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Expression of the Alpha/Beta and Gamma/Delta T-cell Receptors in 57 Cases of Peripheral T-cell Lymphomas

Identification of a Subset of γ/δ T-cell Lymphomas

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Fifty-seven cases of peripheral T-cell lymphoma were studied for cell expression of the T-cell receptor (TCR) chains, using monoclonal antibodies specific for the β chain (βF1) of the α/β TCR, and for the δ chain (anti-TCRδ-1) of the γ/δ TCR. Three different patterns were demonstrated: in 39 cases (69%), the phenotype (CD3+βF1+TCRδ-1−) was that of most normal T cells. A second pattern was found on six cases (10%), which were of CD3+βF1−TCRδ-1+ phenotype, and in which DNA analysis showed a clonal rearrangement of the δ locus in the five cases studied. It is suggested that these cases are the neoplastic counterpart of the small subpopulation of normal T cells that express γδ receptor. It is of considerable interest that these γδ lymphomas had unusual clinicopathologic presentations, as one case corresponded to a lethal midline granuloma and the five others to hepatosplenic lymphomas with a sinusal/sinusoidal infiltration in spleen, marrow, and liver. The fact that the distribution of the neoplastic γδ cells in the splenic red pulp resembles that of normal γδ cells reinforces the concept of a preferential homing of γδ T cells to this tissue. A third pattern (CD3±βF1−TCRδ-1−) was seen in 12 cases (21%), in which, by contrast to normal post-thymic T cells, no evidence of either αβ or γδ T-cell receptor was found. (Am J Pathol 1990, 137:617–628)

Human T cells recognize antigens via a heterodimeric surface receptor that is associated in the cell membrane with the CD3 polypeptide complex. Two forms of the T-cell receptor (TCR) have been identified, one of which, made up of α and β chains, is present on almost all T cells and is responsible for the MHC-restricted antigen recognition; the other, comprising γ and δ chains, is expressed on about 4% of T cells.[1–3] The biologic role of TCR-γδ cells is still the subject of investigation, but there is evidence that most if not all of these cells have a non–MHC (major histocompatibility complex)-restricted cytotoxic capability,[4] are able to respond to certain antigens, and appear to be functionally mature.[5] In contrast to TCR-αβ cells, these cells usually have a CD4−/CD8− phenotype.[6]

Monoclonal antibodies specific for the β chain of TCR-αβ[7] and more recently for the δ chain of TCR-γδ[8] have been produced that can be used for detecting normal and neoplastic T cells in frozen tissue sections.[9–12] In the present article, we report an analysis of the expression of the two types of TCR in more than 50 peripheral T-cell lymphomas, the neoplastic counterpart of post-thymic T cells. In particular, we have investigated how frequently there is discordance between CD3 and TCR expression, and we also have evaluated whether peripheral T-cell lymphomas expressing the γδ-TCR occur and whether they have any distinctive clinicopathologic features.

Materials and Methods

Tissue samples from 57 cases that had been diagnosed as peripheral T-cell lymphomas on the basis of both histologic and immunologic features (excluding lymphoblastic lymphoma and *mycosis fungoides*) were selected from our tissue bank. These accounted for 16% of the non-

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Table 1. *Antibody Panel*

Antigen	Antibody	Pattern of reactivity
CD1	Anti-Leu 6*	Cortical thymocytes
CD2	Anti-Leu 5*	Pan-T lymphocyte
CD3	Anti-Leu 4*	Pan-T lymphocyte
CD4	Anti-Leu 3*	Helper/inducer T lymphocyte
CD5	Anti-Leu 1*	Pan-T lymphocyte
CD7	Anti-Leu 9*	Pan-T lymphocyte
CD8	Anti-Leu 2*	Cytotoxic/suppressor T lymphocyte
CD56	NKH 1†	"Natural killer" cells
TCR β-chain	βF1‡	Most T cells
TCR δ-chain (common epitope)	TCRδ-1§	Minor subpopulation of T cells
TCR δ-chain (variable epitope)	δ-TCS1§	Minor subpopulation of T cells
TCR δ-chain (variable epitope)	Vδ2‖	Minor subpopulation of T cells

* Becton Dickinson, Mountain View, CA.
† Coulter, FL.
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§ T Cell Science, Cambridge, MA.
‖ Dakopatts, Denmark. Kindly supplied by Th. Hercent.

Hodgkin's lymphomas referred to our laboratory, with available frozen tissue for immunophenotyping. The phenotypic and genotypic findings in 19 of these cases have been reported previously.[13]

Tissue Specimens

Fresh tissue samples were obtained for diagnosis from the following sites: lymph nodes (34), spleen (6), skin, (6), gastrointestinal tract (3), bone marrow (4), lung (2), nasopharynx (1), and liver (1). One portion of each sample had been processed for routine histologic analysis; the others had been snap-frozen in liquid nitrogen after 30 minutes' incubation in gum-sucrose[14] and stored for immunohistologic and genotypic studies.

