Hepatosplenic gamma-delta T-Cell Lymphoma as a Late-Onset Posttransplant Lymphoproliferative Disorder in Renal Transplant Recipients

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Abstract

We report 2 cases of renal transplant recipients in whom hepatosplenic gamma-delta T-cell lymphoma (gamma-delta HSTCL) developed 5 and 10 years after transplantation. Both patients had marked hepatosplenomegaly, B symptoms (weight loss, fever, and night sweats), and abnormal peripheral blood findings, including anemia in both, thrombocytopenia and leukoerythroblastic changes in 1, and leukocytosis in the other. Markedly atypical lymphoid infiltrate of intermediate to large cells was observed in the spleen, liver, and bone marrow. The malignant cells showed typical immunophenotype of gamma-delta T cells (CD2+, CD3+, CD4–, CD8–, CD7+, gamma-delta T-cell receptor–positive, and alpha-beta T-cell receptor–negative) with clonal T-cell receptor gene rearrangement and were of the V-delta-1 subset. In addition, the cells contained a cytolytic granule–associated protein, TIA-1, and Fas ligand, indicating cytotoxic T-cell differentiation. The malignant T cells in both cases were of host tissue origin. Both cases were negative for Epstein-Barr virus genome using Southern blot analysis. The patients did not respond to reduction of immunosuppression. Despite initial response to chemotherapy, both patients died within 6 months of diagnosis. Our findings indicate that gamma-delta HSTCL can occur as a late complication in transplant recipients.

In comparison with the general population, organ transplant recipients have markedly increased risk for developing lymphoproliferative disorders.[1] The majority of posttransplant lymphoproliferative disorders (PTLDs) are of B-cell origin, with histologic features ranging from lymphoid hyperplasia to frankly malignant lymphoma. The lesions almost invariably contain Epstein-Barr virus (EBV). The incidence of B-cell PTLDs is related directly to the intensity of immunosuppression, and reduction in immunosuppressive therapy may result in complete regression of the disease. Nevertheless, a small percentage of PTLD cases are of T-cell origin. Although both B- and T-cell PTLDs have a strong predilection for extranodal sites,[2,3] T-cell PTLDs have several distinct features. Most of the reported T-cell PTLD cases manifested as frank lymphoma or leukemia, lacked a clear association with EBV infection, and had a clinically aggressive course.[2,4-8]

Hepatosplenic gamma-delta T cell lymphoma (gamma-delta HSTCL) is a rare subset of peripheral T-cell lymphoma characterized immunologically by expression of gamma-delta T-cell receptor (TCR) and clinically by hepatosplenomegaly (but usually no associated lymphadenopathy), significant cytopenia, and an aggressive course.[9,10] These lymphomas preferentially infiltrate the hepatic sinusoids and splenic red pulp but also may involve lymph node sinuses, bone marrow, and peripheral blood.[9] Normal gamma-delta T cells represent a minor subset of T cells. Based on the use of the TCR delta chain variable region, 2 subsets of gamma-delta T-cells are found in humans; the V-delta-1 subset is distributed preferentially in the thymus and spleen, whereas the V-delta-2 subset is distributed in the peripheral blood, lymph nodes, and tonsils.[9]

We present clinicopathologic, immunophenotypic, serologic, and molecular analyses of 2 cases of T-cell lymphoma that occurred in patients receiving long-term immunosuppressive therapy for renal transplantation. Our studies show that the lymphomas observed in these patients are distinct from the more commonly seen polymorphic or monomorphic B-cell lymphoproliferative disorders that occur in organ transplant recipients and that the lymphomas are clinically and histopathologically similar to the gamma-delta HSTCLs seen in the general population. The tumor cells in both cases were of the V-delta-1 subset, corresponding to the dominant subset of gamma-delta T cells normally present in the spleen. The malignant T cells expressed a cytolytic granule–associated protein, TIA-1, and Fas ligand, indicating cytotoxic T lymphocyte (CTL) differentiation. In both cases, the tumor cells were of host tissue origin. Clinically, the patients did not respond to reduction of immunosuppression and died within 6 months of the initial diagnosis despite administration of chemotherapy.

Case Reports

Case 1

A 46-year-old man with a history of hereditary nephritis that resulted in end-stage renal disease received a cadaveric renal transplant in 1991. Early after transplantation, he required OKT3 treatment for acute rejection on 2 separate occasions. He was receiving cyclosporine and prednisone for immunosuppression.

