Virchows Arch (1995) 426:629–634

CASE REPORT

Corina C. Dommann-Scherrer
Sylvia Baumann Kurer · Dieter R. Zimmermann
Bernhard F. Odermatt
Maria Teresa Dours-Zimmermann
Jakob Briner · Philipp U. Heitz

Occult hepatosplenic T-γδ lymphoma

Value of genotypic analysis in the differential diagnosis

Received: 1 February 1995 / Accepted: 5 April 1995

Abstract We report on a patient with a rare hepatosplenic γδ T-cell lymphoma (γδ TCL) presenting clinically with B-symptoms, hepatosplenomegaly and pancytopenia. During the initial stage of the disease the sparse malignant cells could not be detected histologically. Furthermore, their identification was obscured by massive macrophage proliferation with haemophagocytosis in the spleen. Diagnosis was established by detection of a clonal T-cell receptor (TcR) rearrangement and, retrospectively, by demonstration of rare cells expressing an aberrant T-cell phenotype. The findings in this patient emphasize that minimal neoplastic T-cell infiltrates can lead to severe clinical symptoms. Initial biopsy findings may be misinterpreted as benign. γδ TCL may elaborate lymphokines that suppress haematopoiesis, leading to pancytopenia and macrophage proliferation.

Key words T-cell lymphoma · γδ T-cell receptor · Spleen · Liver · Haemophagocytosis

Introduction

Peripheral T-cell lymphomas (PTCL) account for 15–20% of non-Hodgkin's lymphomas in Western populations and occur more frequently than B-cell lymphomas in extranodal localizations. They usually express a CD4+ CD8- TcR αβ immunophenotype of normal mature helper T-cells with frequent concomitant loss of one or several pan-T-cell antigens in correlation with monoclonality [16]. We describe a patient suffering from a rare and distinct type of PTCL showing a sinusal/sinusoidal infiltration in spleen, liver, bone marrow and subsequent spread to the peripheral blood. In addition, the tumour cells expressed characteristically the rare γδ TcR along with a double negative CD4/CD8 phenotype. An uncommon feature of the present patient's illness at presentation was the discrepancy between the severity of the disease and the relative paucity of the malignant cells, which needed recourse to genotyping to allow their discovery.

The findings described in this report were presented in part in poster form at the 78th meeting of the Deutsche Gesellschaft für Pathologie (German Association of Pathology) in Zürich, April 1994

C.C. Dommann-Scherrer (✉) · D.R. Zimmermann · B.F. Odermatt
M.T. Dours-Zimmermann · J. Briner · P.U. Heitz
Department of Pathology, University Hospital of Zurich, Schmelzbergstrasse 12, CH-8091 Switzerland

S. Baumann Kurer
Department of Medicine, Division of Hematology, University Hospital of Zurich, Switzerland

Clinical course

A 45-year-old man was admitted in May 1990 because of subfebrile temperatures, night sweats and recent weight loss. Physical examination revealed splenomegaly but no palpable lymph nodes. Initial laboratory values showed pancytopenia with a haemoglobin of 9.4 g/dl, a white cell count of 1.4×10^9/l with 68% lymphocytes (mostly showing an irregular and indented nucleus), 11.5% granulocytes, 19% monocytes and a platelet count of 41×10^9/l. A bone marrow biopsy showed stimulated haematopoiesis. Splenectomy, abdominal lymph node biopsies and a liver wedge biopsy were performed. Histological examination of the spleen showed a massive proliferation of macrophages with prominent haemophagocytosis. In the liver only a small number of intrasinusoidal lymphocytes was observed. Immunohistochemical and genotypic analysis of spleen and liver specimens raised the suspicion of an occult lymphoma. The patient subsequently underwent chemotherapy with endoxan 50 mg/day and prednisone. After a transient improvement he was readmitted in March 1991 with fever and hepatomegaly with cholestasis. Throughout the entire course of the illness, the patient showed a variable, ultimately progressive neutropenia, always associated with marked monocytosis (20–70%). The lymphocyte count varied from 20% to 60% with no obvious atypia, although some lymphocytes displayed irregularities of the nucleus. A second liver biopsy revealed an increased number of intrasinusoidal lymphocytes compared with the first. This finding was consistent with malignant lymphoma. The dose of endoxan was increased to 100 mg/day. This regimen resulted in remission of the fever and normalization of liver size and function. After remaining relatively stable for 2 years, the patient's condition deteriorated rapidly with massive hepatomegaly, fever, thrombocytopenia and coagulation abnormalities developed in March 1993. For the first time, not only the liver but also the bone marrow revealed