Morphologic Study

The histologic classification of non-Hodgkin's lymphoma was based on the International Working Formulation.[15] Specimens also were categorized according to the updated Kiel classification.[16] In addition, we used the category of monomorphic medium-sized-cell lymphoma as defined by Watanabe et al.[17]

Monoclonal Antibodies

The antibodies used in this study, their specificity, and commercial sources are shown in Table 1. The NKH-1 antibody, which recognizes the CD56 antigen, was used as a marker for natural killer (NK) cells. TCR-$\alpha\beta$ cells were identified by the βF1 antibody,[7] which recognizes the β chain both in its free form and in the mature α/β heterodimer. TCR-$\gamma\delta$ cells were identified by the anti-TCRδ-1 antibody,[8] which is specific for an invariant epitope on the δ chain, and which thus represents a pan TCR-$\gamma\delta$ marker. TCR-$\gamma\delta$ cells also were analyzed in some cases with the anti-δTCS1 antibody,[18] which reacts with a varaible Vδ1/Jδ1-encoded epitope, and with antibody anti-Vδ2, which recognizes a Vδ2-encoded determinant.[3]

Immunohistochemical Staining

Cryostat sections were stained using the alkaline phosphatase autoalkaline phosphatase (APAAP) immunoalkaline phosphatase technique.[19] Rabbit anti-mouse immunoglobulins and APAAP complexes were obtained from Dako (Dakopatts A/S, Versailles, France). The alkaline phosphatase reaction was demonstrated by incubation in a solution containing Fast Red TR (1 mg/ml; Sigma Chemical CO, St. Louis, MO) and naphtol AS-TR phosphate (0.2 mg/ml, Sigma). Levamisole (0.24 g/ml) was added to block endogenous alkaline phosphatase activity.

Immunophenotypic Criteria

Lymphomas were considered to be of peripheral T-cell derivation if the tumor cells lacked the CD1 cortical thymocyte antigen and expressed one or more T-cell markers (CD3, CD2, CD5, CD7, CD4, CD8). CD3 was expressed by tumor cells in 51 cases. All cases were negative for immunoglobulin light chains and for the pan-B antigens CD19 and CD22. Neoplasms were considered to have lost a pan-T antigen if more than 50% of the tumor cells lacked expression of an antigen that was present on infiltrative small reactive T lymphocytes.[9,13]

Genomic Study

DNA analysis was performed in 33 cases using standard methods detailed elsewhere.[20] Ten micrograms of DNA from each sample was digested with appropriate restriction endonucleases, subjected to electrophoresis in 0.6% to 1% agarose gel, transferred onto a nitrocellulose filter by the method of Southern, and hybridized to 32P-labeled DNA probes. Rearrangements of TCR-β genes were analyzed using a probe[13,21] specific for the Cβ1 and Cβ2 constant regions after DNA digestion with *Eco*RI, *Bam*HI, and *Hind*III endonucleases. Analysis of immunoglobulin gene rearrangement was performed using a JH probe specific for the heavy-chain joining region[22] after *Bam*HI–*Hind*III double digestion. Rearrangement of the TCR-δ genes was analyzed in five cases using the JδS16 probe specific for the Jδ1 region[23,24] after *Bam*HI and *Hind*III digestion (all above probes donated by Th. Rabbits).

Results

The histopathologic, immunohistologic, and genotypic features of the 57 cases of peripheral T-cell lymphoma (PTCL) are detailed in Tables 2 and 3. All but six of the cases were histologically classified according to the Working Formulation and the Kiel updated classification, although it was difficult to assign some cases to a category in the Working Formulation. Most of the cases belonged either to the diffuse mixed or the diffuse large-cell histologic subgroups (23 and 18 cases, respectively). The diffuse mixed subtype included the following categories in the updated Kiel classification: Lennert's lymphoma (five cases), angioimmunoblastic lymphoproliferative disease (AILD)-like lymphoma (five cases), and T-zone lymphoma (one case), each of which are regarded as polymorphous immunoblastic lymphomas in some series.[15,25] Three cases were classified as immunoblastic lymphomas and four as anaplastic large cell lymphomas. Only three cases were classified as small lymphocytic. The six remaining cases were found to correspond to the monomorphic medium-sized type.

Three main patterns of TCR expression by lymphoma cells could be distinguished.

'Common' αβ-TCR Type

Thirty-nine cases (69%), representing all histologic subtypes (Table 2), had the phenotype (CD3+/βF1+/TCRδ-1−) common to most normal T cells, ie, they expressed CD3 and the αβ-TCR and lacked the γδ receptor. The staining pattern for CD3 and TCR β chain were indistinguishable and differed from the labeling for TCR-γδ, which showed only few scattered small lymphocytes (Figure 1). The majority of cases expressed either CD4 (31 cases) or CD8 (two cases), and only four cases (10%) had an 'immature' CD4−/CD8− or CD4+/CD8+ phenotype (Tables 2 and 4). In the two remaining cases (cases 9,28), which were studied on bone marrow biopsies, the expression of CD4 antigen could not be evaluated, because of the presence of numerous CD4+ histiocytes. Twenty-eight of the thirty-nine αβ cases (74%) were found to have an aberrant antigenic profile, consisting of the absence of one or more pan-T antigens. NKH-1 (CD56) was only expressed on one of 15 cases studied.

Rearrangement of one or two TCR-β chain alleles was found in 16 of 18 cases studied (data not illustrated).

TCR-γδ Type

Six cases (10%) expressed the TCR-γδ (CD3+/βF1−/TCRδ-1+) (Table 3). The great majority of tumor cells were stained by the anti-TCRδ-1 antibody, and only a few βF1+ small lymphocytes were seen. The neoplasms all expressed CD2, in addition to CD3, but CD5 and CD7 antigens were either absent or only weakly expressed (Table 3). Four cases could be shown to lack both CD4 and CD8 antigens. In the two remaining samples (the bone-marrow biopsy from case 40 and the palatal granulomatous lesion from case 45), the presence of numerous CD4+ macrophages prevented evaluation of CD4 expression by the neoplastic cells. Four cases expressed the NK cell-associated marker NKH-1 (CD56) (Table 2).

