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## Non-hepatosplenic $\gamma\delta$ T-cell lymphomas represent a spectrum of aggressive cytotoxic T-cell lymphomas with a mainly extranodal presentation

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### Abstract

$\gamma\delta$  T-cells represent a minor T-cell subset mainly distributed in mucosal surfaces. Two distinct lymphomas derived from these cells have been recognized: hepatosplenic  $\gamma\delta$  T-cell lymphoma (HSTL) and primary cutaneous  $\gamma\delta$  T-cell lymphoma (PCGD-TCL). However, whether other anatomic sites may also be involved and whether they represent a spectrum of the same disease is not well studied. The lack of TCR $\beta$  expression has been used to infer a  $\gamma\delta$  origin when other methods are not available.

We studied 35 T-cell tumors suspected to be  $\gamma\delta$  TCL using monoclonal antibodies reactive with TCR  $\delta$  or  $\gamma$  in paraffin sections. We were able to confirm  $\gamma\delta$  chain expression in 22 of 35 cases. We identified 8 PCGD-TCL, 6 HSTL, and 8  $\gamma\delta$  TCL without hepatosplenic or cutaneous involvement involving mainly extranodal sites. Two such cases were classified as enteropathy associated T-cell lymphoma, type II. The other  $\gamma\delta$  TCL presented in the intestine, lung, tongue, orbit and lymph node. In addition we observed 13 cases with mainly extranodal involvement that lacked any TCR expression (“TCR silent”). In all cases an NK origin was excluded.

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In conclusion, the lack of TCR $\beta$  expression does not always predict a  $\gamma\delta$  T-cell derivation since TCR silent cases may be found. The recognition of  $\gamma\delta$  TCL presenting in extranodal sites other than skin and liver/spleen expands the clinical spectrum of these tumors. However, non- HSTL  $\gamma\delta$  TCL do not appear to represent a single entity. The relationship of these tumors to either HSTL or PCGD-TCL requires further study.

### Keywords

$\gamma\delta$  T-cell lymphomas;  $\gamma\delta$  T-cell receptor;  $\alpha\beta$  T-cell receptor; cutaneous  $\gamma\delta$  T-cell lymphoma; hepatosplenic T-cell lymphoma

## INTRODUCTION

Normal  $\gamma\delta$  T-cells comprise an immunologically distinct lymphoid population that correspond to less than 1%–5% of peripheral blood lymphocytes and up to 50% of T-cells in mucosal sites, particularly intestine and skin.<sup>6, 7, 17, 22–24</sup>  $\gamma\delta$  T-cells, along with NK-cells are components of the innate immune system and do not require specialized antigen processing and presentation.<sup>4, 8</sup> Furthermore,  $\gamma\delta$  T-cells are involved in regulation of immune responses including cell recruitment and activation, and tissue repair.<sup>7, 16, 27</sup>

The 2008 WHO classification recognizes two main types of  $\gamma\delta$  TCL,  $\gamma\delta$  hepatosplenic T-cell lymphoma (HSTL)<sup>5, 11</sup> and primary cutaneous  $\gamma\delta$  T-cell lymphoma (PCGD-TCL).<sup>2, 12, 26, 35</sup> Nevertheless, infrequent examples of  $\gamma\delta$  TCL have been reported to present in mucosal sites.<sup>2, 12, 26</sup> The relationship of such lesions to the more common primary cutaneous  $\gamma\delta$  T-cell tumors is unknown.

The investigation of  $\gamma\delta$  TCLs is limited by the rarity of these tumors and by the absence of reliable methodologies to assess  $\gamma\delta$  T-cell origin in routine diagnostic specimens. In this study, we describe the clinical, immunophenotypic and molecular characteristics of 29 non-hepatosplenic T-cell lymphomas suspected of being of  $\gamma\delta$  TCL, and explore new diagnostic markers potentially useful for diagnosis in routinely processed paraffin-embedded material.

## MATERIAL AND METHODS

### Tissue samples

A total of 35 lymphomas were collected from the pathology files of the National Cancer Institute (Bethesda, Maryland), Hospital Clínic (Barcelona, Spain), Chi-Mei Medical Center (Tainan, Taiwan), Hospital Henri Mondor (Créteil, France) and Hospital Verge de la Cinta (Tortosa, Spain). Cases were classified according to the current WHO classification.<sup>32</sup> Six of the cases had proven expression of the  $\gamma\delta$  receptor by flow cytometry or frozen section immunohistochemistry, and were used in part to assist in the validation of the immunohistochemical studies. An additional 29 cases of mature T-cell lymphoma (TCL), suspected of being  $\gamma\delta$  TCL on the basis of absent TCR $\beta$  chain expression and a cytotoxic phenotype were studied. Information regarding sites of disease, therapy, and clinical course were obtained. Five patients were previously reported (cases 15, 17–20).<sup>10</sup> This study was approved by the Intramural Research Boards of the participating institutions.