In October 1996, the patient was examined because of a several-month history of daily fevers with chills, drenching sweats, and headache. Physical examination and a computed tomography scan revealed hepatosplenomegaly without associated lymphadenopathy. Laboratory studies showed the following: lactate dehydrogenase level, 966 U/L; elevated liver function test results, including an aspartate aminotransferase level of 160 U/L, an alanine aminotransferase level of 84 U/L, and a total bilirubin level of 0.9 mg/dL (15 μmol/L); WBC count, 5,000/μL (5.0 · 10⁹/L); platelet count, 45 · 10³/μL (45 · 10⁹/L); and hemoglobin level, 9.0 g/dL (90 g/L). Examination of the peripheral blood smear showed a leukoerythroblastic picture with 27% nucleated RBCs per 100 WBCs.

Results of viral studies, including serologic testing for HIV, EBV, cytomegalovirus, and syphilis and direct fluorescent assays for varicella and herpes simplex virus, were negative.

The patient underwent a diagnostic splenectomy and liver biopsy, which revealed a T-cell lymphoma. A bone marrow biopsy showed scant atypical lymphoid infiltrate. Postoperatively, the patient had immediate resolution of his fevers, and

the platelet count had normalized by postoperative day 4. Initial therapy consisted of withdrawal of immunosuppression, and there was no evidence of graft rejection. Within several weeks, however, the patient developed recurrent fever, malaise, and thrombocytopenia. He then received combination chemotherapy, which consisted of 2 cycles of cyclophosphamide, methylprednisolone, and etoposide, followed by 1 cycle of standard cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy. With each treatment, the patient had brief but unsustained resolution of his systemic symptoms. A subsequent single treatment with high-dose cyclophosphamide resulted in a more prolonged symptomatic response.

After a conditioning therapy, which consisted of cyclophosphamide and total body irradiation, he received an allogeneic bone marrow transplant from an HLA-identical brother. The patient's course after bone marrow transplantation was complicated by veno-occlusive disease, hypercalcemia, hypoxemia, and changes in mental status. A bone marrow biopsy at posttransplant day 20 revealed regenerating marrow without evidence of residual lymphoma. However, multiorgan system failure developed, and the patient died on posttransplant day 21, approximately 5 months after the initial diagnosis of lymphoma. An autopsy was not performed.

Case 2

A 42-year-old woman with a history of scleroderma and CREST (calcinosis cutis, Raynaud phenomenon, esophageal dysfunction, sclerodactyly, and telangiectasia) syndrome complicated by lupus nephritis and renal failure received a renal transplant from a living related male donor in 1985 and was receiving cyclosporine and prednisone for immunosuppression.

The renal graft failed 10 years after transplantation, and the patient underwent hemodialysis in May 1996. The patient also was examined at that time for an 8-month history of persistent fever of unknown origin and recent pain in the left upper quadrant of the abdomen. The physical examination identified marked hepatosplenomegaly with the spleen palpable 5 cm below the costal margin, which was confirmed by a computed tomography scan. There was no associated lymphadenopathy.

Laboratory studies showed the following: lactate dehydrogenase level, 652 U/L; considerably elevated liver function test results, including an alkaline phosphatase level of 362 U/L, a gamma-glutamyltransferase level of 147 U/L, and a total bilirubin level of 3.6 mg/dL (62 μmol/L) with 3.0 mg/dL (51 μmol/L) of direct bilirubin; WBC count, 13,700/μL (13.7 · 10⁹/L); platelet count, of 247 · 10³/μL (247 · 10⁹/L); and hemoglobin level, of 7.6 g/dL (76 g/L). A bone marrow biopsy revealed a T-cell lymphoma.

The cyclosporine was discontinued, and the patient was followed-up closely for 10 days. However, her condition

continued to deteriorate. Combination chemotherapy with cyclophosphamide, doxorubicin, prednisone, and etoposide resulted in symptomatic improvement and decreased hepatosplenomegaly. A repeated bone marrow biopsy after the second cycle of the chemotherapy also showed decreased involvement by lymphoma, to less than 5% of the marrow cellularity. The patient received a total of 6 cycles of chemotherapy. Nevertheless, at the end of the sixth cycle she had persistent fever, splenomegaly, and persistent lymphoma in the bone marrow; overt leukemia developed in the peripheral blood. Her condition deteriorated rapidly, and the patient died 6 months after the initial diagnosis of the lymphoma. An autopsy was not performed.

Materials and Methods

Morphologic and Immunophenotyping Studies

Histologic evaluation of the spleen, liver, and bone marrow was performed on 4-μm-thick, H&E-stained and periodic acid-Schiff–stained, paraffin sections of formalin-fixed or B-5-fixed tissue. Wright-stained peripheral blood and bone marrow aspirate smears also were reviewed.

