Cytotoxic hepatosplenic γδ T-cell lymphoma following acute myeloid leukemia bearing two distinct γ chains of the T-cell receptor. Biological and clinical features

Eckhart Weidmann,* Thomas Hinz*, Stephan Klein,* Daniela K. Schui,* Svetlana Harder,@ Susanne Kriener,° Dieter Kabelitz,# Dieter Hoelzer,* Paris S. Mitrou*

*Department of Medicine III, Hematology/Oncology; °Department of Pathology, University Hospital, J.W. Goethe University, Frankfurt/Main; #Division of Immunology, Paul Ehrlich Institut, Langen; @Institute for Human Genetics, University of Kiel, Germany

ABSTRACT

Background and Objectives. Hepatosplenic γδ T-cell lymphoma is a rare entity of peripheral T-cell lymphomas. We characterized in detail the first case of hepatosplenic γδ -T-cell lymphoma following acute myeloid leukemia.

Design and Methods. Hepatosplenic γδ -T-cell lymphoma was diagnosed in a woman who had been in complete remission (CR) of acute myeloid leukemia (AML) for two years. Improvement but no objective response of the disease was observed after various types of chemotherapy. CR was achieved after related donor stem cell transplantation. Thirteen months later relapse of hepatosplenic γδ T-cell lymphoma was diagnosed. While being prepared for a second transplantation the patient developed meningeal lymphoma and died. The patient's lymphoma cells were studied by immunologic, functional and molecular techniques.

Results. Lymphoma cells expressed the γδ T-cell receptor (TCR), CD2, CD3, CD5, CD7, CD38, CD45, CD161 (NKR-P1), TIA and Ki67. Further analysis revealed expression of Vδ1 and two distinct TCRγ chains, Vγ3 and Vγ9, by the malignant cell clone. The clonality of the T-cells was confirmed by reverse transcriptase polymerase chain reaction (RT-PCR) followed by sequencing of TCR Vγ3, Vγ9 and Vδ1 junctional regions. Clone-specific PCR was negative at diagnosis of AML and was positive at all times during follow-up of the hepatosplenic γδ T-cell lymphoma. The lymphoma cells mediated strong natural killer cell-like cytotoxic activity, possibly explained by expression of CD161 and a lack of killer inhibitory receptor.

Interpretation and Conclusions. Several so far undescribed features were observed in this case of hepatosplenic γδ T-cell lymphoma, such as T-cell lymphoma following AML, expression of two distinct T-cell receptor γ-chains, and an unexpected cytotoxic phenotype.
©2000, Ferrata Storti Foundation

Key words: hepatosplenic γδ-T-cell lymphoma, NK-like cytotoxicity, cytotoxic phenotype, expression of two TCR γ chains

Correspondence: E. Weidmann, M.D., Department of Medicine III, Hematology/Oncology, University Hospital, Theodor-Stern-Kai 7, 60590 Frankfurt/M, Germany. Phone: international +49-069-6301-5779 – Fax: international +49-69-6301-7373 – E-mail: e.weidmann@em.uni-frankfurt.de

Hepatosplenic γδ T-cell lymphoma was reported as a distinct lymphoma entity in 1990[1] and recognized as such in 1994 by the REAL classification[2] because of several characteristic clinicopathologic features. Since the initial description by Farcet *et al.*[1] approximately 45 cases have been published in detail.[3] The disease has a rather aggressive clinical course. The clinical appearance is usually characterized by hepatosplenomegaly with a sinusal infiltration of the liver and a sinusoidal infiltration of the spleen, but no or little lymphadenopathy. In the majority of the cases the disease affects young male adults. Many of the patients present with systemic symptoms and pancytopenia.[3-5] A number of cases of hepatosplenic γδ T-cell lymphoma have been reported following organ transplantation, and 1 case after treatment of Hodgkin's disease.[3] The malignant cells usually appear as small to medium size lymphocytes with agranular, pale blue, somewhat abundant cytoplasm, however, blast-like transformation has been observed, mostly in association with a terminal event.[6-9] Immunologically, most of the cases have been characterized to express CD2, CD3, CD7, γδ T-cell receptor (TCR), Vδ1, and the cytotoxic granule associated protein (TIA-1) and in part CD5, CD8, CD56 and CD11c.[1,3,4,10,11] The predominant chromosomal aberration is an isochromosome 7q often in combination with trisomy 8.[3-5,12] In humans, γδ T-lymphocytes represent 1-10% of peripheral blood T-cells.[13] The TCR γ locus comprises only 6 expressed variable gene segments, i.e. Vγ2/3/4/5/8 belonging to the highly homologous VγI family, and Vγ9 of the single member family VγII. Eight expressed Vδ genes were described of which several can be used to construct functional TCR α chains.[14] Because hepatosplenic γδ T-cell lymphoma is a rarely diagnosed lymphoma entity, the purpose of our study was to characterize the clinical and biological features of this case, which may add information to previous reports.