Table 1 Phenotypic features of the clonal lymphocyte population (*p* predigestion (0.1% pronase))

Antigen	Clone	Results
Frozen sections		
CD1	Na1/34[a]	–
CD2	T9–10[a]	+
CD3	T3-4B5[a]	+
CD5	DK23[a]	–
CD7	DK24[a]	–
CD4	Leu-3[a]	–
CD8	Leu-2[a]	–
CD56	T199[a]	–
CD57	Leu-7[b]	–
TcRβ chain	βF1[c]	(+)
TcRδ chain	TcRδ1[c]	+
CD19	HD37[a]	–
CD20	B1[d]	–
μ Heavy chains	R1/69[a]	–
λ Light chains	λ-1[e]	–
κ Light chains	κ-117[e]	–
CD25	ACT-1[a]	–
CD30	Ber-H2[a]	–
CD74	CR3/43[a]	–
Proliferating cell nuclear antigen	Ki-67[a]	<5% (1990), ~80% (1993)
Paraffin sections		
CD45	PD7/26,22B11[a]	+
CD45RO	UCHL1[a]	+
CD43	MT1[f]	+
CD20	L26[a]	–
CD68 *p*	KP1[a]	–

[a] DAKOPATTS, Glostrup, Denmark
[b] Becton Dickinson Immunocytometry Systems, Mountain View, Calif., USA
[c] T Cell Sciences, Cambridge, Mass., USA
[d] Coulter Immunology, Hialeah, Fla., USA
[e] Biomedicals AG, Angst, Switzerland
[f] Biotest, Dreieich, Germany

a distinct neoplastic lymphoid infiltration. The patient was treated with combined chemotherapy (7 cycles of VACOP-B), and because of poor response, with fludarabine. This treatment led to a short partial remission. Subsequently the disease again progressed rapidly, and the patient died 3 years and 6 months after the first admission. No autopsy was performed.

Materials and methods

Fresh tissue samples were obtained from the following sites: spleen in 1990, liver in 1990 and 1991, and bone marrow in 1993. The samples were either shock frozen or fixed in 4% buffered formalin, embedded in paraffin, cut at 4 μm and stained with haematoxilin-eosin, van Gieson, period acid-Schiff, Giemsa and reticulin stains. Bone marrow biopsies were embedded in methacrylate, cut at 1 μm and stained with May-Grünwald-Giemsa.

Immunohistochemical investigation was performed on formalin-fixed deparaffinized tissue sections and on acetone-fixed cryostat sections. The primary monoclonal mouse antibodies (mAb) used in the study are listed in Table 1. Mouse antibodies were visualized using the ABC/peroxidase method (paraffin sections) or an indirect immunoperoxidase staining procedure (cryostat sections). Paraffin and frozen sections of human tonsils were used as controls. All technical controls were negative.

For genotypic analysis, genomic DNA was extracted from snap-frozen biopsy samples according to standard procedures [2]. Original protocols to analyse immunoglobulin heavy (*IgH*) chain [1] and *TcRβ* [15] gene rearrangements by Southern blotting were slightly modified. To study rearrangements of the *IgH* gene locus, *Hin*dIII and *Bgl*II were used as restriction enzymes, whereas for the *TcRβ* locus EcoRI, EcoRV, HindIII and XbaI restrictions were used. The Southern blots were hybridized with digoxigenin-labelled probes covering a 0.7-kb-long genomic fragment immediately downstream of the JH region and with a TcR $C_{\beta 1}$ cDNA probe, respectively. Detection was carried out by using a digoxigenin nucleic acid detection kit (Boehringer Mannheim, Germany).

