed a severe cutaneous leg ulcer and bilateral vascular mammary skin lesions. Treatment with 2-deoxycoformycin resulted in both clinical and hematological complete responses, including the resolution of vascular skin lesions. Combined immuno-staining with relevant T-cell associated and anti-TCR-Vβ monoclonal antibodies proved to be a sensitive method to assess the therapeutic effect of 2-deoxycoformicin and to evaluate the residual disease. Copyright © 2002 John Wiley & Sons, Ltd.

KEY WORDS: rheumatoid arthritis; large granular lymphocytes; $CD8^+$; skin lesions; autoimmune cytopenias; 2-deoxycoformycin

INTRODUCTION

Lymphoproliferative disorders of large granular lymphocytes (LGL) comprise a large spectrum of polyclonal, oligoclonal or monoclonal expansions of LGL the latter being designated LGL-leukemia.[1,2] The majority of cases are of T-cell origin, mainly with a $CD3^+/TCR\alpha\beta^+/CD8^{+bright}$ immunophenotype, and a minority derived from NK-cells. Arthritis and cytopenias are common findings. Cytopenias, often neutropenia and anemia may be autoimmune in nature or result from a T cell-mediated suppressor effect on the hematopoiesis; thrombocytopenia occurs less frequently. Splenomegaly and hepatomegaly are relatively frequent while

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lymphadenopathy and skin involvement are extremely rare (E. Granjo *et al.*, unpublished data).[1-3]

Several agents, including corticosteroids, hematopoietic growth factors, high dose intravenous immunoglobulins, low dose methotrexate, cyclosporin A and cyclophosphamide have been used with variable success in the treatment of LGL-leukemia and associated conditions.[4–9] Recently, purine analogues such as fludarabine, 2-chlorodeoxyadenosine and 2-deoxycoformycin (2-DCF), have also been proved to have potent activity in the treatment of chronic T-cell leukemias, including a few cases of LGL-leukemia.[10–12]

We describe a patient with a $CD3^+/TCR\alpha\beta^+/CD8^{+bright}/V\beta5.1^+$ LGL leukemia, who presented with arthritis, mild lymphocytosis, autoimmune cytopenias (neutropenia and thrombocytopenia), recurrent infections and vascular mammary skin lesions that were successfully treated with 2-DCF.

CASE REPORT

A 71-year-old female was referred to the Department of Hematology (Hospital S. João, Porto, Portugal) in October 1991 for investigation of lymphocytosis, severe neutropenia and thrombocytopenia detected in a routine blood analysis. She had a previous history for 5 years of arthralgias and synovitis of the wrists and the second and third metacarpo-phalangeal joints. Hepatosplenomegaly and lymphadenopathy were not present. The main clinical and laboratory features at presentation and during the disease course are shown in Table 1. Peripheral blood counts at diagnosis were: white blood cells (WBC) 7.2×10^9/l, neutrophils 0.5×10^9/l, lymphocytes 6.3×10^9/l, platelets (PLT) 88×10^9/l and hemoglobin (HGB) 12.3 g/dl. The majority of circulating lymphocytes were LGL and flow cytometry analysis showed a $CD3^+/TCR\alpha\beta^+/CD8^{+bright}/CD4^-$ phenotype in 90% of cells. The expanded $CD8^+$ LGL population expressed CD11c, was negative for NK-associated markers ($CD11c^+$, $CD16^-$, $CD56^-$, $CD57^-$) and, unlike normal circulating $CD3^+/TCR\alpha\beta^+/CD8^{+bright}$ LGL, were CD5 negative. Like normal $CD8^+$ LGL, the cells expressed activation-markers ($CD11a^{+bright}/CD25^-/CD122^+/HLA\text{-}Dr^+$). Neutrophil-associated and platelet-associated IgG antibodies were detected by flow cytometry, whereas the direct anti-human immunoglobulin Coomb's test was negative. There was a polyclonal hypergammaglobulinemia (IgA 1043 mg/dl, with a normal range of 90 to 450 mg/dl and IgG 1955 mg/dl with normal range of 80 to 1800 mg/dl). Serum rheumatoid factor was positive and X-ray of the joints showed soft tissue swelling, bone erosions of the hand small joints and narrowing of the joint spaces. HLA studies showed A2,24; B6,2; Cw2,3; DR4,6; DQw1,3. There was no serological evidence for recent or persistent infection by cytomegalovirus, *Herpes simplex*, *Herpes zoster*, Epstein-Barr, human hepatitis B and C, human immunodeficiency type I and II or human T-cell lymphotropic type 1 and 2 viruses. The bone marrow (BM) aspirate showed infiltration (25%) by LGL and the BM trephine biopsy revealed an interstitial lymphocytic infiltrate and increased numbers of plasma cells. Cytogenetic studies in the BM cells revealed a normal karyotype. TCR-beta chain variable region (TCR-Vβ) repertoire analysis using a panel of antibodies against the variable domains of the TCR beta chain (Immunotech, Marseille, France) showed that 92% of the blood $CD8^+$ T-cells expressed the TCR-Vβ5.1 family, as compared to a value of $3.0 \pm 1.2\%$ (mean $\pm$ standard deviation) in normal peripheral blood $CD8^+$ T-cells. Southern blotting using specific probes for the TCR-beta gene region (Cb, TCRBC and TCRBJ2) and ECO RI and HIND III restriction enzymes showed a rearranged pattern of the TCR-beta chain gene compatible with a clonal T-cell expansion.