Case Report

The 41-year old patient was admitted to our hospital in December 1994. She presented in the 21st week of pregnancy with severe anemia and thrombocytopenia, weakness and fatigue. Acute myeloid leukemia FAB M2 with a normal karyotype (46, XX) was diagnosed by cytology, cytochemistry, histology and immunophenotype (CD34+CD13+DR+). The patient decided not to interrupt her pregnancy. Two induction chemotherapies were needed to achieve complete remission (CR). Cesarean section was performed during the 27th week of pregnancy. The child is alive, but has a history of recurrent pneumonia. Consolidation therapy using intermediate-high dose cytarabine and amsacrine with peripheral stem cell apheresis followed by late consolidation with high dose cytarabine and daunorubicin was administered. In June 1997, the patient presented with marked lymphocytosis with partly enlarged cytoplasm at a routine check up. Because she had suffered from a strong viral infection immediately before, lymphocytosis was interpreted as reactive following the infection. However, in August, the patient had a leukocytosis of 80×10⁹/L, anemia (hemoglobin 9.9 g/dL) and thrombocytopenia (32×10⁹/L). Lactate dehydrogenase (LDH) concentration was 873 U/L, aspartase transferase (AST) 29 U/L, and alanine transferase (ALT) 34 U/L; all other routine parameters were normal. Epstein Barr virus (EBV), cytomegalovirus and human immunodeficiency virus titers were negative. Blood and bone marrow smears demonstrated the presence of blast cells within a population of small and medium sized lymphoid cells. Because of the patient's history and the evident presence of blast cells, relapse of AML was assumed. During pancytopenia following treatment with intermediate-high dose cytarabine/etoposide, a population of 4-6×10⁹/L predominantly small lymphocytes persisted. Immunophenotyping these lymphocytes showed that they expressed the γδ-T-cell receptor (Table 1). Because of the immunophenotype in combination with moderate hepato- and marked splenomegaly (longitudinal diameter 16 cm), anemia, thrombocytopenia and elevated LDH, at that point hepatosplenic γδ-T-cell lymphoma was diagnosed, even though a fine-needle liver biopsy demonstrated that portal infiltration was more pronounced than sinusal infiltration. Because of the diagnosis of a T-cell lymphoma, a bone marrow biopsy was performed. Due to chemotherapy the marrow was hypocellular, but was diffusely infiltrated by CD3 positive T-cells. After further chemotherapy consisting of fludarabine, cytarabine, granulocyte colony-stimulating factor (G-CSF), and idarubicin (FLAG-

Table 1. Immunophenotype (% positive cells) of the patient's peripheral blood or bone marrow lymphocytes during the course of hepatosplenic γδ T-cell lymphoma.

Antigen	cell type/protein	A (PB)	B (PB)	C (PB)	D (PB)	E (PB)
HLA-DR	MHC-class II	0	42	0	33	6
CD2	T-NK-cells	88		37		90
CD3	T-cells	98	26	94	36	93
CD5	T-cells	95		68		94
CD7	early T-cells	98				93
CD4	T-helper cells	5	8	4	27	1
CD8	T-suppressor cells	2	12	2	13	13
CD10	lymphoid progenitor cells	0				4
CD19	B-cells	0				3
CD20	B-cells	0			2	2
CD38	early B-, activated T/NK, plasma cells	96				99
CD45	common leukocyte antigen	99				
CD34	hematopoietic precursor cell	0		0		0
CD33	myeloid cells	0		1		1
CD11c	monocytes, activated T- and B-cells, NK-cells	39				26
CD13	myeloid cells	30				1
CD25	IL-2 receptor α chain	2		2		3
IL-2Rβ	IL-2 receptor β chain	19				3
CD16	NK-cells, cytotoxic T cells	7				5
CD56	NK-cells, cytotoxic T cells	8		2		1
CD57	NK-cells	6				1
CD161	natural-killer receptor-protein 1	95				95
KIR	killer-inhibitory receptor	0				0
TIA	cytotoxic-granule associated protein	83				29
perforin	cytotoxic-granule associated protein	0				0
Ki67	Ki67, proliferation marker	99				99
Fas ligand	Fas ligand, apoptosis inducing protein	9				6
TdT	lymphoid progenitor cells	0				0
TCαβ	T-cell receptor αβ chain	6	13	5	36	7
TCRγδ	T-cell receptor γδ chain	92	12	88	0	89
δ-TCS1	TCR Vδ1	89	19	89	1	92
Vγ2/3/4	TCR Vγ2/	94				
Vγ9	TCR Vγ9	94				

The percentage (%) of positive mononuclear cells are shown. A: at diagnosis of hepatosplenic γδ T-cell lymphoma; B: after FLAG-Ida; C: before related donor stem cell transplantation; D: after transplantation; E: in relapse. PB = peripheral blood; BM = bone marrow; empty spaces = not done. For comparison see the Case report section.