The six cases that expressed γδ-TCR all had unusual clinicopathologic presentation. One case (case 45), which has been reported previously, presented as a lethal midline granuloma; histology was consistent with a polymorphic reticulosis evolving into overt lymphoma, and genotypic study demonstrated a T-cell monoclonal proliferation with rearrangement of the β-TCR gene.[26] The five other patients presented with splenomegaly and hepatomegaly without lymphadenopathy. The histologic pattern of liver and spleen involvement was very similar in each of these five patients and has already been detailed in a previous report of three of these patients.[27] Briefly, in the spleen there was a diffuse tumoral infiltration of both the cords and sinuses of the red pulp (Figure 2A through D), whereas in the liver (Figure 2E, F) the pattern of involvement was predominantly sinusoidal. Tumor cells were of medium size, with a relatively regular nucleus, and all cases were classified as being of monomorphic medium-sized type. Erythrophagocytosis by benign histiocytes was detected in the bone marrow in case 40 and in the spleen in case 44. In the bone marrow, a discrete sinusal infiltration was morphologically detectable in three patients, but in the two remaining cases (cases 41, 44), its demonstration required the immunohistochemical detec-

Table 2. *Histologic, Immunohistochemical, and Genotypic Findings in 57 Cases of Peripheral T Cell Lymphoma*

Case	WF	UK	CD3	βF1	TCRδ-1	CD4	CD8	CD7	CD5	CD2	CD56	JH	Cβ
1	DM	PML	+	+	−	+	−	−	−	+	−	G	R
2	DM	AIL	+	+	−	+	−	−	+	+	/	/	/
3	DM	PML	+	+	−	+	−	−	+	+	−	/	/
4	IBL	IBL	+	+	−	+	−	−	+	−	/	G	R
5	DM	PML	+	+	−	+	−	−	+	+	−	G	R
6	DM	LeL	+	+	−	+	−	−	+	+	−	G	R
7	DM	LeL	+	+	−	+	−	−	−	+	NI	G	G
8	Uncl*	Uncl*	+	+	−	−	−	−	−	+	−	G	R
9	DM	AIL	+	+	−	NI	+	−	+	+	/	/	/
10	DM	LeL	+	+	−	+	−	−	+	−	/	G	R
11	ALC	ALC	+	+	−	+	−	−	−	−	−	G	R
12	DM	LeL	+	+	−	+	−	+	+	+	−	G	R
13	SL	PSC	+	+	/	+	−	−	+	+	/	G	R
14	DM	PSC	+	+	/	+	−	−	+	+	−	G	R
15	DM	PML	+	+	−	+	−	−	+	+	−	G	R
16	DM	AIL	+	+	−	+	+	+	+	+	+†	/	/
17	DLC	PML	+	+	−	+	−	−	+	+	/	G	R
18	DLC	PML	+	+	−	+	−	−	+	+	−	R	R
19	ALC	ALC	+	+	−	+	−	+	+	+	−	G	G
20	DM	PML	+	+	−	+	−	−	−	+	/	G	R
21	DLC	PML	+	+	−	+	−	+	+	+	/	/	/
22	DM	AIL	+	+	−	+	−	+	+	+	−	/	/
23	IBL	IBL	+	+	−	+	−	−	+	+	/	/	/
24	DLC	PML	+	+	−	+	−	−	−	+	/	/	/
25	DLC	PML	+	+	−	+	−	+	+	+	/	/	/
26	DM	AIL	+	+	−	+	−	+	+	+	/	/	/
27	DLC	PML	+	+	−	−	−	−	−	+	/	G	R
28	DM	PSC	+	+	−	NI	+	+	+	+	/	/	/
29	DM	PML	+	+	−	+	−	−	+	+	/	/	/
30	DLC	PML	+	+	−	+	−	−	+	−	/	/	/
31	DLC	PML	+	+	−	+	−	−	+	−	/	/	/
32	DM	PML	+	+	−	+	−	−	+	−	/	/	/
33	DM	PML	+	+	−	+	−	+	+	−	/	/	/
34	DLC	PML	+	+	−	+	−	+	+	+	/	G	R
35	IBL	IBL	+	+	−	−	+	+	−	−	−	/	/
36	DLC	PML	+	+	−	+	−	+	+	+	−	/	/
37	DM	PML	+	+	−	−	+	−	+	+	−	/	/
38	DLC	PML	+	+	−	+	+	−	+	+	/	/	/
39	DLC	PML	+	+	−	+	−	+	+	+	/	/	/
40	Uncl*	Uncl*	+	−	+	NI	−	+†	−	+	+†	/	/
41	Uncl*	Uncl*	+	−	+	−	−	−	−	+	−	G	R
42	Uncl*	Uncl*	+	−	+	−	−	−	−	+	−	G	G
43	Uncl*	Uncl*	+	−	+	−	−	−	−	+	+	G	G
44	Uncl*	Uncl*	+	−	+	−	−	+†	−	+	+	G	G
45	DM	PML	+	−	+	NI	−	−	+	+	+	G	R
46	DLC	PML	+	−	−	−	−	+	+	+	−	G	R
47	DLC	PML	+	−	−	+	+	−	+	+	−	G	G
48	DM	LeL	+	−	−	+	−	−	+	+	−	G	R
49	DLC	PML	+	−	−	+	−	−	+	−	−	G	R
50	SL	SL	+	−	−	+	−	+	+	+	−	R	R
51	DM	Tzone	+	−	−	+	−	−	+	+	/	G	R
52	DLC	PML	−	−	−	−	−	−	−	+	+	/	/
53	SL	PSC	−	−	−	−	−	−	−	+	−	/	/
54	DLC	PML	−	−	−	+	−	−	−	+	+	G	G
55	DLC	PML	−	−	−	+	−	+	−	−	+	G	R
56	ALC	ALC	−	−	−	+	−	−	−	−	−	G	R
57	ALC	ALC	−	−	−	+	−	−	−	−	−	G	R

* Monomorphic medium-sized cells.