### Immunohistochemistry and hybridization studies

Immunohistochemical analysis was performed using a large panel of monoclonal antibodies detecting CD3, CD2, CD4, CD5, CD7, CD8, CD56, CD30, TIA-1 and granzyme B, performed as previously reported.<sup>3, 19</sup> Detection of the TCR- $\delta$  chain was performed using a

primary monoclonal antibody raised against the human TCR $\delta$  constant region (Human Pan TCR $\gamma\delta$ 1, clone 5A6.E9, Thermo Scientific, IL) (see supplemental information, online only). Only membranous staining was considered positive, irrespective of the intensity. For TCR $\beta$  we used a mouse monoclonal antibody (TCR 1151, clone 8A3, Pierce Endogen, IL). In cases with no detectable expression of either  $\delta$  or  $\beta$  chain and with additional sections available, TCR $\gamma$  chain detection was performed using the monoclonal antibody TCR 1153 (clone  $\gamma$ 3.20, Thermo Scientific, IL).<sup>31</sup> Epstein-Barr virus (EBV) was detected using in situ hybridization with EBER probes (INFORM EBER, Benchmark XT; Ventana Medical Systems, Tucson, AZ).

To determine whether the 5A6.E9 anti TCR $\delta$ 1 antibody could recognize  $\gamma\delta$  T-cells with a wide repertoire of TCR $\delta$  chains in formalin-fixed paraffin embedded cells we obtained an enriched population of  $\gamma\delta$  T-cells from peripheral blood mononuclear cells of healthy donors (see supplemental information, online only). After enrichment, the purity of the  $\gamma\delta$  T-cells was assessed by flow cytometry. A pellet of these cells was formalin fixed and embedded in paraffin and the sections stained with the 5A6.E9 anti TCR $\delta$ 1 antibody (Supplemental Figure 1, online only). As a control group we also studied normal tissues (Supplemental Figure 2, online only), 8 cytotoxic TCR $\beta$  chain positive TCL, NOS, one subcutaneous panniculitis-like TCL, and 6 EBV-positive NK/T-cell malignancies. In all of them, the tumor cells were negative for TCR $\delta$  chain expression although some reactive cells were easily identified in the reactive background.

### TCR gene rearrangements

Polymerase chain reaction analysis of the *TCR $\gamma$*  gene was performed using BIOMED2 protocol as described elsewhere<sup>14, 19, 36</sup> and DNA extracted from formalin-fixed tissues using the QIAamp DNA mini kit (Qiagen, Hilden, Germany). Cases from the NCI were characterized for *TCR $\gamma$*  rearrangement as previously described.<sup>15</sup>

## RESULTS

The main clinical and pathologic features are summarized in Tables 1 and 2 respectively. Based on review, 8 cases were classified as PCGD-TCL and 6 cases as HSTL. Eight additional cases were proven to be of  $\gamma\delta$  origin, 2 of which had features of enteropathy-associated T-cell lymphoma (EATL), Type II, following WHO criteria for enteropathy (villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis).<sup>32</sup> The remaining 6  $\gamma\delta$  TCL involved mainly extranodal sites, including one intestinal case without features of enteropathy. Thirteen cases were classified as TCL, “silent TCR”, and involved mainly extranodal sites. The clinical and pathological features are detailed below.

### Primary cutaneous $\gamma\delta$ T-cell lymphoma

Eight cases were classified as PCGD-TCL. This tumor occurred mainly in adults (median age, 46 years; age range, 16–63 years). The male to female ratio was 3:5. A mean follow-up of 14 months was recorded (range, 1–25). Six patients were treated with multiagent systemic chemotherapy and two cases received additional radiotherapy. One patient was treated with corticosteroids alone. Most patients experienced an aggressive clinical course with extranodal and nodal dissemination. Six patients died as a result of the disease between 1 and 22 months after the initial diagnosis. One patient (case 5) underwent allogeneic bone marrow transplantation and had no evidence of disease 25 months after diagnosis. One patient (case 7) experienced persistence of the disease at the last follow-up.