Ida), the number of lymphocytes decreased to 0.5×10⁹/L with the proportion of γδ-T cells being 12% (Table 1). The patient received related donor peripheral stem cell transplantation after conditioning with total body irradiation (TBI) (12.5 Gy) and 600 mg thiotepa, without major complications. Thirteen months after transplantation the patient relapsed from γδ T-cell lymphoma with marked lymphocytosis (40×10⁹/L), anemia, thrombocytopenia, and elevated LDH and aminotransferases, and mild systemic symptoms. While a donor related non-

myeloablative transplant in combination with radioimmunotherapy was prepared, the patient presented with right-sided Bell's pulsy. Cranial computed tomography was unremarkable. A cytospin of the cerebrospinal fluid revealed close to 100% small to medium sized agranular lymphoid cells, 15% of them with a blast like appearance and irregular nuclei. By flow cytometry, these cells were found to express the γδ T-cell receptor (data not shown). The diagnosis of meningeosis lymphomatosa was, therefore, established. The patient died shortly thereafter, 2 years after diagnosis of hepatosplenic γδ T-cell lymphoma. A *post mortem* autopsy was refused by the husband.

Design and Methods

Immunophenotyping

Surface expression of the following antigens on the patient's lymphocytes was analyzed by flow cytometry (FACScalibur, Becton-Dickinson, Heidelberg, Germany) using the FITC- or PE-labeled monoclonal antibodies: HLA-DR, CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD11c, CD13, CD16, CD19, CD20, CD25, CD33, CD34, CD38, CD45, CD56, CD57, CD161, TCRαβ-1, TCRγδ-1, purchased from Becton Dickinson, δ-TCS1 (T-cell Diagnostics, Cambridge, MA, USA), 23D12 (Vγ 2/3/4),[15] 7A5 (Vγ9),[16] Tu27 (IL-2 receptor β chain, Endogen, St Woburn, MA, USA), NKB1 (killer inhibitory receptor, Pharmingen, Düsseldorf, Germany) FasL (Alexis, San Diego, CA, USA). Flow cytometric analysis of cytoplasmic proteins was performed after permeabilization of the cells using a Fix and Perm kit (An der Grub Bio Research, Kaumberg, Austria) according to the manufacturer's instruction and staining the cells with TIA, perforin (Pharmingen), Ki67 (Dianova, Hamburg, Germany), or TdT (Coulter Immunotech, Krefeld, Germany).

Polymerase chain reaction (PCR)

RT-PCR amplification of Vδ1, Vγ3, and Vγ9 junctional sequences was performed as described elsewhere.[17] Clonality of γδ TCR rearrangements was determined by using the following junction-specific oligonucleotides: 5′-GCTACTTCTGAT-TCCCTC-3′ (sense, specific for the Vδ1 junction); 5′-TTCTTATGCCATAGGTGC-3′ (antisense, specific for the Vγ9 junction).

Cloning and sequencing of PCR products

PCR products were loaded onto 1% agarose gels, isolated with QiaEx II (Qiagen, Hilden, Germany) and ligated into pCR2.1 TOPO (Invitrogen, De Schelp, The Netherlands). Competent *E. coli* (XL2-Blue, Stratagene, Heidelberg, Germany) were transformed with the ligation mixture. Recombinant plasmids were isolated with QIAwell 8 Plus Plasmid Kit (Qiagen). They were sequenced on 373 and 377 DNA Sequencers (Applied Biosystems, Darmstadt, Germany) by using the Dye Terminator Cycle Sequencing Kit with Ampli Taq, FS (Applied Biosystems).

Immunofluorescent cytotoxicity assay using PKH67 target labeling

Cytotoxic activity of the patient's γδ T-cells was analyzed against autologous blast cells from the time of the initial diagnosis of AML and against cultured Daudi (Burkitt's lymphoma), K562 (CML blast crisis) and KG-1 (AML) cell lines. The target cells were labeled with PKH67 dye according to the instructions provided by the manufacturer (Sigma, Deisenhofen, Germany). The patient's γδ T-cells were used as effector cells without and after incubation with 500 IU/mL interleukin-2 (IL-2) for 3 days. Effector and target cells were incubated in complete RPMI 1640 media at effector:target ratios of 20:1, 10:1, 5:1, and 1:1 for 3 hours. Subsequently, the cells were pelleted and resuspended in 300 μL propidium iodide (PI) (Sigma) at a concentration of 10 μg/mL. Percentages of lysed target cells were analyzed by flow cytometry using a FACScan and the following equation:

$$\text{Lysis (\%)} = \frac{E - S}{100 - S} \times 100$$

where:
E = percentage of PI positive cells in the effector-target-cell co-culture; S = spontaneous percentage of PI positive cells.