Table 1. Main clinical and hematological findings at diagnosis and during the evolution of the disease

Date	Symptoms	Treatment	HGB (g/dl)	PLT ($\times 10^9$/l)	WBC ($\times 10^9$/l)	% Neutrophils ($\times 10^9$/l)	% Lymphocytes ($\times 10^9$/l)	$CD8^+$ % Lymphocytes ($\times 10^6$/l)	$V\beta5.1^+/CD5^-$ % CD8 ($\times 10^6$/l)	$V\beta17.1^+/CD5^{+dim}$ % CD8 ($\times 10^6$/l)
1991	Arthralgias Arthritis	None	12.3	8.8	7.2	6.9 (0.5)	87.5 (6.3)	90 (5670)	ND	ND
1992		None	11.8	101	5.2	2.0 (0.1)	92.5 (4.8)	ND	ND	ND
1993	Recurrent respiratory infections	IVIg G-CSF	12.3	90	7.2	1.4 (0.1)	89.4 (6.4)	90 (5793)	92 (5330)	ND
1994			12.2	90	5.3	1.9 (0.1)	90.6 (4.8)	ND	ND	ND
		Prednisone	11.6	31	4.1	20.1 (0.8)	79.9 (3.3)	ND	ND	ND
1995	Leg ulcer		11.5	90	5.4	1.9 (0.1)	90.7 (4.9)	ND	ND	ND
	Mammary skin lesions	2-DCF	11.6	49	4.4	6.8 (0.3)	86.4 (3.8)	ND	ND	ND
	Pneumonia, upper respiratory infections		10.7	50	3.1	51.6 (1.6)	25.8 (0.8)	ND	ND	ND
			8.4	39	2.3	73.0 (2.3)	19.2 (0.6)	58 (345)	ND	ND
			13.1	104	3.3	55.6 (1.8)	28.1 (0.9)	ND	ND	ND
1996	*Herpes zoster*	None	11.3	99	4.1	80.5 (3.3)	14.6 (0.6)	ND	ND	ND
1997	None	None	13.9	144	5.0	61.4 (3.1)	25.4 (1.3)	58 (737)	53 (390)	15 (110)
1998		None	12.9	125	4.2	61.9 (2.6)	23.8 (1.0)	53 (530)	39 (207)	20 (106)
1999		None	11.7	139	4.8	59.1 (2.8)	27.4 (1.3)	55 (723)	43 (311)	14 (100)
2000		None	11.4	134	5.8	63.8 (3.7)	25.9 (1.5)	50 (751)	9 (68)	17 (128)

ND, not done.