Results

Morphological findings

The spleen was enlarged, weighing 2.5 kg and measuring 29×19×8 cm. The cut surface was dark red and soft. On microscopic examination the normal structure was effaced by a population of normal-looking activated histiocytes mainly located in enlarged sinuses and showing a massive phagocytosis of erythrocytes, and a small number of mononuclear cells (Fig. 1). The residual splenic cell population consisted of a few small, sometimes medium-sized lymphocytes without obvious atypia. Some of these latter cells corresponded to the morphologically undetectable neoplastic cell population to be shown and discussed later. No mitoses were seen. Small foci of reactive plasma cells were scattered in the red pulp. The white pulp was reduced to a few residual areas. Lymph nodes in the hilum of the spleen exhibited erythrophagocytizing macrophages and plasmocytosis but an intact architecture.

The liver wedge biopsy performed at splenectomy appeared normal apart from discretely proliferating macrophages, some displaying erythrophagocytosis, and few small to medium-sized lymphocytes in the sinusoids (Fig. 2a). Two needle biopsies of the liver, performed 10 months and 3 years (Fig. 2b) after splenectomy, showed progressively distended sinusoids with increased tumour cell infiltration. The tumour cells were slightly larger than small lymphocytes. The nucleus was round or had slightly irregular contours and contained dense chromatin and only occasionally a nucleolus. The cytoplasmatic rim was clear and slim. A few mitotic figures could be detected. Macrophages were slightly increased and occasionally showed erythrophagocytosis. Portal infiltration was absent.

The bone marrow aspirate and the trephine biopsy performed at admission showed a hypercellular marrow with stimulated erythro- and megacaryopoiesis. Two months later the bone marrow revealed progressive monocytosis. Just before fulminating illness developed (March 1993), the hypercellular marrow (15% monocytes) showed atypical lymphocytes (20%) (Fig. 3). Two months later massive infiltration of the bone marrow by neoplastic cells (35–60%) was present, now showing a blast-like change.

Phenotyping

In paraffin sections the tumour cells expressed CD45, CD45RO, CD43 and occasionally the activation antigen

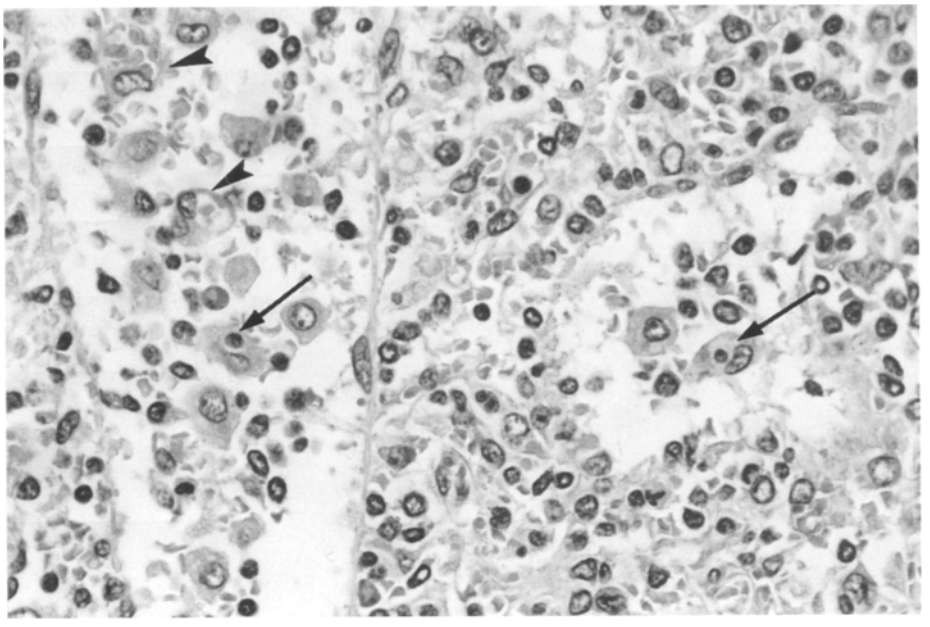


Fig. 1 Spleen showing expanded sinuses with phagocytic histiocytes (*arrows* lymphocytephagocytosis, *arrowheads* erythrophagocytosis) and few lymphoid cells without atypia