† Weak positivity.

SL, small lymphocytic; PSC, pleomorphic small-cell; DM, diffuse mixed; AIL, AIL-like lymphoma (angio-immunoblastic); T-zone, T-zone lymphoma; LeL, lympho-epithelioid lymphoma (Lennert's lymphoma); DLC, diffuse large-cell; IBL, immunoblastic; ALC, anaplastic large-cell; PML, pleomorphic medium and large; WF, International Working Formulation; UK, updated Kiel classification; Uncl, unclassifiable; NI, noninterpretable; /, not done; G, germ-line; R, rearranged.

tion of γδ+ cells in frozen sections. By using the anti-δTCS1 and anti-Vδ2 monoclonal antibodies, which react with variable epitopes of the δ chain, the tumor cells were shown to express the variable Vδ1/Jδ1-encoded epitope in three cases, whereas none of the six cases expressed the Vδ2-encoded epitope (Table 3).

Table 3. *Clinical, Histologic, Phenotypic, and Genomic Features in Peripheral T-Cell Lymphoma Expressing γδ T-Cell Receptor*

Case	Sites of involvement at presentation	Histology	Phenotype							Genotype	
			CD3	CD4	CD8	βF1	TCRδ1	δTCS1	Vδ2	Cβ	Jδ1
40	Spleen, bone marrow	MMS	+	NI	−	−	+	−	−	NA	NA
41	Spleen, liver, bone marrow <	MMS	+	−	−	−	+	+	−	R	R
42	Spleen, liver, bone marrow	MMS	+	−	−	−	+	+	−	G	R
43	Spleen, liver, bone marrow	MMS	+	−	−	−	+*	−	−	G	R
44	Spleen, liver, bone marrow <	MMS	+	−	−	−	+	+	−	G	R
45	Nasal septum (lethal midline granuloma)	PR	+	NI	−	−	+	−	−	R	R

* Weak positivity.

MMS, monomorphic medium-sized cell; bone marrow <. minimal sinusal infiltration demonstrated by immunohistology; PR, polymorphic reticulosis evolving in pleomorphic lymphoma; NI, not interpretable; NA, not available.

In five of the six TCR-γδ lymphomas, including the case of lethal midline granuloma (case 45), and in four of the five cases with hepatosplenic presentation (case 41 to 44), material was available for DNA analysis. Hybridization with a Jδ1 probe disclosed clonal rearrangements of the TCR-δ locus, as shown on Figure 3. The latter was associated with a clonal rearrangement of the TCR-β gene in cases 41 and 45 (Table 3).

'TCR Silent' Type

The third group consisted of 12 cases of lymphomas in which there was no detectable expression of either αβ or γδ T-cell receptor (βF1−/TCRδ-1−). In each case, the presence of scattered βF1+, and TCRδ-1+-reactive T cells among the tumor cells acted as an internal positive control and confirmed that the immunohistologic reaction was functioning normally. Six of these cases expressed CD3. Of the six CD3-negative cases, a T-cell origin was deduced on the basis of lack of B-cell antigens and expression of CD2 antigen in three cases (cases 52 to 54), and on the basis of CD4 expression, absence of myelomonocytic antigens, and clonal Cβ gene rearrangement in the other three cases (cases 55 to 57), one of which also was CD7 positive. Three of these cases in which TCR expression was undetectable carried the NKH-1 NK cell-associated antigen (CD56). Four cases had either a CD4+/CD8+ (one case) or a CD4−/CD8− (three cases) 'immature' phenotype (Table 4).

Clonal Cβ gene rearrangement was demonstrated in eight of 10 cases studied for genomic analysis.

Discussion

The present study indicates that three major patterns of TCR expression are found in peripheral T-cell lymphomas.

The most frequent pattern, present in 69% of cases, was expression of TCR-αβ in association with CD3. This finding is in keeping with recent immunophenotypic studies and suggests that these cases are the neoplastic equivalent of the major population of normal T cells. In most of these cases, as in normal αβ T cells, either CD4 or CD8 was expressed, but a few cases expressed either both or neither of these molecules.

A second group, comprising six cases (10%), represents a novel type of T-cell lymphoma in which the γδ receptor is present and that therefore are likely to arise from the very small population of peripheral T cells that express this form of receptor in thymus, blood, and lymphoid organs.[28] To the best of our knowledge, this is the first report on the occurrence of TCR-γδ neoplasms in a large series of peripheral T-cell lymphomas. Thus, in a recent study,[12] no cases of a series of 41 peripheral T-cell lymphomas, (including seven cases with a CD3+/βF1− phenotype), expressed the TCRδ-1 epitope, which has only been found in two of 22 lymphoblastic thymic lymphomas.