The clinical course was stable for 1 year, when there was a decrease in the neutrophil count concomitantly to recurrent respiratory infections. From 1992 to 1995 she received high dose intravenous immunoglobulin (IVIg), prednisone and granulocyte-colony stimulating factor (G-CSF) with only minor transient benefit. In December 1994, she manifested with a severe cutaneous leg ulcer that required treatment with broad-spectrum antibiotics and G-CSF. At that time she also developed B symptoms, hepatosplenomegaly and bilateral vascular mammary skin lesions (Figure 1A). Mammary skin biopsy showed capillary ectasies in the dermis and a perivascular infiltrate by T-lymphocytes (Figure 1B). Because of the low benefit obtained with corticosteroids, G-CSF and IVIg and persistence of lymphocytosis and cytopenias, she was started on 2-DCF 7 mg i.v., twice a month for 10 courses (March 95 to November 95). During this period she was hospitalized for pneumonia and developed a transient pancytopenia reaching minimum values of WBC of 0.8×10^9/l, HGB of 7.4 g/dl, PLT of 39×10^9/l and a lymphopenia with both low $CD4^+$ (0.143×10^9/l) and $CD8^+$ (0.345×10^9/l) lymphocyte counts. Clinical and hematological re-evaluation after 10 courses of 2-DCF showed a complete resolution of the cutaneous lesions and hepatosplenomegaly, a rise in the neutrophil and PLT counts and the anti-platelet and anti-neutrophil autoantibodies were no longer detected. By this time the WBC count was 3.2×10^9/l with 56% neutrophils and 28% lymphocytes, the PLT count was 104×10^9/l and HGB was 13.1 g/dl. The patient remained lymphopenic for 1 year and developed a severe perianal herpetic infection that was successfully treated with acyclovir. The blood counts continued to recover progressively until reaching normal values. In December 1997, 2 years after treatment, she had: WBC of 5.0×10^9/l (neutrophils 61%, lymphocytes 25%), PLT 144×10^9/l and HGB 13.9 g/dl. Combined four-colour staining with anti-CD8, anti-CD5, anti-CD28 and TCR-Vβ5.1 MAb revealed that the abnormal $CD8^+/V\beta5.1^+/CD5^-/CD11c^+/CD57^-$ LGL clone had decreased markedly, representing 53% of the $CD8^+$ circulating lymphocytes. Additional flow cytometric studies detected a new $CD8^+/V\beta17.1^+$ LGL expansion, with different immunophenotypic properties, representing 15% of $CD8^+$ lymphocytes (normal value $4.7 \pm 2.4\%$). In contrast to $CD8^+/V\beta5.1^+$ leukemic LGL, $CD8^+/V\beta17.1^+$ LGL were $CD5^{+dim}/CD11c^-/CD57^+$; other immunophenotypic findings were similar. A BM aspirate showed 12% lymphocytes, 53% of which were $CD8^+$ LGL. At the last reassessment carried out in November 2000 blood counts were normal and the abnormal $CD8^+/V\beta5.1^+$ LGL clone was reduced to minimal residual levels (9% of blood $CD8^+$ T-cells) whereas $CD8^+/V\beta17.1^+$ LGL remained stable (17% of $CD8^+$ T-cells). Although the abnormal $CD8^+/V\beta5.1^+$ LGL clone was still clearly detected by flow cytometry, Southern blot-based molecular biology studies revealed a germ-line pattern of the TCR-β chain gene.

DISCUSSION

We described a case of a $CD8^+$ LGL leukemia associated with autoimmune cytopenias, arthritis and recurrent infections and who developed vascular mammary skin lesions during the disease course. The patient achieved a complete clinical and hematological response with 2-DCF including resolution of the vascular breast lesions.

Unusual features in our patient were the vascular mammary skin lesions and the therapeutic effect obtained with 2-DCF while other manifestations were those typically found in LGL leukemia.

Although association of LGL lymphoproliferative disorders with cytopenias and arthritis is well established, reports on the association of LGL-leukemia and cutaneous lesions are scarce (E. Granjo *et al.*, unpublished data).[3] To the best of our knowledge to date only one case of