Histologically, six of these tumors were composed of small/medium-sized atypical lymphocytes. In all cases, the tumor involved the subcutis and in six extended into the

dermis. All tumors had an activated cytotoxic phenotype. Six cases were double negative for CD4 and CD8, whereas two cases expressed CD8. Expression of CD56 was observed only in 3 of 7 patients. In all cases  $\gamma\delta$  TCR expression was convincingly demonstrated by positive staining for TCR $\delta$  chain. In two cases,  $\gamma\delta$  TCR expression was further confirmed, by flow cytometry in one case, and by immunohistochemistry on frozen sections in a second case.

A clonal *TCR $\gamma$*  gene rearrangement was observed in 6 cases, whereas 1 case showed a germline configuration of the *TCR $\gamma$*  gene. Epstein-Barr virus infection was observed in two cases (cases 6 and 7) in which the strong  $\delta$  chain expression excluded a NK-cell origin (Figure 1). In one EBV-positive case *TCR $\gamma$*  gene rearrangement confirmed a T-cell origin, but PCR studies could not be performed in the second case.

### Hepatosplenic T-cell lymphoma

Six cases were classified as HSTL following the criteria of the WHO classification. No male predominance was observed; two patients were men while four were women. The median age was 44 years (range 24–62). Follow up was available in four patients. Two patients achieved a complete remission (CR) following chemotherapy, which included allogeneic stem-cell transplantation in one patient (case 11) and autologous stem-cell transplantation in other case (case 12). Median overall survival was 25 months (range 15 to 35 months), and one patient was still alive 35 months from the initial diagnosis. Case 13 did not receive any adjuvant therapy and was alive and in remission 32 months following splenectomy. The remaining two patients, after initial response to therapy, died as result of progression of lymphoma.

In all cases the tumor cells exhibited a CD3+, CD56+, CD4- and CD8- phenotype with expression of TIA-1; two cases were also positive for granzyme B (Figure 2). Staining for TCR $\delta$  chain was positive in all cases and expression was confirmed in two by flow cytometry. In situ hybridization for EBV (EBER) was performed in five cases and was negative in all cases. Five cases had *TCR $\gamma$*  gene rearrangement.

### T-cell lymphomas with intestinal involvement

There were six male patients with a median age of 58 years old (range: 49 to 72). None of them had prior history of celiac disease and five patients were Asian and one Hispanic. All patients underwent surgical resection and adjuvant chemotherapy. The median follow-up duration was 14 months. Five patients died of disease within 2 to 34 months.

Tumor cells were small to medium in all cases, and extended through the submucosa, with perforation in five cases. Histologically, all tumors showed a diffuse monotonous lymphoid infiltrate, as described in the monomorphic or Type II variant of EATL. However, features of enteropathy with increased intraepithelial lymphocytes in the mucosa adjacent to the tumor were only clearly seen in two cases (cases 15, 16), one Asian and one Hispanic, both males. All tumors expressed CD3, CD7, CD8, CD56 and cytotoxic granule associated proteins (Figure 3), while stains for CD4 (6/6), CD5 (6/6) and CD30 (2/3) were negative. Three cases showed unequivocal TCR $\gamma\delta$  derivation with strong TCR $\delta$  chain expression (Figure 3H), including the two cases of EATL, Type II. Three cases were negative for both TCR $\delta$  and TCR $\beta$  chain. Admixed reactive TCR $\gamma\delta$  and TCR $\alpha\beta$  tumor infiltrating T-cells were present confirming the validity of the techniques. In addition, immunohistochemistry for the TCR $\gamma$  chain was performed and the tumor cells also lacked TCR $\gamma$  chain expression. All cases had a clonal *TCR $\gamma$*  gene rearrangement and were EBV negative by in situ hybridization.

### Other $\gamma\delta$ T-cell lymphomas

Five patients presented with  $\gamma\delta$  T-cell tumors exhibiting strong  $\delta$  chain expression that did not correspond to any of the WHO categories; these were designated as  $\gamma\delta$  TCL, not otherwise specified. There were two males and three females with a median age of 44 years (range, 29 to 68). None of them had cutaneous or hepatosplenic involvement at diagnosis. Two cases had primary nodal involvement (Figure 4). The remaining three patients showed extranodal disease involving lung (case 21), orbit (case 22) and tongue (case 24). The patients were treated with chemotherapy (cases 21, 23 and 25) or only underwent surgical excision (cases 22 and 24). During follow-up one patient developed skin lesions and liver involvement (case 21). At the time of last follow-up (median follow-up 17 months, range, 5 to 48 months), 2 patients died of disease, while 3 patients were in complete remission.