Results

At diagnosis of hepatosplenic γδ T-cell lymphoma a lymphoid population of mature lymphocytes with a somewhat abundant pale blue cytoplasm predominated in the peripheral blood smear. The bone marrow smear demonstrated hypocellular normal hematopoiesis and the above mentioned lymphoid population (not shown). Retrospectively, the blast cells noted when AML relapse was suspected were probably blast-like transformed γδ-lymphoma cells. Unfortunately, no material was preserved for further analysis. At the time when hepatosplenic γδ T-cell lymphoma was diagnosed, approximately 90% of peripheral and bone marrow mononuclear cells expressed the γδ T-cell receptor (Table 1). The cells co-expressed CD2, CD3, CD5, CD7, CD38, CD45, CD161 (NKR-P1), TIA, Ki67, and in part CD11c, CD13, and Tu27 (Table 1). B-cell markers, CD4, CD8, CD10, CD25, CD33, CD34, HLA-DR, perforin, NKB1 (KIR), TdT, and the αβ T-cell receptor were negative (Table 1). Expression of CD16, CD56, CD57 and Fas ligand was
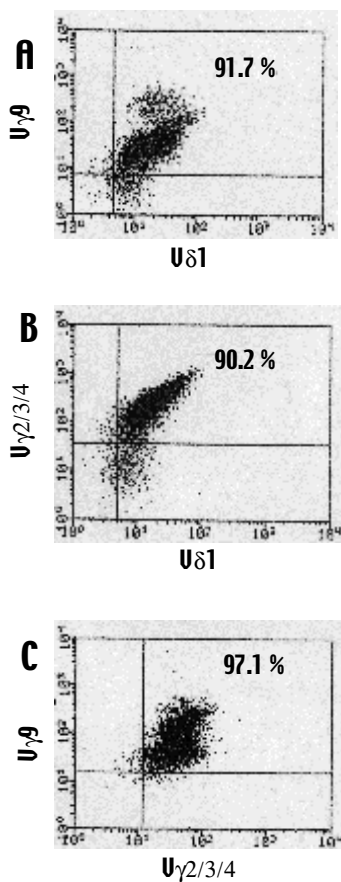
Figure 1. Demonstration of the presence of two distinct TCR γ regions on the malignant clone. FACS analysis of the patient's peripheral blood mononuclear cells using A: monoclonal antibodies directed towards Vδ1 (dTCS1) and Vγ9 (7A5), and B: Vδ1 and Vγ2/3/4 (23D12). C: For direct demonstration of the two Vγ chains, the lymphoma cells were stained with Vγ9 and Vγ2/3/4 specific antibodies.

low (Table 1). Virtually the same expression pattern was found in relapse of the disease. Only expression of TIA had decreased to 29% (Table 1). Expression of TIA, granzyme B and perforin was additionally analyzed by immunohistochemistry of a bone marrow biopsy. According to the results obtained by FACS, the cells did express TIA but not perforin. Granzyme B was likewise not detectable (data not shown). Further flow cytometric characterization of the γδ T-cell receptor using Vγ- and Vδ-specific monoclonal antibodies revealed that almost all peripheral blood mononuclear cells were 23D12+ (recognizing Vγ2/3/4) and 7A5+ (recognizing Vγ9) (Table 1, Figure 1). At the same time the lymphoma cells were Vδ1+/Vγ9+ and Vδ1+/Vγ2/3/4+. Amplification of the Vγ1 family junctional region followed by cloning and sequencing revealed the presence of a single in-frame Vγ3 junctional sequence (Fig-