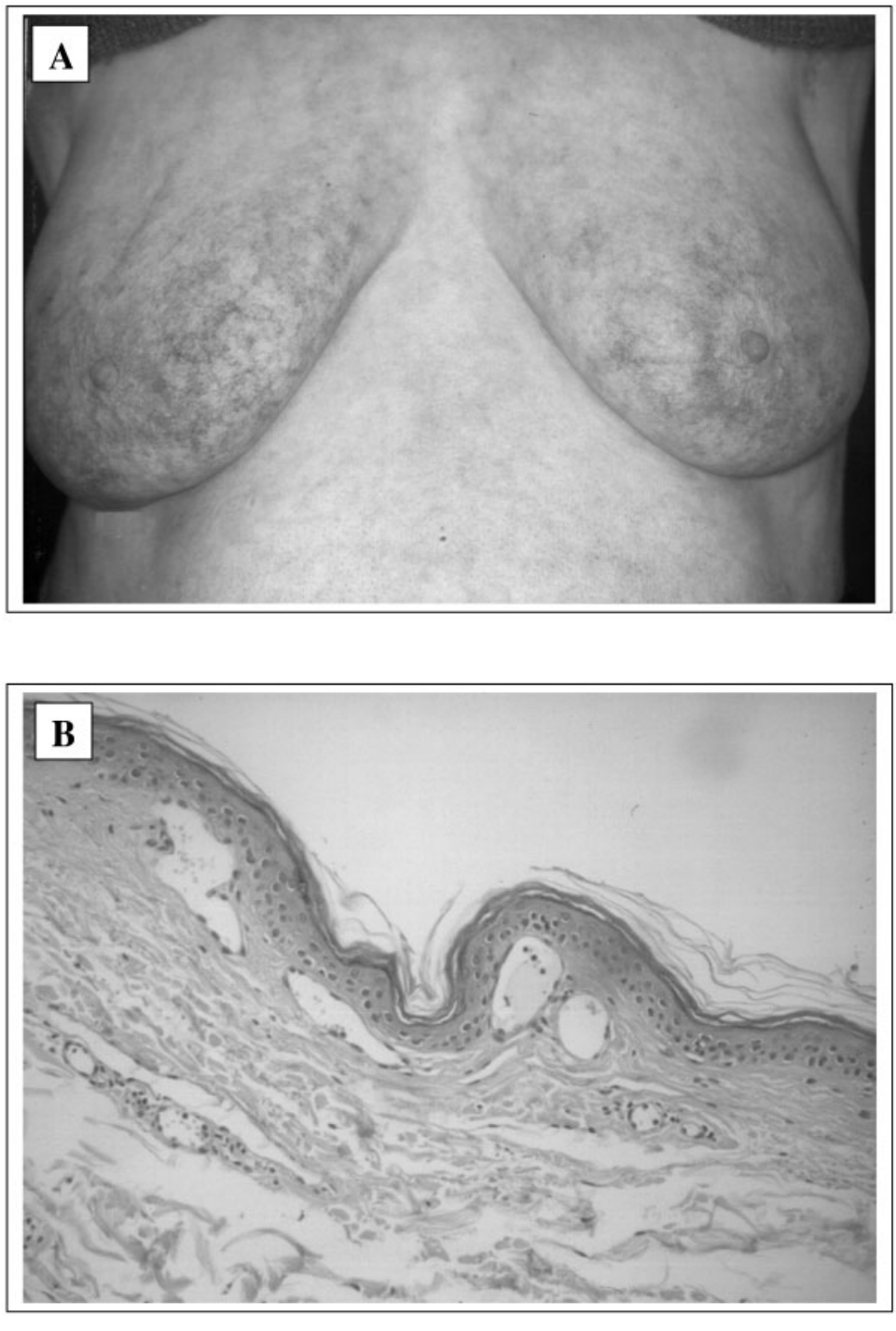

Figure 1. Bilateral mammary skin lesions (A) whose biopsy revealed vascular ectasies and a perivascular lymphocytic infiltrate (B)

$CD8^+$ LGL with cutaneous infiltration has been described[3] and we have recently seen a case with a $CD4^+/CD8^{+dim}/CD56^+$ LGL leukemia and skin lesions, the latter diagnosed as disseminated granuloma annulare.[20] Although in our patient skin histology revealed a vascular proliferation suggestive of a reactive condition, the presence of a lymphoid infiltrate with a T-cell phenotype and the response to 2-DCF would suggest that the cutaneous lesions were associated with the LGL-leukemia. It has been shown that LGL exhibit cytotoxic activity against endothelial cells in certain experimental conditions[13,14] and may also induce vascular proliferation and endothelial damage by means of secreting a variety of cytokines and growth factors, such as tumour necrosis factor alpha (TNF-alpha) and gamma interferon (IFN-gamma), that interfere with endothelial proliferation and adhesion[15–17] and may be responsible for vascular damage.[18,19] In line with these observations, it could be speculated that the vascular mammary skin lesions were the result of direct or indirect damage of the endothelial cells by the expanded LGL population.

The response to 2-DCF in our patient was remarkable on the organomegaly, skin lesions and cytopenias as well as on lymphocytosis with the almost complete disappearance of the circulating leukemic LGL clone. 2-DCF was well tolerated except for an increased risk of infections by opportunistic organisms. Therefore, 2-DCF should be used only if LGL-leukemias have frank progression and anti-viral and anti-fungal prophylaxis should always be considered. There have been a few other cases of T-LGL leukemia achieving a long-term clinical and hematological remission with 2-DCF, without evidence of relapse up to 4 years after finishing therapy.[11,12] Similar responses were previously reported with cyclosphosphamide[8,9] whereas treatment with cyclosporin A may result in a clinical and hematological responses but with persistence of the abnormal LGL clone that circulates in the blood, supporting the proposal that cyclosporin A acts only at the functional level by inhibiting T-cell activation.[6,7] Our case also illustrates that combined staining with relevant T-cell associated and anti-TCR-Vβ MAb is a very sensitive method for assessing the therapeutic effect and evaluating the residual disease at levels that were undetectable by molecular analysis of TCR-beta chain gene using conventional Southern blotting. The value of Southern blot for determining T-cell clonality is considerably limited when the clonal T-cell population represent a minor proportion of the total nucleated cells (e.g. less than 10%) in the sample.

ACKNOWLEDGEMENTS

This work was partially supported by the following grants: Comissão de Fomento da Investigação em Cuidados de Saúde, Ministério da Educação, Portugal (PI 51/99) and Acções Integradas Luso-Espanholas do Conselho de Reitores das Universidades Portuguesas (E-31/99).

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