Three-color flow cytometric studies using a FACScan instrument (Becton Dickinson, San Jose, CA) were performed to phenotype single-cell suspension of the spleen in case 1 and the bone marrow aspirate in case 2. Antibodies used included fluorescein isothiocyanate–, phycoerythrin-, or peridin-chlorophyll–conjugated monoclonal antibodies to T-cell–associated antigens CD2, CD3, CD4, CD5, CD7, CD8, alpha-beta TCR (WT31), gamma-delta TCR (TCR delta-1), V-delta-1, V-delta-2, and V-delta-3; natural killer (NK) cell–associated antigens CD11c, CD16, CD56, and CD57; B-cell associated antigen CD10; and HLA-DR (all antibodies were from Becton Dickinson except the V-delta-1, V-delta-2 and V-delta-3 monoclonal antibodies, which were from T Cell Diagnostics, Woburn, MA). The expression of cytolytic granule proteins perforin, granzyme, and TIA-1 was analyzed by indirect immunofluorescence as previously described.[9] The Fas ligand expression was studied by flow cytometric analysis using a polyclonal antibody to human CD95L (Santa Cruz Biotechnology, Santa Cruz, CA) and indirect immunofluorescence. Terminal deoxynucleotidyl transferase was evaluated as previously described.[9] The percentage of positive cells was determined by gating on the lymphoid population. Isotype controls for IgG1 and IgG2 were used to set thresholds for positive fluorescence. Antigen expression by less than 25% of the gated cells was considered negative. Paraffin immunohistochemistry was performed on sections of the spleen in case 1 and the bone marrow in case

2, using a streptavidin-biotin complex technique (Research Genetics, Huntsville, AL) and polyclonal antibody against CD3 (DAKO, Carpinteria, CA).

TCR Rearrangements, HLA Typing, and Viral Studies

Genomic DNA was isolated from resuspended cells from the spleen in case 1 and the bone marrow aspirate in case 2. For Southern blot analysis, 10 μg of the genomic DNA was digested with *Eco*RI, *Bam*HI, and *Hin*dIII restriction endonucleases, separated by electrophoresis on 0.8% agarose gels, and transferred to nylon membranes (Hybond-N, Amersham, Baton Rouge, LA) according to the method of Southern.[11] Digoxigenin-labeled DNA probes specific for the TCR delta gene J-delta-1 region (TCRDJ1 Probe) (DAKO) were used for hybridization.[12] Detection was performed using the DIG High Prime DNA Labeling and Detection Starter Kit II (Boehringer-Mannheim, Indianapolis, IN) according to the manufacturer's recommendation. The molecular component of the rearranged TCR delta gene was analyzed by polymerase chain reaction (PCR) using a serial combination of 5′ primers representing 6 different V-delta regions and 3′ primers representing J-delta-1, as previously described.[13]

In case 1, the HLA types of host and cadaver donor were determined by using standard serologic tests. The HLA class I alleles were determined by using genomic DNA isolated from the splenic cell suspension that contained greater than 75% of malignant T cells, with the sequence-specific oligonucleotides PCR method according to the manufacturer's recommendation (Lifecodes, Stamford, CT).

Southern blot analysis was performed for the detection and clonality study of the EBV genome. Genomic DNA from both cases was isolated as previously described. Ten micrograms of the genomic DNA was digested with *Bam*HI restriction endonuclease, separated, and transferred to a nylon membrane as previously described. A digoxigenin-labeled DNA probe corresponding to the unique viral sequence adjacent to the terminal repeat region (*Eco*RI I fragment of the EBV genome, provided by Nancy Raab-Traub, MD, Johns Hopkins University, Baltimore, MD) was used for hybridization.[14] Detection was performed as previously described. Positive, negative, and sensitivity controls were run and gave the appropriate results. In situ hybridization for the detection of the EBV-encoded RNA using commercially available reagents (DAKO) was performed on involved paraffin-embedded tissues from both cases according to the manufacturer's recommendation.

Cytogenetics and Fluorescence In Situ Hybridization

Cytogenetic analysis was performed on unstimulated cultures of the cells from the spleen (case 1) and the bone marrow (case 2). Trypsin Giemsa–banded metaphases were

prepared using standard techniques of colchicine arrest, hypotonic treatment, and 3:1 of methanol/acetic acid fixation. For fluorescence in situ hybridization (FISH), the following probes from VYSIS (Downers Grove, IL) were used according to the manufacturer's recommendation: probe CEP8 for enumerating the centromere of chromosome 8, probe LSI D7S486/CEP7 (a 2-color probe composition) for simultaneous detection of the centromere of chromosome 7 and band 7q31 on the long arm of chromosome 7, and probe CEPY for detection of Y chromosome. FISH analysis was performed on cells from the spleen (case 1) and the peripheral blood (case 2), which previously were stored frozen and then appropriately thawed, incubated overnight at 37 C, and prepared using the cytogenetic method as described.