ure 2). Additionally, single Vδ1 and Vγ9 junctional sequences were detected (Figure 2). These results demonstrated clonality of the cells and proved expression of two distinct TCR-Vγ chains: 10 out of 10 TCR δ-chain cDNA clones showed an identical in-frame sequence with a Vδ1-Dδ2-Jδ1 rearrangement including N-region diversity (Figure 2). Regarding the TCR γ chain, in 18 out of 18 sequenced Vγ3 cDNA clones we found the same in-frame junctional region. Similarly, all of the Vγ9 cDNA clones (23/23) harbored the same junctional in-frame sequence displaying N-region diversity (Figure 2). From these nucleotide sequences, clone-specific primers annealing to the junctional regions were designed to monitor, by reverse transcriptase-PCR, the presence of the clonal γδ-cell population at diagnosis of AML, at the time of stem cell apheresis and during follow-up of the hepatosplenic γδ T-cell lymphoma. In bone marrow from diagnosis of AML and in the leukapheresis product after the first consolidation therapy neither specific Vγ9 nor Vδ1 rearrangements were detected (Figure 3). Specific PCR products for TCR Vδ1 and TCR Vδ9 were detected at the time when relapse of AML was assumed, at diagnosis of hepatosplenic γδ T-cell lymphoma, after two different chemotherapy regimens and in relapse of the disease (Figure 3). Three attempts were made to perform cytogenetic analysis from frozen cells from diagnosis of the disease and from relapse. Unfortunately the cells did not grow in culture. Fluorescent *in situ* hybridization (FISH) analysis was performed and excluded the presence of isochromosome 7 and trisomy 8 (data not shown). Cytotoxic activity of the patient's γδ T-cell lymphoma cells was tested against autologous AML blasts or 3 cell lines. The cells did not mediate lysis of autologous AML blasts and the KG1 (myeloid) cell line (Figure 4). Of the natural-killer cell sensitive cell line K562 (myeloid), more than 70% of the cells were killed by the γδ T-cells at effector:target ratios of 20:1 and 10:1 (Figure 4). Some cytotoxic activity was also detected against the Daudi (Burkitt's lymphoma) cell line (Figure 4). Incubation of the lymphoma cells with interleukin-2 for 3 days did not result in any difference regarding cytotoxicity as compared to the γδ T-cells not incubated with IL-2 (data not shown).

Discussion

Peripheral T-cell lymphomas account for approximately 10-15% of all non-Hodgkin's lymphomas.[5] A rare entity of these is hepatosplenic γδ T-cell lymphoma, a disease with a number of common clinicopathologic features, such as hepatosplenomegaly with a typical infiltration pattern, presentation with systemic symptoms, pancytopenia, predominant occurrence in

Vδ1	N	Dδ2	N	Jδ1	n	IF
<u>TGTGCTCTTGGGGACT</u> TGTGCTCTTGGGGA	TCCCTTGCTA	<u>CCTTCCTAC</u> CTTC	TGATTCCCTC	<u>ACACCGATAAACTCATC</u> TAAACTCATC	10/10	+

Vγ	N	Jγ2.3	n	IF	Vγ
<u>TGTGCCACCTGGGACAGG</u> TGTGCCACCTGGGAC	TGTC	<u>GAATTATTATAAGAAA</u> ATTATAAGAAA	18/18	+	3
<u>TGTGCCTTGTGGGAGG</u> TGTGCCTTGTGGGAGG	TGCACCTATGGC	<u>GAATTATTATAAGAAA</u> ATAAGAAA	23/23	+	9

Figure 2. Junctional sequences of Vγ3, Vγ9, and Vδ1 γδ-TCR rearrangements. The 3' ends of Vγ3, Vγ9, and Vδ1 beginning at the highly conserved cysteine (TGT) are shown. N: non-germline encoded nucleotides; D: diversity segment; J: joining segment; n: number of identical cDNA clones/total number of sequenced clones; IF+: in-frame sequence. Germline sequences are underlined.
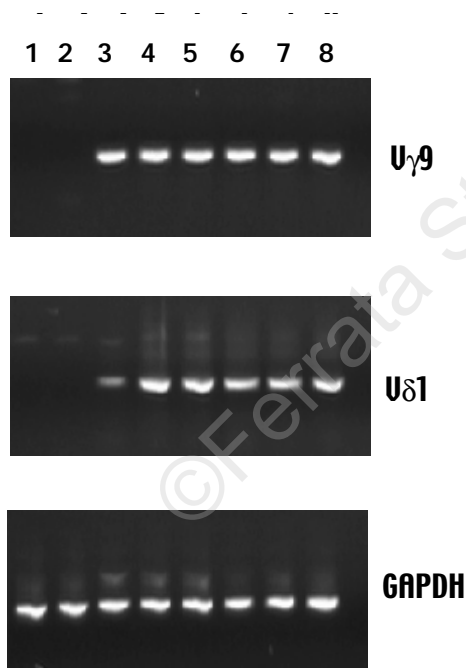
Figure 3. Clone-specific PCR at different times of follow-up. The figure shows specific PCR products of Vγ9 and Vδ1, and of GAPDH to monitor the integrity of the cDNA. Lanes 1: at diagnosis of AML (bone marrow); 2: leukapheresis product after first consolidation therapy of AML; 3: at the time of suspected relapse of AML (bone marrow); 4: at diagnosis of hepatosplenic γδ T-cell lymphoma (peripheral blood); 5: after the second treatment with intermediate-high dose cytarabine/etoposide (peripheral blood); 6: after FLAG-Ida (peripheral blood), 7: before allogeneic stem cell transplantation (bone marrow); 8: at diagnosis of relapse of hepatosplenic γδ T-cell lymphoma (bone marrow). For comparison see the Case report section.