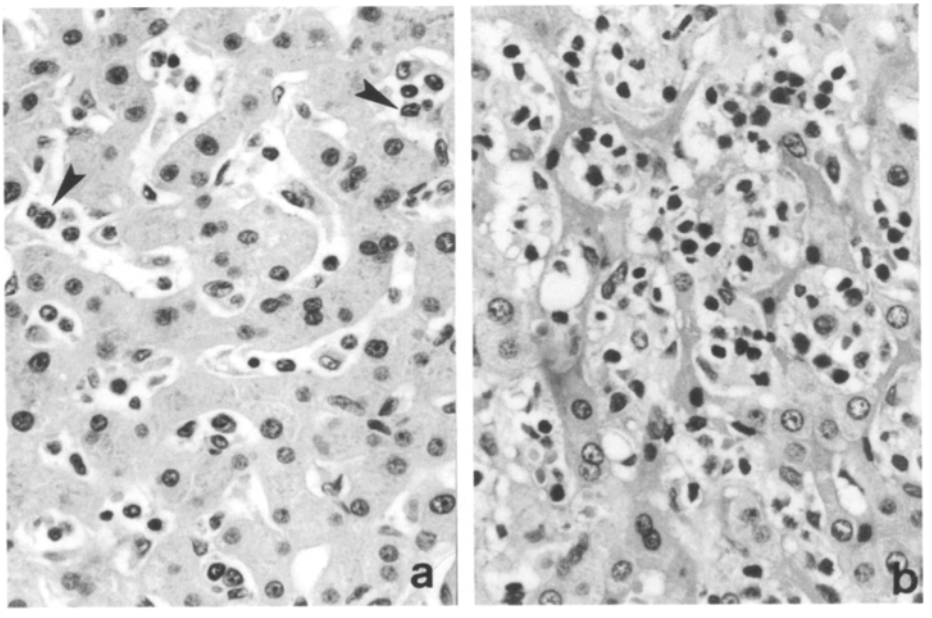


Fig. 2 **a** Initial liver biopsy with scant sinusoidal infiltration by macrophages and lymphoid cells, some corresponding to neoplastic cells (*arrowheads*). **b** During progression of the disease, massively proliferated intrasinusoidal tumour cells and atrophic hepatocytes

HLA-DR (CD74). The reactions for B-cell antigens (CD20, immunoglobulins), CD30 and CD15 were negative.

On frozen sections (liver, spleen, bone marrow) the neoplastic lymphoid T-cell population expressed a CD2+, CD3+, γδ TcR + (Fig. 4b) phenotype. CD1, CD5, CD7, CD4, CD8, CD56, CD57 and αβ TcR (Fig. 4a) were not expressed. The proliferation rate of neoplastic cells, estimated with the mAb Ki-67, progressed from 5–10% positive cells at the beginning of the disease to 80% in the last months before death (Table 1). Surface marker analysis by FACS of peripheral blood mononuclear cells performed 5 months before death demonstrated CD2+ 53%, CD3+ 69%, CD4+ 7%, CD8+ 5%, all values compatible with γδ TCL.