Measurement of the Serum Soluble IL-2 Receptor

The serum soluble interleukin-2 receptor (sIL-2R) enzyme-linked immunosorbent assay plates were obtained from T Cell Diagnostics (Woburn, MA), and the assay was performed as recommended by the manufacturer and as previously described[15,16] on serum samples stored frozen at –70°C. The data are shown as a mean of the duplicate samples (SD <10%). The mean normal value as determined in the study of 50 healthy subjects (see IL-2R test kit instruction manual) and confirmed by Wasik et al[16] was 573 U/mL (upper limit of normal, 919 U/mL; mean + 2 SD).

Results

Morphologic Features

For case 1, sections of the spleen showed attenuated white pulp and markedly expanded red pulp filled with highly atypical, intermediate to large lymphoid cells with increased nuclear/cytoplasmic ratio and irregular nuclei ❙**Image 1A**❙ and ❙**Image 1B**❙. A biopsy of the liver from the same patient revealed a sinusoidal infiltrate of similarly atypical lymphoid cells ❙**Image 1C**❙. A bone marrow biopsy revealed scant atypical lymphoid infiltrate.

A bone marrow biopsy from case 2 showed a hypercellular marrow with patchy infiltrates of atypical lymphoid cells ❙**Image 1D**❙. The infiltrates were mostly in the interstitium, without forming large lymphoid aggregates. On bone marrow aspirate smears, the atypical lymphoid cells showed an increased nuclear/cytoplasmic ratio and contained convoluted, markedly irregular, intermediate to large nuclei with nucleoli and a moderate amount of pale cytoplasm ❙**Image 1E**❙. Occasional cells also contained cytoplasmic azurophilic granules. The same cells also were present in the peripheral blood specimen.

Immunophenotyping

As shown in ❙**Table 1**❙, both cases showed similar immunophenotyping results. The malignant cells expressed T-cell antigens CD2, CD3, and CD7. Importantly, the cells showed expression of gamma-delta TCR and no significant expression of CD4, CD8, or alpha-beta TCR. Moreover, the gamma-delta TCR that was expressed on the cell surface represented the V-delta-1 TCR subtype. The malignant T cells also expressed NK-cell–associated markers CD16 and CD56, as well as CTL-associated markers TIA-1 and Fas ligand. The tumor cells were negative for B-cell–associated markers (CD19, CD20, CD22, and CD23).

Molecular Analyses

As shown in ❙**Table 2**❙, in both cases, the malignant cells showed clonal rearrangement of the TCR delta gene, as demonstrated by Southern blot analysis (data not shown) and PCR analysis ❙**Image 2**❙. Furthermore, the PCR analysis of the rearranged V-delta gene showed clonal V-delta-1–J-delta-1 rearrangement (Image 2), corresponding to the V-delta-1 surface expression shown by immunophenotyping (Table 2). In addition, clonal rearrangement of the TCR gamma- and beta-chain genes also was shown in case 2 (Table 2). In both cases, Southern blot analysis revealed no evidence of EBV infection in the neoplastic T cells (data not shown).

Cytogenetic and FISH Analyses

Clonal cytogenetic abnormalities were found by FISH analysis in both lymphomas, using a probe for the centromere of chromosome 8 and a 2-color probe composition for the centromere and band 7q31 of chromosome 7. The spleen cells of case 1 and the peripheral blood cells of case 2 showed isochromosome 7q [i (7)(q10)] and trisomy 8 (8+), which are the most commonly reported chromosomal abnormalities in gamma-delta HSTCL.[9] Specifically, 54.0% (108/200) of the nuclei were positive for i(7)(q10), and 64.3% (257/400) were positive for trisomy 8 in case 2; 18.8% (39/207) of the nuclei were positive for i(7)(q10) and 21.5% (86/400) were positive for trisomy 8 in case 1. Standard cytogenetic analysis for case 2 obtained 25 metaphases from a 24-hour unstimulated culture of the bone marrow cells and yielded the normal 46,XX karyotype. For case 1, 7 metaphases were obtained from a 96-hour unstimulated culture of the spleen and showed chromosomal breakage without evident clonal abnormality.