young men, and the frequent presence of isochromosome 7q often in combination with trisomy 8.[3-5, 12] Our patient represents the first case with secondary hepatosplenic γδ T-cell lymphoma following AML. Secondary disease accounts for approximately 20% of all cases of hepatosplenic γδ T-cell lymphoma, however, the majority of those have been documented after organ transplantation. In a recent review on 45 cases, 6 patients were described to have a history of renal transplantation and 1 patient a history of heart transplantation. One patient had previously had Hodgkin's disease.[3] There was
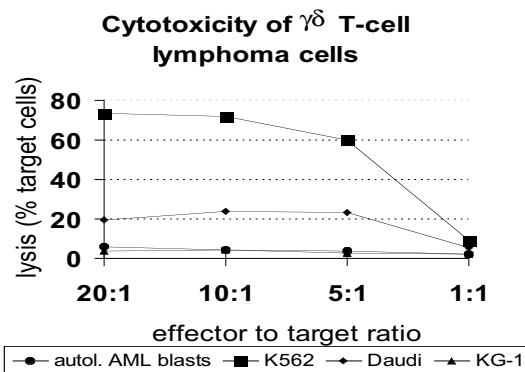

Figure 4. Cytotoxic activity of γδ T-cell lymphoma cells. Autologous AML blasts and the cell lines K562, Daudi, and KG-1 were used as target cells.

been a recent report of an additional case after renal transplantation in a patient who experienced EBV infection, most likely as a late event following transformation of the γδ T-cells.[18] At presentation with marked leukocytosis, thrombocytopenia and anemia, relapse of AML was assumed because of the history of AML, the unfavorable prognostic factor of not having achieved CR after induction therapy and the presence of blast cells even though the majority of the cells were small lymphocytes. Retrospectively, clone-specific PCR demonstrated the presence of the lymphoma. Thus the blast cells must be interpreted as blast-like transformed γδ T-cells, as described in other cases.[3,9] In accordance with most of the reported cases with hepatosplenic γδ T-cell lymphoma the present patient shared features such as hepatosplenomegaly, bone marrow hypocellularity with anemia and thrombocytopenia, increased LDH serum level, mild systemic symptoms, lack of lymphadenopathy, and presence of Vδ1. Clinical features, which have not been described before in hepatosplenic γδ T-cell lymphoma were marked lymphocytosis at presentation, secondary disease after AML, and meningeosis lymphomatosa (see the *Case report* section). Pronounced or exclusively portal infiltration of the liver has been described only in a few patients.[1,19,20] In view of the marked leukemic spread at presentation, hepatosplenomegaly and the aggressive clinical course, the present case resembles the aggressive variant of T-LGL (large granular lymphocyte) leukemia described by Gentile *et al.*[21] However these cases have been described to present with extensive lymphadenopathy. Furthermore, the T-LGL cells expressed CD56 and the αβ T-cell receptor.[21] The clinical course of the patient is just another example of this very aggressive type of peripheral T-cell lymphoma. Even though a wide variety of therapies have been tried, the median survival of the cases reported so far is less then one year.[3] A few patients in CR after bone marrow transplantation have been described; the observation time was, however, short.[10,22] Thus, a curative treatment of hepatosplenic γδ T-cell lymphoma has not been established.

A phenotype which has been accepted to be common in hepatosplenic γδ T-cell lymphoma is CD2+ CD3+ CD4- CD5- CD7+ CD8- CD38+ CD45+ TCRγδ+ TdT-.[3,9,10,20,23] NK-related antigens CD16 and CD56 are frequently expressed.[3,10,18,20,23] There are exceptions to this phenotype since expression of at least CD5, CD7, CD8, CD16 and CD56 is variable.[3,10,22] Therefore, expression of the T-cell associated marker CD5 and the low frequency of NK-cell markers CD16 and CD56 may, as in our patient, occur. In analogy with