Genotyping

In all tissues examined (spleen, liver, bone marrow aspirate), Southern blot hybridizations with the TcR-C_β probe demonstrated the presence of a clonal T-cell population from the early course of the disease (Fig. 5). The *TcRβ* gene of the clonal cell population had undergone a biallelic rearrangement, both of the $C\beta_1$ region, as revealed by the appearance of two bands in addition to the germline fragments in the EcoRI and the EcoRV digest. The similar size differences between the rearranged fragments and the germline $C\beta_1$-band in both restrictions were suggestive of only partial D-J-rearrangements of both alleles. Since a partially rearranged *TcRβ* gene did not give rise to the expression of a functional αβ TcR,

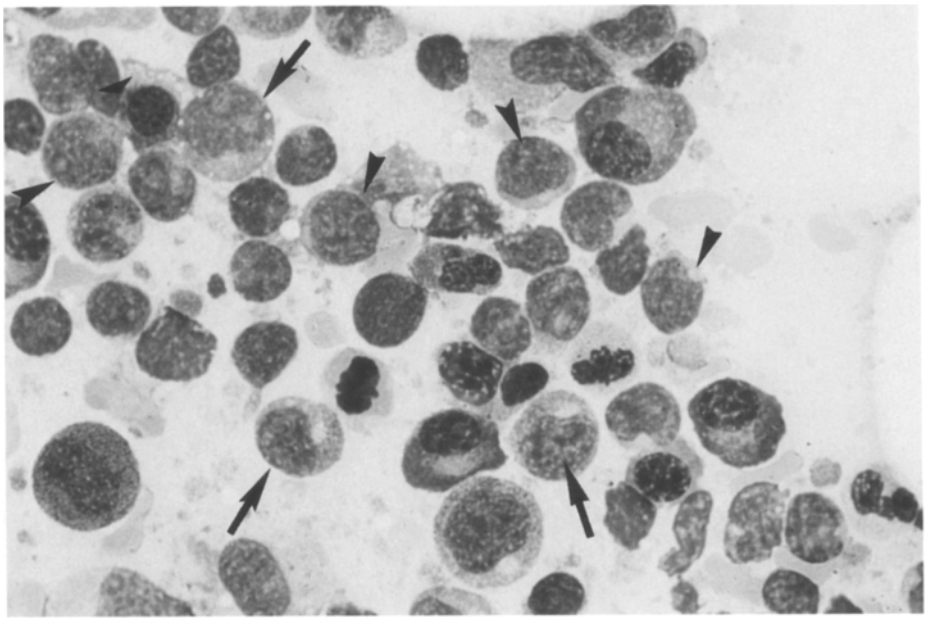
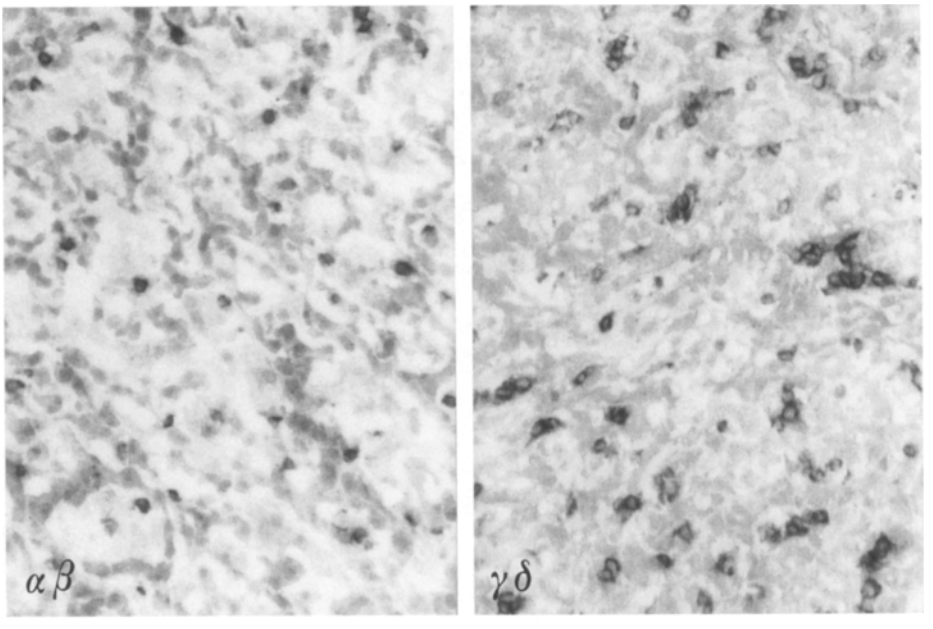