Host Origin of Lymphoma Cells

In case 1, the allelic phenotype of HLA class I antigens of the tumor cells was identical to that of the host tissue (HLA A25, 26, B18, 51) and was different from that of the donor tissue (HLA A2, B38). In case 2, FISH analysis was
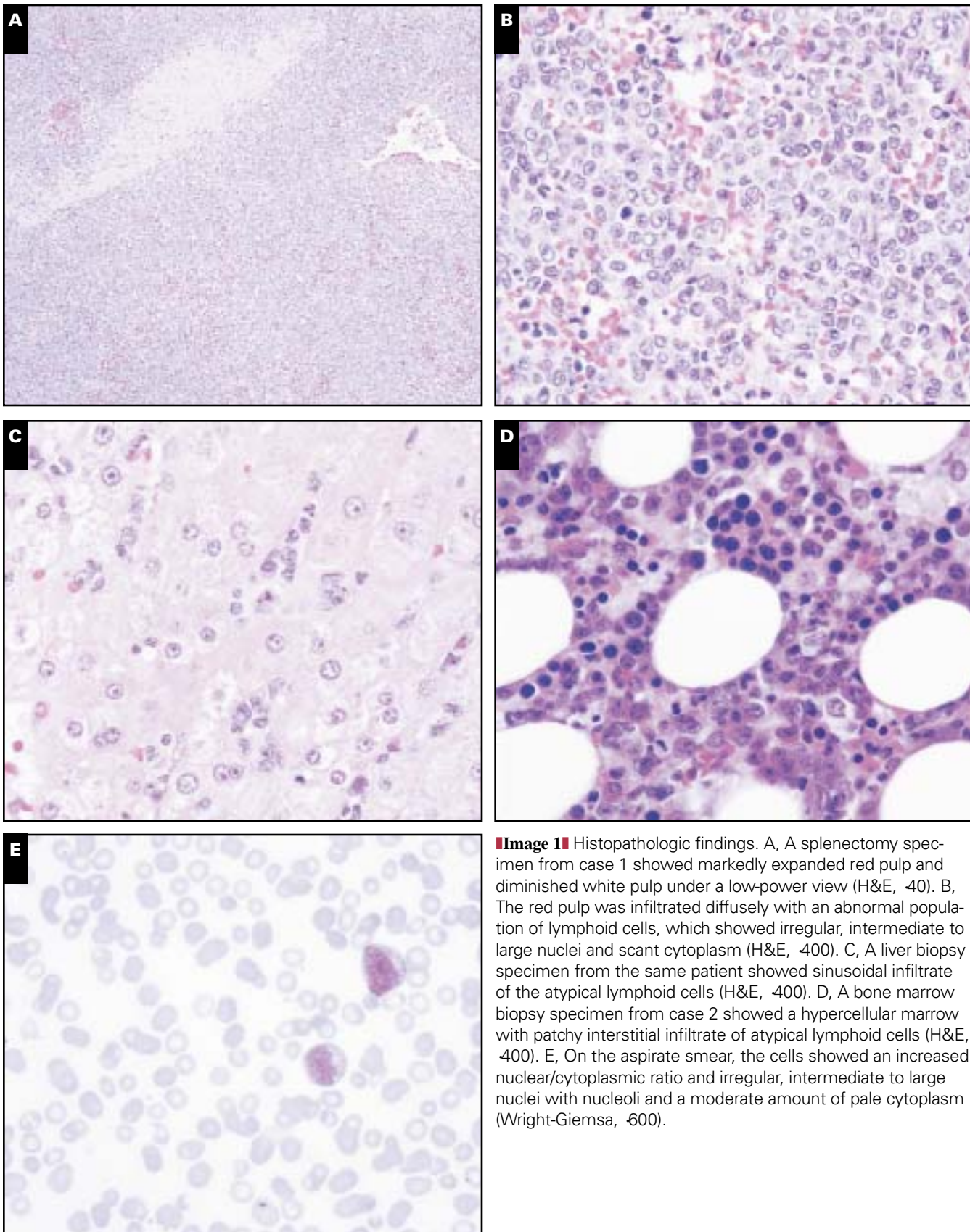
⬛Image 1⬛ Histopathologic findings. A, A splenectomy specimen from case 1 showed markedly expanded red pulp and diminished white pulp under a low-power view (H&E, ×40). B, The red pulp was infiltrated diffusely with an abnormal population of lymphoid cells, which showed irregular, intermediate to large nuclei and scant cytoplasm (H&E, ×400). C, A liver biopsy specimen from the same patient showed sinusoidal infiltrate of the atypical lymphoid cells (H&E, ×400). D, A bone marrow biopsy specimen from case 2 showed a hypercellular marrow with patchy interstitial infiltrate of atypical lymphoid cells (H&E, ×400). E, On the aspirate smear, the cells showed an increased nuclear/cytoplasmic ratio and irregular, intermediate to large nuclei with nucleoli and a moderate amount of pale cytoplasm (Wright-Giemsa, ×600).