The present data on six cases of γδ-positive peripheral T-cell lymphoma clearly demonstrates that these cases have features in common with the normal TCR-γδ population, eg, they had a CD4−/CD8− phenotype, as has been described for normal TCR-γδ cells.[6] Four cases expressed the NK cell-associated marker, NKH-1 (CD56), consistent with the recent description of human CD3+/TCR-γδ+ clones with a NKH-1+ phenotype. Interestingly, the latter correlates with the NK-like cytotoxic potential of these clones.[29] The major difference antigenically was that the neoplasms (unlike normal cells) expressed little or no CD5 and CD7 antigens, but this may reflect the phenomenon of pan-T antigen loss seen in other types of T-cell lymphomas.[9,13]

The organization of the TCR-δ and β chain genes was studied in five of the six γδ lymphomas cases. In each case, DNA analysis demonstrated that the δ locus was rearranged, and in cases 41 and 45 this evidence of clonality was further supported by the detection of a β chain gene rearrangement. This latter rearrangement must be interpreted as nonproductive in view of the βF1- pheno-

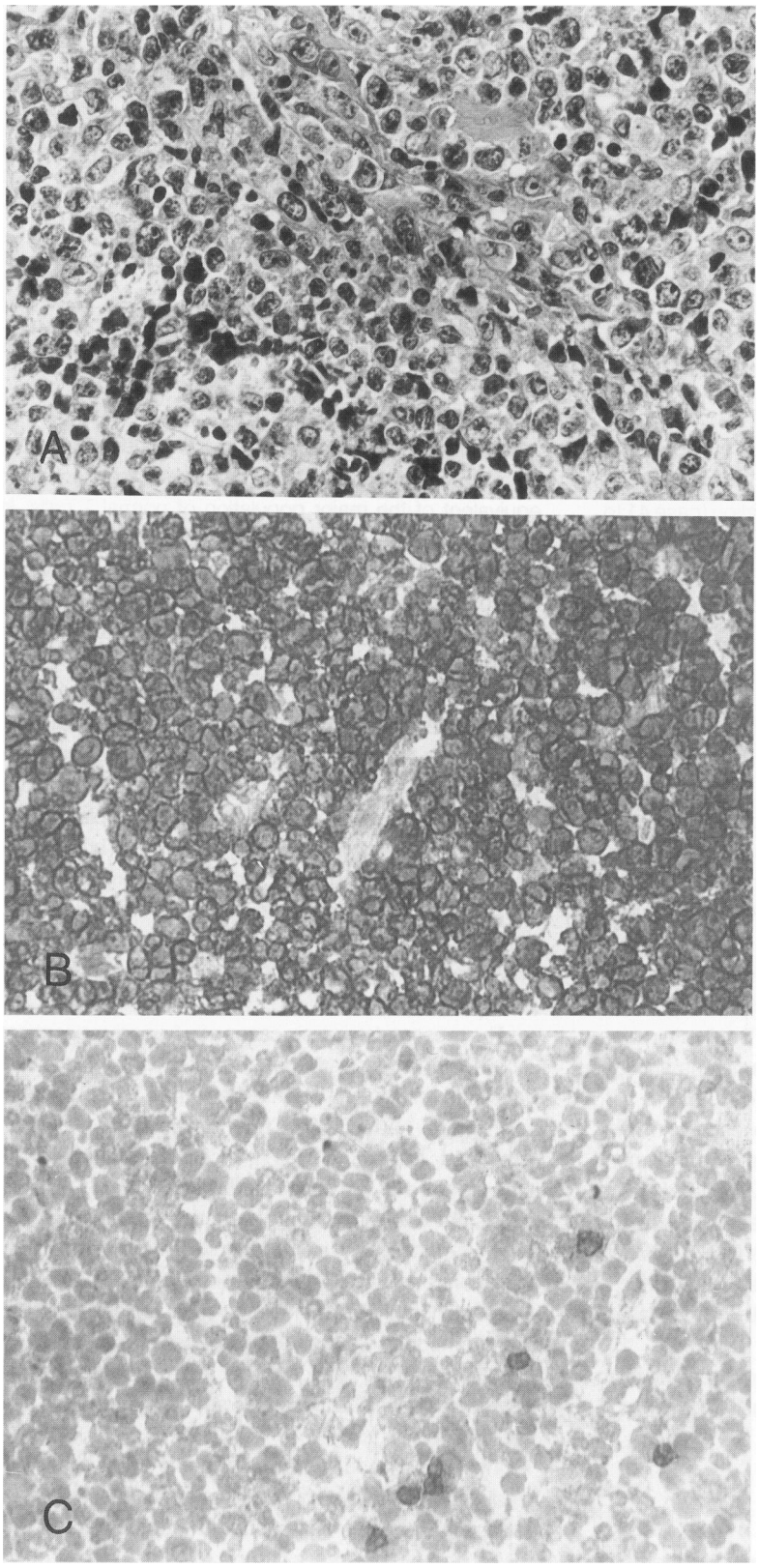

Figure 1. *Peripheral T-cell lymphoma expressing the αβTCR.* **A:** *Pleomorphic medium and large cell type (H&E, original magnification ×320).* **B:** *Neoplastic cells express TCR-β (antibody βF1, APAAP method, original magnification ×320).* **C:** *Scattered TCR-δ+ cells within the neoplasm (antibody TCRδ-1, APAAP method, original magnification ×320).*

Table 4. *Expression of CD4 and CD8 Antigens in the Three Subtypes of Peripheral T Cell Lymphoma*

	No. of cases	CD4+	CD8+*	Immature phenotype CD4+/CD8+ or CD4−/CD8− (%)
CD3+/βF1+/TCR δ-1−	37	31	2	4 (11%)
CD3+/βF1−/TCR δ-1+	4	0	0	4 (100%)
CD3+−/βF1−/TCR δ-1−	12	8	0	4 (33%)

* In four cases, CD4/CD8 expression was uninterpretable because of the presence of numerous CD4+ macrophages.

type, resembling the nonproductive rearrangements that have been described in other lymphoid tumors and leukemias, eg, rearrangements of immunoglobulins and T-cell receptor genes seen in up to 10% of malignant lymphomas,[13,30] and the clonal rearrangement of the δ locus observed in a series of malignant lymphomas, including a few B-cell neoplasms.[31] This is reminiscent of the frequent TCR γ and δ genes rearrangement observed in acute B-cell leukemias.[32] Thus, a conclusive lineage definition can be reached by the immunophenotypic detection of the products of rearranging genes, eg, the epitopes recognized by the βF1 and anti-TCRδ-1 monoclonal antibodies.