the vast majority of the cases[4,10] analysis of the γδ TCR V regions revealed expression of Vδ1. Surprisingly, however, the tumor cells were reactive with both monoclonal antibodies, 23D12 (Vγ2/3/4) and 7A5 (Vγ9). This finding of surface expression of two distinct Vγ chains was confirmed by PCR using a panel of Vγ specific oligonucleotides and subsequent cloning and sequencing of the PCR products. The data indicate that allelic exclusion did not take place in this malignant clone. The phenomenon of not undergoing allelic exclusion of normal, mature T-cells has been observed for all TCR chains,[15,24,25] although its frequency is low in normal γδ T-cells.[15,24] In malignant γδ T-cells expression of two distinct γ chains has been described only once[17] and never yet recognized in hepatosplenic γδ T-cell lymphoma. CDR3-region specific primers were designed to monitor presence of the disease at different times during follow-up. At diagnosis of AML and in a sample of leukapheresed cells after treatment of AML, specific TCRγδ transcripts were not detectable. Because of the possibility of primary splenic disease this does not exclude the presence of hepatosplenic γδ T-cell lymphoma, but the sensitivity of the method indicates that the lymphoma occurred secondary to AML. Strong signals for Vδ1 and Vγ9 appeared at all times after diagnosis of hepatosplenic γδ T-cell lymphoma. Despite several approaches, we were unable to karyotype the lymphoma cells of our patient. Isochromosome 7 or trisomy 8, shown to be present in approximately 50% of the cases, was not detected by FISH. However, this does not exclude hepatosplenic γδ T-cell lymphoma, because other chromosomal aberrations and even normal karyotypes occur.[3,23]

Regarding the cytotoxic phenotype in hepatosplenic γδ T-cell lymphoma, expression of TIA but neither perforin nor granzyme B in 10 cases has been described by Boulland *et al.*[11] These authors concluded that this lymphoma entity represents a tumor consisting of more immature, non-activated γδ T-cells. However, because of the paucity of functional studies, there are few experimental data to confirm this hypothesis. Furthermore, perforin and granzyme B immunopositivity has been reported in hepatosplenic γδ T-cell lymphoma by others.[9,10,20] Functional data have been obtained in two previous studies.[6,9] Falcao *et al.*[6] demonstrated NK-like cytotoxicity. Salhany *et al.*[9] observed quite unusual features in one of their cases: cytotoxicity was mediated by the lymphoma cells after prestimulation with CD3 and CD16 antibodies, suggesting TCR-mediated- or antibody-dependent cellular cytotoxicity. In our case, despite a lack of perforin expression, NK-like cytotoxicity

was clearly demonstrated by strong lysis of K562 cells implicating mature NK-cell function by the γδ T-cells. Autologous AML blasts were not lysed, even after incubation with IL-2. This may be related to low expression of CD25 or to strong resistance to lymphokine-activated killer-cell cytotoxicity by AML blasts in some cases (own observation, not shown). Whether this observation has an *in vivo* relevance remains unclear. Because of the low frequency of expression of CD16 and CD56 in the present case, NK-like cytotoxicity of the lymphoma cells may be somewhat surprising, but is supported by expression of CD161 (NKR-P1, an NK-cell activating receptor) and absence of NKB1 (KIR, killer inhibitory receptor), neither of which has been previously studied in hepatosplenic γδ T-cell lymphoma cells. These receptors belong to receptor families (CD161: lectin-like receptors; NKB1: immunoglobulin-like receptors) that control different activating and inhibiting pathways and whose balance determines the behavior of cytotoxic lymphocytes. Killer inhibitory receptors are activated by HLA-class I molecules.[26] The potentially activating capacity by NKR-P1 in the absence of this inhibitory pathway may explain the strong NK-like activity in our particular case. The reason for the lack of perforin and granzyme B expression remains unclear. Possibly the cytotoxicity by the malignant γδ T-cells was mediated through TIA or some other cytotoxic mechanism. One may speculate that these cells, despite an immature cytotoxic phenotype, may exhibit cytotoxic activity or that they represent functionally mature cytotoxic cells with defective granzyme B or perforin expression. In conclusion, despite an atypical infiltration pattern of the liver and absence of typical cytogenetic features, extranodal lymphoma with hepatosplenomegaly, TCR γδ expression and characteristic routine laboratory parameters in our patient indicated the presence of hepatosplenic γδ T-cell lymphoma. Previous acute myeloid leukemia, the expression of two TCR γ-chains by the malignant clone and the unusual cytotoxic phenotype are so far undescribed features of this disease.

Contributions and Acknowledgments

Funding

Disclosures

Manuscript processing

Potential implications for clinical practice

♦ Hepatosplenic γδ T-cell lymphoma may occur following acute myeloid leukemia Meningeosis lymphomatosa may develop as a complication of this disease.