■**Table 1**■

Immunophenotypic and T-Cell Receptor Analysis of Hepatosplenic gamma-delta T-Cell Lymphomas[*]

Antigen/Gene	Case 1	Case 2	Ross et al[4]	Francois et al[6]
T-cell–associated				
CD2	95	94	94.6	Positive
CD3	89	90	79	Positive
CD4	4	17	1.6	Negative
CD5	6	19	11.2	Negative
CD7	87	89	43.4	NR
CD8	5	29	10.0	Positive
alpha-beta TCR (WT31)	4	19	6.8	NR
gamma-delta TCR (TCR delta-1)	79	72	69.3	Positive
V-delta-1	73	78	NR	NR
V-delta-2	2	3	NR	NR
V-delta-3	1	ND	NR	NR
Natural killer–associated				
CD11c	17	ND	22.6	NR
CD16	65	71	92.2	NR
CD56	75	68	51.1	Positive
CD57	2	3	0.7	NR
CTL-associated				
Perforin	2	0	ND	NR
Granzyme B	0	ND	ND	NR
TIA-1	66	76	ND	NR
FasL (CD95L)	73	>75	ND	NR
Other				
TdT	ND	0	ND	NR
CD10	1	3	11.3	NR
CD25	ND	ND	0.8	NR
CD30	ND	ND	ND	NR
HLA-DR	13	32	ND	NR

CTL, cytotoxic T lymphocyte; FasL, Fas ligand; ND, not done; NR, not reported; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase.

[*] Data for the cases and Ross et al[4] are given as percentages. All percentages were derived from 3-color flow cytometric analysis. Antigens expressed by less than 25% of cells were considered negative.

■**Table 2**■

Genotyping and Viral Status for Posttransplant Patients With Hepatosplenic gamma-delta T-Cell Lymphoma

Case No.	TCR delta Gene/TCR delta Gene Subtype Rearrangement	TCR gamma Gene	TCR beta Gene	EBV Genome
1	R/V-delta-1	ND	ND	Negative
2	R/V-delta-1	R	R	Negative

EBV, Epstein-Barr virus; ND, not done; R, clonal rearrangement; TCR, T-cell receptor.

performed using a probe for the Y chromosome on the peripheral blood cells in which lymphoma cells constituted at least 50% of the cellularity, as shown by the percentage of cells with clonal chromosomal abnormalities (see "Cytogenetic and FISH Analyses" section) and by immunophenotyping (data not shown). None of 500 nuclei were positive for the Y chromosome, indicating that the tumor cells were of the host (female) instead of the donor (male) origin.

Serum Soluble IL-2 Receptor Levels

An analysis of the serum concentration of sIL-2R ■**Figure 1**■ in case 1 showed an elevated level of 4,630 U/mL, which decreased substantially after the splenectomy.

The level declined to within the reference range, to 480 U/mL, when the patient achieved a complete remission in response to intense chemotherapy in preparation for the bone marrow transplantation. For case 2, the serum sIL-2R concentration was strikingly elevated (40 times of the mean normal value) at the time of the diagnosis. The sIL-2R level gradually decreased during the course of chemotherapy, but it never reached the normal value (Figure 1). This correlated with the clinical partial response to the chemotherapy and histologic evidence of persistent disease. These findings indicate that monitoring of sIL-2R concentration might be useful in the management of patients with T-cell PTLD, despite marked differences in the baseline values between these 2 cases.
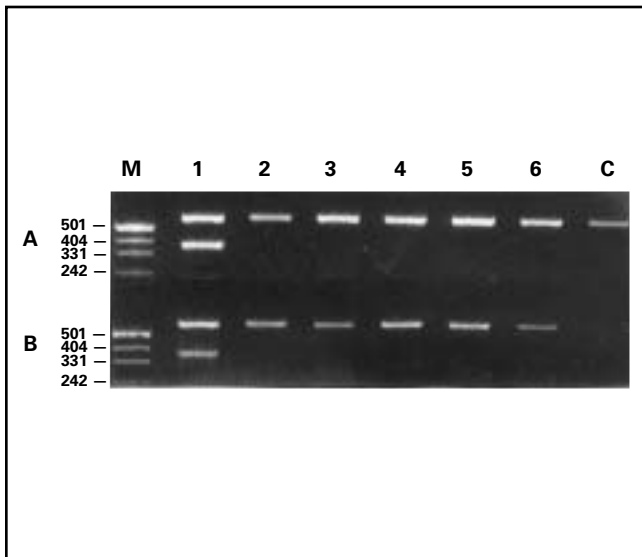
∎Image 2∎ T-cell receptor V-delta–J-delta polymerase chain reaction (PCR). PCR analysis of the DNA from case 2 (A) and case 1 (B) showing clonal V-delta–J-delta rearrangements in lane 1 (350-kd band) for both tumors. Lanes 2 through 6 show negative reactions for V-delta-2-J-delta-1 through V-delta-6–J-delta-1, respectively. The 600-kd upper band in all lanes is the internal RAG-1 positive control. Lane C is the negative control.
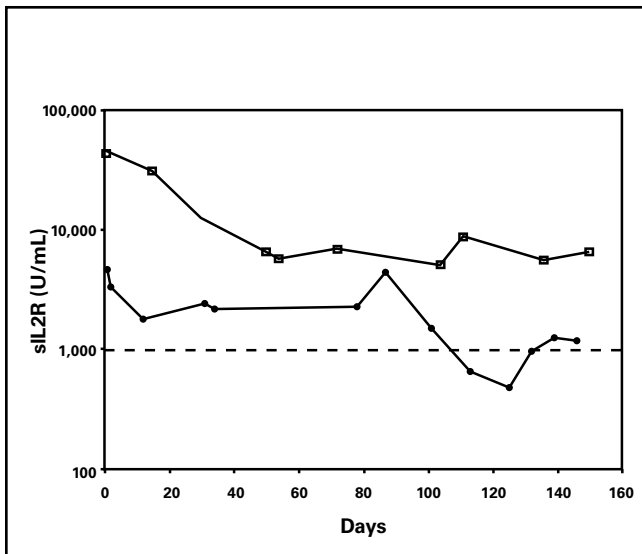