Interestingly, the 6 γδ neoplasms had very peculiar clinicopathologic features. One case, previously reported by us, was a polymorphic reticulosis presenting as lethal midline granuloma.[26] Immunophenotypic and genomic studies support the view that such lesions arising in the facial tissues, similar in their histopathologic features to lymphomatoid granulomatosis,[33,34] are clonal T-cell lymphoproliferative disorders.[26,35,36] The present report indicates that at least some cases represent γδ-T-cell neoplasms. A recent study[11] reported that three among four cases of CD3+/βF1− lymphomas (one of them being NKH-1+) were nasal T lymphomas, and suggested that such cases might be γδ-T-cell lymphomas. The five other cases had the peculiar form of hepatosplenic PTCL that we described previously, before the availability of anti-TCR monoclonal antibodies, as a clinicopathologic entity in three of these patients.[27] In a recent report, we detailed the phenotypic and genomic characteristics of two cases and proposed they could constitute a distinct entity among peripheral T-cell lymphomas.[24] This is further confirmed by the present study, in which the five cases shared common histopathologic, immunophenotypic, and genotypic features: tumor cells were monomorphic, medium-sized, and infiltrated the sinusoids of the liver, the sinuses, and the cords of the splenic red pulp, and to a lesser extent the sinuses of the bone marrow. In the present series, such a sinusal/sinusoidal pattern of infiltration in the spleen and bone marrow, and in the liver, was only found in γδ T-cell lymphomas. This striking tendency to localize to liver and spleen resembles the pattern described in 'erythrophagocytic T lymphomas resembling malignant histiocytosis' by Kadin et al,[37] before the availability of antibodies against TCR. However, our cases differed in that erythrophagocytosis was either not a feature or was restricted to apparently reactive histiocytes, no cases showing convincing evidence of red cell ingestion by the tumor cells themselves.

The reason why such γδ lymphomas seem to involve either preferentially nasal tissue or spleen and liver in a sinusal/sinusoidal pattern remains to be determined. A tropism of TCR-γδ cells for intestinal epithelium[38] and epidermis[39] has been described in mouse and chicken and has led some to propose that TCR-γδ cells constitute a class of T cells involved in surveillance of epithelial surfaces, although the general validity of these findings has been challenged by recent data in humans showing no such tropism of γδ cells in man.[6] However, we are not aware of any studies of γδ cells in human nasal tissue. By contrast, in homology to the chicken, human TCR-γδ cells seem to localise preferentially in anatomically distinct regions of the organized lymphoid tissue, ie, the splenic red pulp.[28,40] It is therefore tempting to propose that the neoplastic γδ cells might interact with endothelial cells of the sinuses of spleen, bone marrow, and the sinusoids of the liver. In this way, it is of note that the course of patient with lethal midline granuloma (case 45) was characterized by the development of hepatosplenomegaly, and that a liver biopsy disclosed a massive sinusoidal infiltration by lymphoma cells.[26]

The third pattern (CD3±/βF1−/TCRδ-1−), which was found in 12 cases (21%), is of interest. The same pattern has been demonstrated in 30% of T-cell lymphomas in a recent study using both βF1 and TCRδ-1 antibodies.[12] Because the immunohistochemical value of both βF1 and anti TCRδ-1 antibodies has been demonstrated in normal reactive tissues, lymphomas, and T-cell clones and lines,[7,8,41] it is unlikely that a significant number of lymphomas expressing either TCR-αβ or TCR-γδ are missed by using both antibodies. Therefore, a first explanation could be that the βF1−/TCRδ-1− cases represent a more 'immature' step, before or during cytoplasmic CD3 expression. Indeed, it is tempting to propose that, despite their non lymphoblastic morphology, the CD4−/CD8− or CD4+/CD8+ cases reflect transformation of either thymocytes that have yet to express CD3, or of thymocytes caught at an intermediate stage between cytoplasmic expression of CD3 and expression of TCR (αβ or γδ). It is