All cases exhibited an activated cytotoxic phenotype, with expression of TIA-1 and granzyme B in all tested cases. Three cases were double negative for CD4 and CD8, whereas 2 cases were CD8 positive. CD5 was lost in 5/7 tested cases and all cases were CD56-positive. TCR $\delta$  expression was confirmed in two cases by flow cytometry. A clonal peak for TCR $\gamma$  was observed in all cases. Epstein-Barr virus infection was detected in one case (case 23), which presented in lymph node.

### Peripheral T-cell lymphoma, TCR silent

Lack of any form of TCR expression was observed in 13 cases: three were classified as TCL with intestinal involvement, and are discussed with the other intestinal neoplasms. The remaining 10 cases were grouped as TCL, TCR silent. There was a marked male predominance (8/10 were males). The median age of the patients in this category was 50 years, range 22–84. At initial presentation, six cases had cutaneous lesions (cases 27–32), two cases had features of HSTL (cases 26 and 33) and the remaining cases involved lymph node and central nervous system (cases 34 and 35, respectively). All but one patient was treated with chemotherapy, and in 2 of the 5 patients with available clinical records radiotherapy was added. Mean follow-up was 20 months. Most patients experienced an aggressive clinical course and 5 patients died with progression of the disease.

The tumor cells were negative for TCR $\delta$  and TCR $\beta$  chain expression by immunohistochemistry, although some reactive lymphocytes expressing both TCR receptors were easily identified in the reactive background. In four cases with additional available sections, immunohistochemistry for the TCR $\gamma$  chain was performed and the tumor cells also lacked TCR $\gamma$  chain expression. The tumor cells expressed CD3 but not CD4 or CD5. Three cases showed CD8 expression. In all cases a diagnosis of NK/T-cell lymphoma, nasal type was excluded, either by a monoclonal T-cell gene rearrangement (8 cases) or absence of EBV infection (8 cases). Interestingly, case 26 was originally diagnosed as an  $\alpha\beta$  variant of HSTL (Figure 5). This case was negative for TCR $\delta$  chain and positive for TCR $\beta$  chain by both immunohistochemistry and flow cytometry of the splenectomy specimen. In subsequent studies following splenectomy, an abnormal T-cell population with an identical neoplastic phenotype but lacking TCR $\beta$  chain expression was demonstrated in the bone marrow and peripheral blood compartments by flow cytometry. A molecular study for TCR $\gamma$  chain gene rearrangement demonstrated a clone of identical size in the spleen, bone marrow and peripheral blood examined both before and after surgery (Figure 5).

## DISCUSSION

T-cell lymphomas of  $\gamma\delta$  cell origin represent a rare form of lymphoma with aggressive behavior and poor outcome.<sup>5,38</sup> HSTL and PCGD-TCL are recognized in the 2008 WHO classification of lymphoid neoplasms as the two main types of TCL that express a  $\gamma\delta$

TCR.<sup>32,32</sup> In the absence of flow cytometry studies or frozen material these cases are diagnosed based on clinicopathological features and the expression of a typical CD3+, CD56+ phenotype with absence of the TCR $\beta$  chain and CD5, and usually a double negative (CD4/CD8) phenotype. Some cases with uncommon clinical presentations or not fulfilling this phenotype are more difficult to categorize, and may not be appreciated as being of  $\gamma\delta$  T-cell origin.

Several studies have described the presence and the distribution of  $\gamma\delta$  TCR's in frozen tissue sections<sup>17, 20, 22</sup> but their characterization in routinely processed tissues has been less successful.<sup>29,31</sup> The detection of a  $\gamma\delta$  TCR in formalin-fixed paraffin-embedded tissues is currently a technological challenge that hampers the diagnosis and understanding of  $\gamma\delta$  T-cell lymphoproliferative disorders. In this study, we have demonstrated the mutually exclusive expression of the TCR $\beta$  and TCR $\delta$  chains in several types of TCL in routinely processed tissues.

TCR $\delta$  chain expression was demonstrated in 8 of 14 tumors initially diagnosed as PCGD-TCL and 6 of 8 HSTL. Of note, TCR $\delta$  chain expression was observed in 8 cases of TCR $\beta$  negative TCL without hepatosplenic or cutaneous involvement at diagnosis. Three of these patients presented with intestinal involvement, two of which had features of EATL, Type II. The remaining cases had the characteristic phenotype of PCGD-TCL but presented with pulmonary, orbital, nodal, and tongue involvement. In contrast to most  $\gamma\delta$  TCL, three of the non-intestinal cases had an indolent clinical course. This heterogeneity in histological and clinical features suggests that non-hepatosplenic  $\gamma\delta$  TCL is not a homogeneous clinical entity<sup>2</sup>.