References

1. Farcet JP, Gaulard P, Marolleau JP, et al. Hepatosplenic T-cell lymphoma: sinusal/sinusoidal localization of malignant cells expressing the T-cell receptor γδ. Blood 1990; 75:2213-9.
2. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms. A proposal from the International Lymphoma Study Group. Blood 1994; 84:1361-92.
3. Weidmann E. Hepatosplenic T-cell lymphoma. A review on 45 cases since the first report describing the disease as a distinct lymphoma entity in 1990. Leukemia 2000; 14:991-7.
4. Chang KL, Arber DA. Hepatosplenic γδ T-cell lymphoma - not just alphabet soup. Adv Anat Pathol 1998; 5:21-9.
5. Jaffe ES, Krenacs L, Kumar S, Kingma DW, Raffeld M. Extranodal T-cell and NK-cell neoplasms. Am J Clin Pathol 1999; 111:46-55.
6. Falcao RP, Voltarelli JC, Simoes BP, et al. Malignant Tγ/δ lymphoproliferative disease with natural killer lytic activity. Am J Hematol 1992; 41:128-31.
7. Mastovich S, Ratech H, Ware RE, Moore JO, Borowitz MJ. Hepatosplenic T-cell lymphoma: an unusual case of γδ T-cell lymphoma with a blast like terminal transformation. Human Pathol 1994; 25:102-8.
8. Garcia-Sanchez F, Menarguez J, Cristobal E, et al. Hepatosplenic γδ T-cell malignant lymphoma: report of the first case in childhood, including molecular minimal residual disease follow-up. Br J Haematol 1995;

90:943-6.

9. Salhany KE, Feldman M, Kahn MJ, et al. Hepato-splenic γδ T-cell lymphoma: ultrastructural, immunophenotypic, and functional evidence for cytotoxic T lymphocyte differentiation. Hum Pathol 1997; 28: 574-85.

10. Cooke CB, Krenacs L, Stetler-Stevenson M, et al. Hepatosplenic T-cell lymphoma: a distinct clinico-pathological entity of cytotoxic γδ T-cell origin. Blood 1996; 88:4265-74.

11. Boulland ML, Kanavaros P, Wechsler J, Casiraghi O, Gaulard P. Cytotoxic protein expression in natural killer cell lymphomas and in αβ and γδ peripheral T-cell lymphomas. J Pathol 1997; 183:432-9.

12. Kinney MC. The role of morphologic features, phenotype, genotype, and anatomic site in defining extra-nodal T-cell or NK-cell neoplasms. Am J Clin Pathol 1999; 111:104-18.

13. Kabelitz D, Wesch D, Hinz T. γδ T cells, their receptor usage and role in human diseases. Semin Immuno-pathol 1999; 21:55-75.

14. Arden B, Clark SP, Kabelitz D, Mak TW. Human T-cell receptor variable gene segment families. Immuno-genetics 1995; 41:455-500.

15. Kabelitz D, Ackermann T, Hinz T, et al. New mono-clonal antibody (23D12) recognizing three different Vγ elements of the human γδ T-cell receptor: 23D12+ cells comprise a major subpopulation of γδ T-cells in postnatal thymus. J Immunol 1994; 152:3128-36.

16. Jannsen O, Wesselborg S, Heckl-Östereicher B, et al. T-cell receptor/CD3 signalling induces death by apoptosis in human T-cell receptor γδ+ cells. J Immunol 1991; 146:35-9.

17. Hinz T, Marx S, Nerl C, Kabelitz D. Clonal expansion of γδ T cells expressing two distinct T-cell receptors.

Brit J Haematol 1996; 94:62-4.

18. Roncella S, Cutrona G, Truini M, et al. Late Epstein-Barr virus infection of a hepatosplenic γδ T-cell lymphoma arising in a kidney transplant recipient. Haematologica 2000; 85: 256-62.

19. Gaulard P, Zafrani ES, Mavier P, et al. Peripheral T-cell lymphoma presenting as predominant liver disease: a report of three cases. Hepatology 1986; 6:864-8.

20. Yamaguchi M, Ohno T, Nakamine H, et al. γδ T-cell lymphoma: a clinicopathologic study of 6 cases including extrahepatosplenic type. Int J Hematol 1999; 69:186-95.

21. Gentile TC, Uner AH, Hutchison RE, et al. CD3+, CD56+ aggressive variant of large granular lympho-cyte leukemia. Blood 1994; 84:2315-21.

22. Jonveaux P, Daniel MT, Martel V, Maarek O, Berger R. Isochromosome 7q and trisomy 8 are consistent pri-mary, non-random chromosomal abnormalities asso-ciated with hepatosplenic T γδ lymphoma. Leukemia 1996; 10:1453-5.

23. Alonsozana ELC, Stamberg J, Kumar D, et al. Isochro-mosome 7q, the primary cytogenetic abnormality in hepatosplenic γδ T-cell lymphoma. Leukemia 1997; 11:1367-72.

24. Davodeau F, Peyrat MA, Houde L, et al. Surface expression of two distinct functional antigen recep-tors on γδ T-cells. Science 1993; 260:1800-2.

25. Davodeau F, Peyrat MA, Romagné F, et al. Dual T-cell receptor β chain expression on human T lympho-cytes. J Exp Med 1995; 181:1391-8.

26. Valez-Gomez M, Reyburn H, Strominger J. Molecular analyses of the interactions between human NK recep-tors and their HLA ligands. Hum Immunol 2000; 61: 28-38.