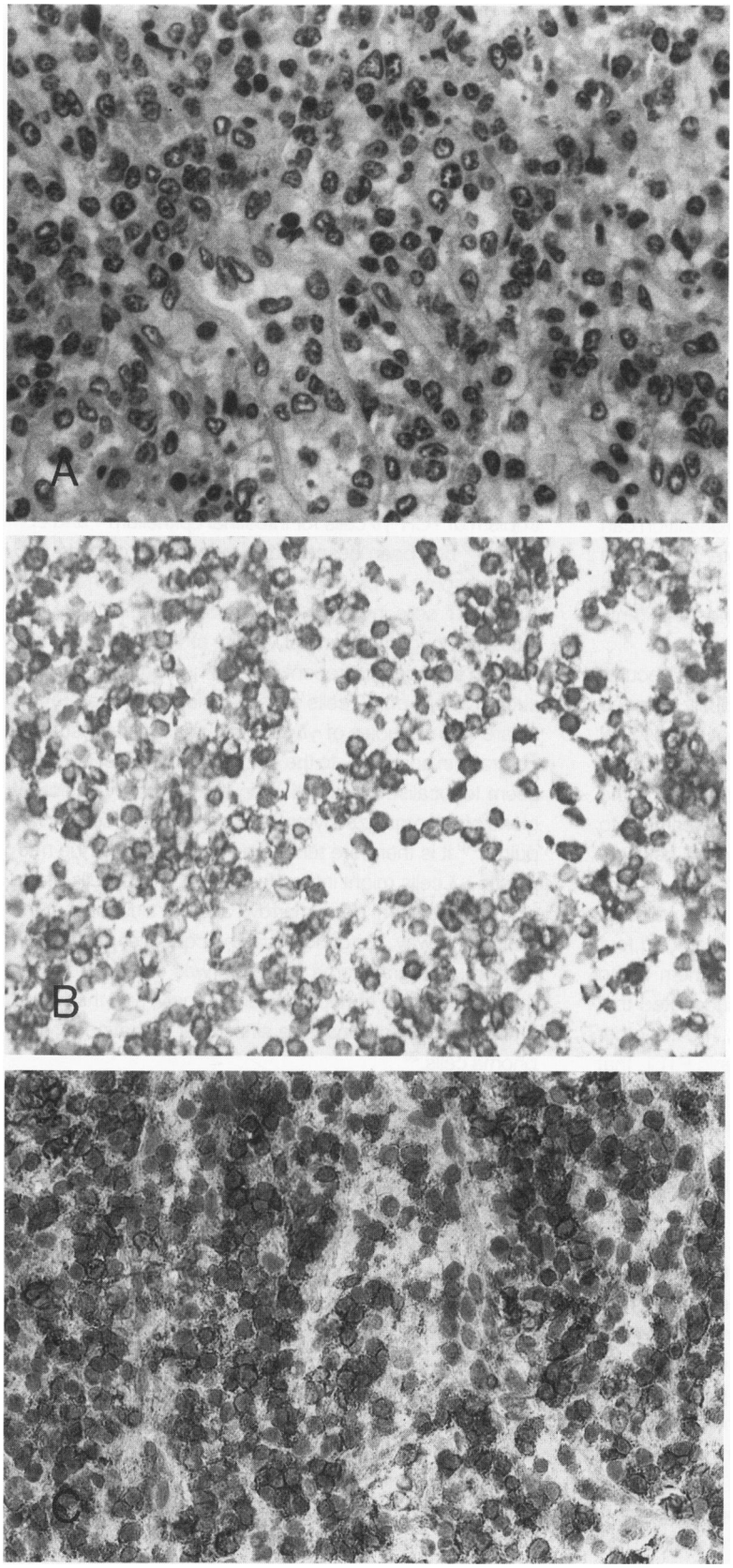

Figure 2. *Hepatosplenic γδ T cell lymphoma.* **A:** *Infiltration of the cords and the sinuses of the splenic red pulp by medium-sized tumor cells (H&E, original magnification ×320).* **B, C:** *Staining of tumor cells for CD3 (antibody leu 4)* **(B)** *and δ chain (antibody TCRδ-1)* **(C)** *(APAAP method, original magnification ×320).* **D:** *Neoplastic cells do not stain for TCR-β chain (antibody βF1, APAAP method, original magnification ×320).* **E:** *Sinusoïdal infiltration by tumor cells in the liver (H&E, original magnification ×160).* **F:** *Sinusoïdal neoplastic cells express TCRδ chain (antibody TCRδ-1, APAAP method, original magnification ×320).*