∎Figure 1∎ Serum concentration of serum soluble interleukin-2 receptor (sIL-2R). Case 1 (circles) and case 2 (squares) showed elevated sIL-2R levels before any therapy. For case 1, the level decreased substantially after splenectomy (days 2-12) and fell within the normal range after chemotherapy in preparation for bone marrow transplantation (around day 110). For patient 2, the level decreased gradually during the course of chemotherapy (days15-150) but never reached the normal value. The horizontal line indicates the upper normal value of approximately 1,000 U/mL.

Discussion

PTLDs represent a major complication of the immunosuppressive therapy after solid organ transplantation. The most frequent and best characterized form of PTLD is a polyclonal or monoclonal B-cell proliferation associated with EBV infection.[3] T-cell PTLDs were described mostly as occasional case reports.[2,4,6-8,17-31] In the largest series to date, Hanson et al[8] reported 6 cases of T-cell PTLD.

The present study describes 2 cases of gamma-delta HSTCL as an unusual form of T-cell PTLD in long-term renal transplant recipients. We have shown that the malignant T cells expressed TIA-1 and Fas ligand, indicating CTL differentiation. Based on delta-chain variable region use, the tumor cells in both cases were of the V-delta-1 subset, corresponding to the dominant subset of gamma-delta T cells normally present in the spleen. In addition, the malignant T cells were of host origin in both cases. FISH analysis revealed, in both cases, clonal cytogenetic abnormalities i(7)(q10) and trisomy 8(8+), which are the most common cytogenetic abnormalities in gamma-delta HSTCL.[9]

As illustrated by the cases reported in the literature and our 2 cases, the distinctions between B- and T-cell PTLDs have important clinical and prognostic implications. The exact incidence of T-cell PTLDs is uncertain; however, based on reports from several large centers, they may constitute up to 14% of all PTLD cases.[1,5,31]

A common feature shared by T- and B-cell PTLDs is a strong predilection for extranodal sites.[2,3] The primary locations of the lymphomas are extranodal in more than 80% of the reported T-cell PTLD cases.

Although there is a wide range in the time interval between organ transplantation and the occurrence of T-cell PTLD, this disease tends to occur in patients who have received prolonged immunosuppression. The interval from transplantation to diagnosis of T-cell PTLD ranged from 4 to 26 years with a median of 15 years in the report by Hanson et al.[8] Similarly, in our 2 cases, the lymphomas were diagnosed 5 and 10 years after organ transplantation.

The incidence of PTLD is known to vary among different types of organ transplants, presumably owing to the different intensities of the immunosuppression. For B-cell PTLD, renal transplant recipients have the lowest incidence of the disease, and the incidence increases sequentially in heart, liver, and combined heart-lung transplantation.[1,32] In comparison, the reported T-cell PTLD cases seem to be disproportionately concentrated in renal transplant recipients. Based on our review of the literature, 26 (76%) of 34 reported cases, including the present cases, occurred in long-term renal transplant recipients.[2,4,6-8,17-31] In contrast to the unequivocal association between B-cell PTLD and EBV infection, the recently described T-cell PTLD cases,[8]

including ours, showed no evidence of EBV infection in the malignant T cells. Clinically, at the time of diagnosis, most of the T-cell PTLD cases were fully developed T-cell malignant neoplasms. None of the patients in the series of Hanson et al[8] and neither of our patients responded to reduction in immunosuppression alone. The long-term survival of T-cell PTLD cases is generally poor, as found in the present study and by others.[2,4,6-8,17-30] Most of the patients died within 1 year of the diagnosis.