Fig. 3 Bone marow smear during progression of the disease showing small to medium-sized atypical lymphoid cells (*arrowheads*), with beginning blast-like transformation (*arrows*)



a b

Fig. 4a, b Frozen tissue sections of liver (1991) immunostained with mAb for TcRγδ and αβ. The malignant intrasinusoidal cells are TcRa1 positive (**b**) and bF1 negative (**a**). The few cells expressing bF1 correspond to reactive lymphocytes (**a**)

we suspected that the clonal cells express a γδ TcR on the cell surface. This rearrangement was subsequently confirmed by immunohistochemistry as the majority of lymphoid cells displayed surface staining with the anti-TcR-d1 mAb, identifying them as tumour cells.

Discussion

To date, two different TcR types have been described. While approximately 95% of normal T-cells circulating in the peripheral blood express the αβ TcR, 2–5% of these cells bear the γδ TcR. Mature αβ T-cells express either CD4 or CD8 [17], whereas most thymic αβ T-cells express both CD4 and CD8. In contrast, γδ T-cells are primarily CD4- CD8- during thymic differentiation and later in peripheral blood, while often expressing CD8 in tissues such as the spleen and the intestine [3,10]. Although still extremely rare, a growing number of γδ TCL are identified in tissues as the spleen and the skin, where normal γδ T-cells usually home.

The present hepatosplenic γδ TCL with its unusual histological presentation caused problems in both clinical and histological differential diagnosis. The patient's initial clinical presentation suggested a haemophagocytic syndrome (HS). Although splenectomy revealed proliferated macrophages with erythrophagocytosis, evidence of an HS could not be provided because erythrophagocyto-

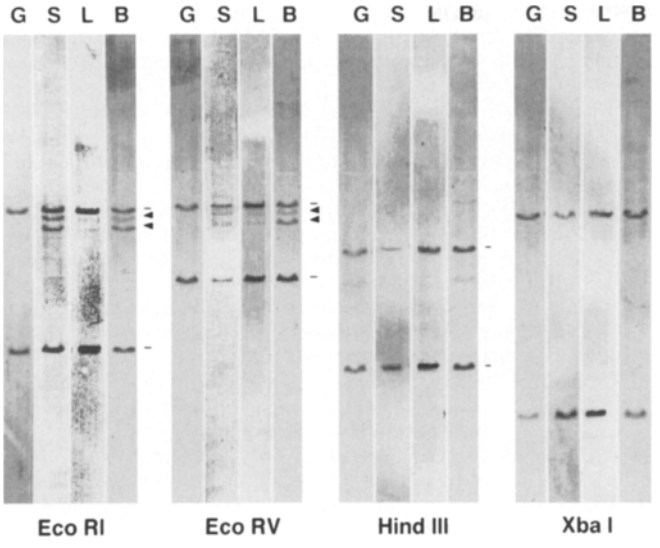


Fig. 5 TcR β gene rearrangement patterns of spleen (*S*), liver (*L*) and bone marrow (*B*) biopsies taken in May 90, April 91 and June 93, respectively, are compared with the germline fragments (*G*) from a fibroblast cell line on a non-radioactive Southern blot. Bands representing clonal rearrangement products are marked with an *arrowhead*, germline bands with a *dash*. The restriction digestions with *Eco*RI and *Eco*RV cover rearrangements of the *Cb1* locus, *Hin*dIII and *Xba*I rearrangements of the *Cb2* region

sis was absent in bone marrow and only minimal in the liver. Owing to the lack of any pre-existing disease, this incomplete form of HS was suggestive of an occult T-cell lymphoma. However, due to their scantiness and their small size, the tumour cells could not be identified on conventional histological sections or bone marrow smears. Furthermore, they resembled activated lymphoid cells in the context of a massive erythrophagocytosis in the spleen. Immunohistochemistry was difficult to interpret: the splenic T-cell population expressed CD2 and CD3 but intermingled proliferating and partially erythrophagocytizing macrophages expressing CD45 and CD4 blurred the picture. Genotyping finally established clonality by demonstrating the presence of a partially rearranged, non-functional TcRβ gene in both alleles. By immunoreactivity with TcRδ1 we confirmed that the small number of neoplastic cells in fact expressed a functional γδ TcR.