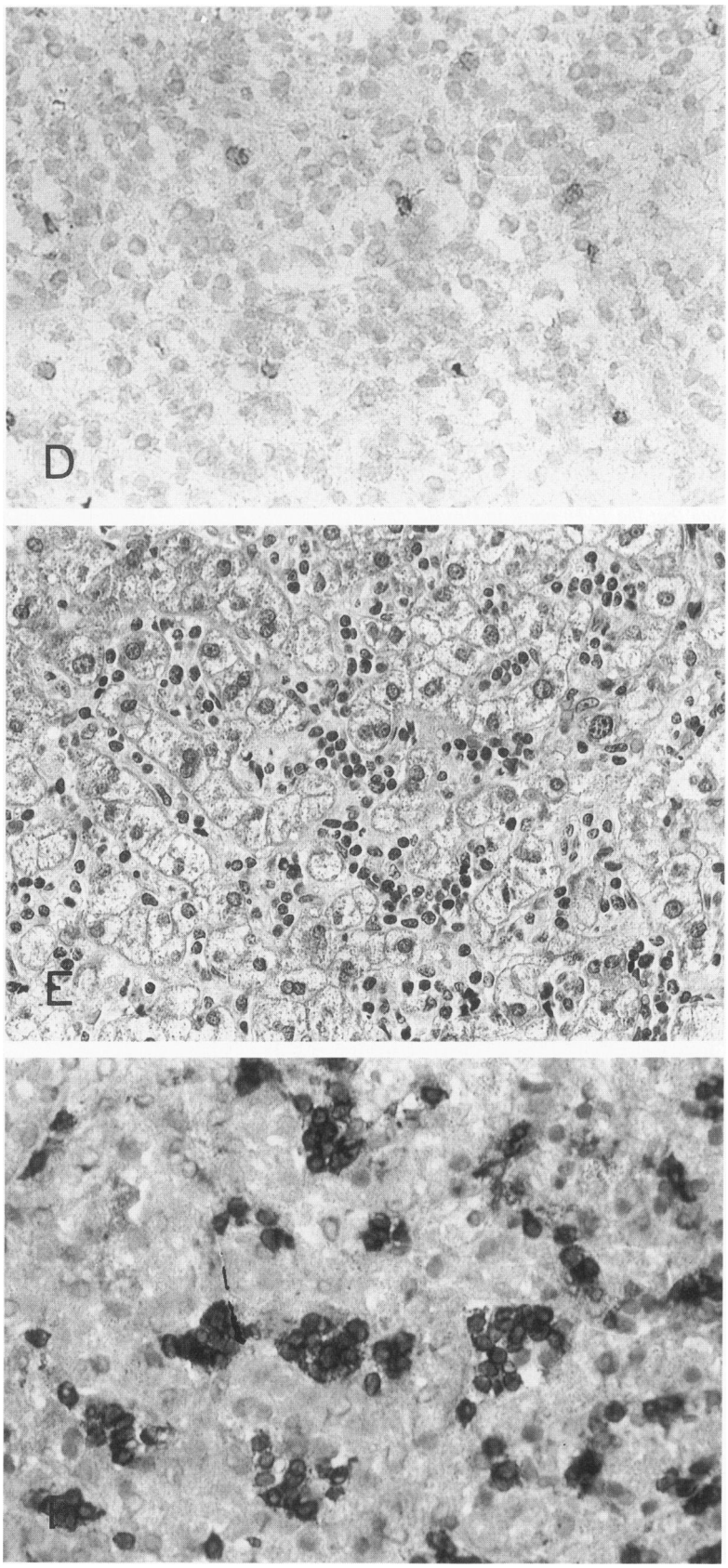

Figure 2 *(Continued)*.

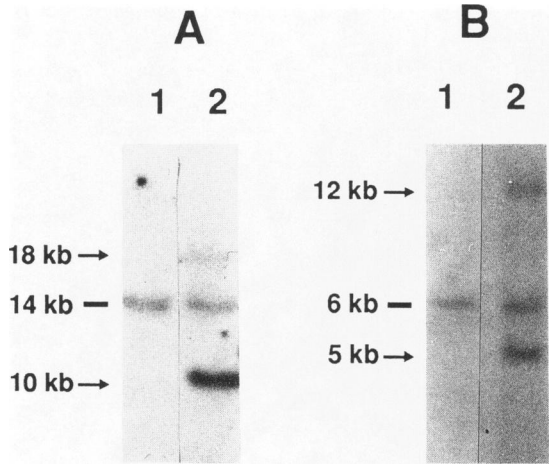

Figure 3. *Hepatosplenic γδ T cell lymphoma. TCRδ rearrangements were demonstrated by using the JδS16 probe in* Bam*HI* **(A)** *and* Hin*dIII* **(B)** *digests of cellular DNA (spleen, case 44). Note that the 12-kb band in* Hin*dIII digest corresponds to a Vδ1/Jδ1 joining, which is in concordance with the δTCS1+ phenotype of this case.[24] Lane 1, control, granulocytes; lane 2, Case 44, spleen; arrows, rearranged bands; dashes, germline bands.*

shown in this study that this 'immature' CD4/CD8 antigen expression is much more frequent in these 'TCR-silent' cases than in the group in which TCR-αβ is expressed. The failure to demonstrate a clonal Cβ rearrangement in two cases could fit with this hypothesis, as previously suggested from studies on lymphoblastic leukemias[42] and on some βF1-peripheral T-cell lymphomas.[43] A second hypothesis, based on the positivity for NKH-1 observed in three CD3-negative lymphomas, is that some cases may represent neoplasms of NK cells. This is supported by another report showing that NKH-1 is preferentially expressed on peripheral T-cell lymphomas of CD3−/βF1− phenotype,[11] which seems to be the usual phenotype of "true" NK cells.[44] Functional studies are needed to confirm this hypothesis, especially as one tumor with a CD3−/NKH-1+ phenotype had clonal TCR-Cβ rearrangement. A third hypothesis that we cannot exclude is that CD3+/βF1−/TCRδ-1− lymphomas express a third CD3-associated TCR, as recently demonstrated in the chicken.[45] Finally, it is tempting to speculate that such cases reflect abnormal antigen expression. In a recent paper, loss of TCR-β and CD3 antigen also was found to be frequent in post-thymic activated T-cell clones.[41] This could correspond to different levels of T-cell activation, depending on diverse regulatory control mechanisms, or to structural abnormalities of the genes, occurring in association with neoplastic transformation or alteration.[41] These mechanisms can result in nonproductive rearrangement of the TCR β chain gene, as we observed in eight cases. This last hypothesis is supported by the lack of the other T-cell antigens (CD2, CD5, CD7) that we observed with the same frequency in all three subgroups, as well as by the absence of any histologic pattern peculiar in the βF1−/TCRδ-1− lymphomas. However, it is of interest that no case with a CD3−/βF1+ discordant phenotype was found. Such a phenotypic pattern is likely to be rare, as CD3 antigen appears in the cytoplasm before TCR-β chain, and has only been described in rare cases of peripheral T-cell lymphoma, in which it probably represented loss of antigen associated with neoplastic transformation.[10,11]

In summary, three different patterns of TCR expression could be defined in the present study. In addition to the most frequent pattern (CD3+/βF1+), representing transformation of TCR-αβ lymphocytes, occasional cases had a CD3+/TCRδ-1+ phenotype and reflects TCR-γδ lymphomas. Interestingly, these γδ-lymphomas have a peculiar clinicopathologic presentation, as one case was an example of lethal midline granuloma and the five others were hepatosplenic lymphomas with a peculiar sinusal/sinusoidal pattern of infiltration. The special relation of the γδ tumor cells with some endothelial cells (in spleen, liver, bone-marrow), similar to the distribution of the normal cells in the sinuses of the splenic red pulp is of interest and reinforces the concept of a preferential homing for γδ T cells to these tissues. However, further studies are needed to explain the high number of cases (21%) of so-called peripheral T-cell lymphomas that lack expression of both αβ and γδ TCR.

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