The gamma-delta HSTCL is a rare, clinically aggressive subtype of peripheral T-cell lymphoma. The 2 present cases showed many of the characteristic clinical and pathologic features of gamma-delta HSTCLs, including B symptoms (weight loss, unexplained fever, night sweats), hepatosplenomegaly without marked lymphadenopathy, an interstitial infiltrate pattern in the bone marrow, an immunophenotype of "double-negative" T cells (CD4–, CD8–), and expression of gamma-delta TCR.

Besides the 2 present cases, there have been only a few reported single cases of well-documented gamma-delta HSTCL in posttransplant patients.[4,6] The clinical manifestations, histopathologic findings, and immunophenotypes are similar to the 2 present cases (Table 1). Importantly, there was also no evidence of viral infection in the malignant cells. Similar to our cases, the patients in both reports responded initially to chemotherapy, but died of recurrence of the disease within 6 months of the diagnosis.[4,6] One other report also described a case of gamma-delta HSTCLs with isochromosome 7q and trisomy 8 in a renal transplant recipient.[33] An interesting case of gamma-delta HSTCL has been reported in a pediatric heart transplant recipient following the occurrence of B-cell PTLD.[34]

The analysis of several CTL-associated markers is unique for the present study. We have shown that the malignant T cells contained TIA-1 and Fas ligand. These findings provide strong evidence that the malignant gamma-delta T-cells have CTL differentiation and suggest that they may be functionally active cytotoxic effector cells. Interestingly, 2 studies have reported T-cell lymphomas with immunophenotypic or ultrastructural features of CTLs that have occurred in patients receiving long-term immunosuppression, and many patients are solid-organ transplant recipients.[29,30] Despite the variations in the precise classification of the T-cell subtypes based on the expression of the T-cell markers CD4 or CD8 and the use of alpha-beta or gamma-delta subtypes of TCR, these additional reported cases[29,30] share many common features with our cases. All of these cases manifested as a late complication of solid-organ transplantation, particularly of renal transplantation. In most of these cases, malignant cells displayed cytologic and immunologic features of NK-like T cells or CTL cells and express alpha-beta TCR. Clinically, the patients

presented with disseminated disease, which pursued an aggressive clinical course.[6,29]

In contrast with the clear association between B-cell PTLD and EBV infection, serologic and molecular diagnostic studies of the reported T-cell PTLD cases,[8] including ours, failed to show a direct viral cause. The viruses that have been studied so far include EBV, human T-cell lymphotropic virus-1 (HTLV-1), HIV, and human herpesvirus types 6 and 8.[6,29] In the present study, Southern blot analyses showed no evidence of EBV genome in either case, with adequate sensitivity controls.

This raises a challenging question about the cause of T-cell PTLDs and, in particular, of gamma-delta HSTCLs, since the number of cases observed in transplant recipients is surprisingly high for such a rare type of lymphoma. Normal gamma-delta T cells are thought to function primarily as CTLs with NK-like or lymphokine-activated killer activity. In transplant recipients, the allograft may constitute a long-term alloimmune stimulation to the CTLs, including gamma-delta T cells. Several studies have shown that human gamma-delta T cells have an alloreactive response to HLA molecules.[35,36] Characterization of T-cell populations in renal allografts revealed a substantial infiltrate of gamma-delta T cells in allografts at various stages of rejection.[37]

Of equal importance, the absence of viruses in the malignant T cells does not necessarily exclude the possibility of a causative role of viral infection.[38] The spleen is 1 of the sites with a high prevalence of latent EBV infection. Evidence supporting the role of EBV infection in expansion of gamma-delta T cells came from a study of an unusual case of a young child with EBV-associated systemic B-cell PTLD associated with prominent inflammatory brain lesions. In this case, a high proportion of gamma-delta T cells was found in both the blood and brain lesions. Furthermore, these gamma-delta T-cells showed potent major histocompatibility complex–unrestricted cytotoxic activity against EBV-infected target cells.[39] Therefore, although we did not detect evidence of EBV infection in the tumor tissue, it remains possible that stimulation of the gamma-delta T cells by the virus might have a role at an early stage of the malignant transformation. Future functional studies of the malignant T cells may provide insights into the cause of T-cell PTLDs.

In summary, gamma-delta HSTCL can occur as a late complication of renal transplantation. This rare type of lymphoma should be considered in patients receiving long-term immunosuppression who have B symptoms, hepatosplenomegaly (but no substantial lymphadenopathy), and abnormal peripheral blood findings. The pathologist's awareness of this entity also is very important, because without complete immunophenotyping, the histopathologic findings can be difficult to interpret. In particular, the interstitial bone marrow infiltrate can be rather subtle to recognize.

An accurate diagnosis of T-cell PTLD is important for therapeutic reasons, as it seems that the disease does not respond to reduction in immunosuppression alone and should be treated with chemotherapy.

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