In type and distribution, the lymphoma described resembles the 11 hepatosplenic γδ TCL reported in the literature [4,5,6,7,13,14]. In 1986 Gaulard et al. [6] first recognized this form of PTCL infiltrating the spleen, liver and bone marrow without nodal involvement. Subsequently, Gaulard's group [7] suggested that an interaction between neoplastic γδ cells and endothelial cells of the spleen, liver and bone marrow could explain the preferential homing of γδ cells to these organs. Further reports documented the γδ form of the TcR by immunohistochemical and genotypic analysis [5,7,8,11,13,14]. Only one lymphoma expressed CD8 [4] in contrast to a CD4-CD8- phenotype of the other ten lymphomas, including the tumour described here. In the majority of γδ TCL the marker for natural killer cells NKH-1 (CD56) was demonstrated on tumour cells [4,7,14], but this marker was absent in the present tumour. The last bone marrow biopsy performed shortly before death showed a blastic transformation of the neoplastic T-cells. This morphological change during the terminal phase of the disease has also been described by others [14]. Of the other 11 patients with hepatosplenic γδ TCL reported in the literature, only 1 had a seemingly slight erythrophagocytosis in the spleen [13].

Cytopenias have been observed in the previously reported patients with hepatosplenic γδ TCL [6,13,14] and seem to be a characteristic haematological feature of this clinicopathological entity. During the course of the disease of this patient, a variable degree of neutropenia was observed. The association of neutropenia with T-cell lymphomas has been described previously and was related in part to the presence of anti-neutrophil antibodies [12]. However, suppression of granulopoiesis associated with T-cell malignancies by the production of lymphokines by the tumour cells is possibly more common. Recently, Wilhelm's group [18] showed a cutaneous γδ TCL elaborated high serum levels of interferon-γ, and they demonstrated its suppressive effect on myeloid/monocyte colony formation units, leading to progressive neutropenia.

The association of haemophagocytosis with PTCL is well known. Cytokines secreted by tumour cells have been incriminated in stimulation of the monocyte/macrophage line. In our patient haemophagocytosis was present, being observed mainly in the spleen in the initial stage of the disease. It subsided after splenectomy, although the disease and thus the tumour load progressed. It may be suggested that lymphoma cells secreted a low amount of cytokines that may have had a stimulating effect only on a macrophage-rich organ such as the spleen.

References

1. Arnold A, Cossman J, Bakhshi A, Jaffe ES, Waldman TA, Korsmeyer SJ (1993) Immunoglobulin-gene rearrangements as unique clonal markers in human lymphoid neoplasms. N Engl J Med 309: 1593–1599
2. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (eds) (1990) Current protocols in molecular biology, vol 1. Green Publishing Associates/Wiley, New York, pp 2.2.1–2.2.3
3. Bucy RP, Chen-Lo HC, Cihak J, Lösch V, Cooper MD (1988) Avian T cells expressing gamma delta receptors localize in the splenic sinusoid and the intestinal epithelium. J Immunol 141: 2200–2205
4. Cooke CB, Greiner T, Raffeld M, Kingma D, Statler-Stevenson M, Jaffe E (1994) γδ T cell lymphoma: a distinct clinicopathological entity. Mod Pathol 7: 106A
5. Farcet JP, Gaulard P, Marolleau JP, Le Couedic JP, Henni T, Gourdin MF, Divine M, Haioun C, Zafrani S, Goossens M, Hercend T, Reyos F (1990) Hepatosplenic T-cell lymphoma: sinusal/sinusoidal localization of malignant cells expressing the T-cell receptor γδ. Blood 75: 2213–2219
6. Gaulard P, Zafrani ES, Mavier P, Rocha FD, Farcet JP, Divine M, Haioun C, Pinaudeau Y (1986) Peripheral T-cell lymphoma presenting as predominant liver disease: a report of three cases. Hepatology 6: 864–868

7. Gaulard P, Bourquelot P, Kanavaros P, Haioun C, Le Couedic JP, Divine M, Goossens M, Zafrani ES, Farcet J-P, Reyes F (1990) Expression of the αβ and γδ T-cell receptors in 57 cases of peripheral T-cell lymphomas. Identification of a subset of γδ T-cell lymphomas. Am J Pathol 137: 617–628
8. Haioun C, Gaulard P, Bourquelot P, Roudot-Thoraval F, Divine M, Lavaud A, Bagot M, Vasile N, Farcet J-P, Reyes F (1992) Clinical and biological analysis of peripheral T-cell lymphomas: a single institution study. Leuk Lymphoma 7: 449–455
9. Hsu SM, Raine L, Fauger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabled antibodies (PAP) procedures. J Histochem Cytochem 29: 577–580
10 Janeway CA (1988) Frontiers of the immune system. Nature 333: 804–806
11. Kanavaros P, Farcet JP, Gaulard P, Haioun C, Divine M, Le Couedic JP, Lefranc MP, Reyes F (1991) Recombinative events of the T cell antigen receptor δ gene in peripheral T cell lymphomas. J Clin Invest 87: 666–672
12. Kruskall MS, Weitzmann SA, Stossel TP, Harris N, Robinson SH (1982) Lymphoma with autoimmune neutropenia and hepatic sinusoidal infiltration: a syndrome. Ann Intern Med 97: 202–205
13. Lin MT, Shen MC, Tien HF, Chen YC, Hsu PN, Chang CS, Wang CH (1991) Peripheral T gd lymphoma presenting with idiopathic thrombocytopenic purpura-like picture. Br J Haematol 78: 280–282
14. Mastovich S, Ratcj H, Ware RE, Moore JO, Borowitz MJ (1994) Hepatosplenic T-cell lymphoma: an unusual case of a γδ T-cell lymphoma with a blast-like terminal transformation. Hum Pathol 25: 102–108
15. Minden MD, Toyonaga B, Ha K, Yanagi Y, Chin B, Gelfand E, Mak T (1985) Somatic rearrangement of T-cell antigen receptor gene in human T-cell malignancies. Proc Natl Acad Sci USA 82: 1224–1227
16. Picker LJ, Weiss LM, Medeiros JL, Wood GS, Warnke RA (1987) Immunophenotypic criteria for diagnosis of non-Hodgkin's lymphoma. Am J Pathol 128: 181–201
17. Weiss A, Imboden S, Hardy K, Manger B, Terhorst C, Stobos S (1986) The role of T3 antigen receptor complex in T-cell activation. Annu Rev Immunol 4: 593–619
18. Wilhelm M, Meyer P, Batram C, Tony HP, Dummer R, Nestle F, Burg G, Wilms K (1992) γ/δ Receptor-expressing T-cell clones from a cutaneous T-cell lymphoma suppress hematopoiesis. Ann Hematol 65: 111–115

Note added in proof

Since the submission of this manuscript for publication we observed a second patient suffering from a splenic blastic γδ TCL. Bone marrow and blood did not show lymphoma cell infiltration (also controlled by genotypic analysis). So far no liver biopsy was performed. Interestingly, a monoclonal biallelic B-cell population was identified by Southern blot (IgH gene rearrangement) and FACS analysis, probably corresponding a small-sized B-cell population in blood and spleen. In the June 95.issue of The American Journal of Surgical Pathology there is a further description of a hepatosplenic γδ TCL by Wong F.K. et al. (Am J Surg Pathol 19(6):718–726).