Six cases of TCL in our study primarily involved the small bowel and showed a monomorphous small-medium lymphoid infiltrate with CD3+, CD8+, CD56+, CD7+ and CD5-,  $\beta$ F1-immunophenotype. In three cases a  $\gamma\delta$  T-cell origin was proven. However, only two of them showed intraepithelial lymphocytosis fulfilling the criteria for type II EATL. EATL is defined as an intestinal tumor of intraepithelial lymphocytes (IEL) with villous atrophy and crypt hyperplasia in the adjacent, non-neoplastic small intestinal mucosa. The monomorphic variant (type II EATL) may occur sporadically, without risk factors for celiac disease. Our two EATL type II cases were from patients of Asian and Hispanic ethnicities, in whom celiac disease and enteropathy-associated TCL have rarely been reported.<sup>10</sup> EATL Type II has been suggested to be derived from IELs with a  $\gamma\delta$  phenotype.<sup>13, 21, 28</sup> The fact that the two cases of EATL Type II in our series had a  $\gamma\delta$  phenotype may support this theory.<sup>1, 9, 37</sup> Notably three additional TCL with intestinal involvement lacking diagnostic criteria for EATL had similar clinical, cytological and immunophenotypic features but lacked TCR $\beta$ , TCR $\delta$  and TCR $\gamma$  chain expression and were defined as TCR silent. Recently a benign gastrointestinal NK-cell enteropathy has been reported that may closely mimic EATL.<sup>30, 33</sup> The cells of NK cell enteropathy are CD8-negative, lack TCR expression and evidence of TCR rearrangement. In contrast, the intestinal TCR silent cases were TCR $\gamma$  clonal and CD8 positive.

We identified a total of 13 TCL that were negative for both TCR $\delta$  and TCR $\beta$  chains. In 7 of these cases, we performed immunostaining for TCR $\gamma$  chain which was negative as well. Rare TCL with a lack of TCR expression had been previously recognized in cases studied by frozen section immunohistochemistry and had been designated as TCR silent TCL.<sup>20</sup> Six of our TCR silent TCL cases had clinical and pathological features resembling PCGD-TCL while two mimicked a HSTL. As noted above, three presented with intestinal disease. We believe it is most likely that the TCR silent phenotype represents a common phenomenon of "TCR instability" in different tumor types, rather than representing a distinct clinicopathological entity. In favor of this hypothesis was the observation of a TCR  $\alpha\beta$



HSTL that evolved to a TCR silent phenotype with an identical TCR gene rearrangement pattern after splenectomy. A similar phenomenon had been reported in  $\gamma\delta$  HSTL associated with histological progression and in enteropathy-associated T-cell lymphomas evolving from refractory celiac disease.<sup>18, 34</sup> Thus, the lack of TCR expression may represent a phenotypic aberration in TCL, usually encountered at the time of progression. This finding indicates further that the lack of TCR $\beta$  chain expression cannot be used to infer a  $\gamma\delta$  T-cell origin with certainty.

Although most of our cases were unrelated to EBV infection, EBV-encoded RNA was detected in the neoplastic cells of three cases of  $\gamma\delta$  TCL. This phenomenon has been observed previously in rare examples of HSTL and non-hepatosplenic  $\gamma\delta$  TCL.<sup>2, 5</sup> Whether such cases should be classified as extranodal NK/T-cell lymphomas of true T-cell derivation, or  $\gamma\delta$  TCL, EBV-positive is difficult to resolve in the absence of other data, since even gene expression profiles may be similar.<sup>25</sup> The absence of angiocentricity, the strong TCR $\delta$  chain expression and the evidence of a clonal TCR gene rearrangement favored a  $\gamma\delta$  T-cell origin. Moreover, EBV positivity is more common in individuals of Asian origin and Native American descent in Central and South America, and one of our cases was diagnosed in a patient from Mexico.

In summary, our observations indicate that the spectrum of  $\gamma\delta$  derived TCL is broader than previously appreciated. Although HSTL and PC-GDTCL are recognized in the 2008 WHO classification, we identified a number of other  $\gamma\delta$  TCL presenting in other mainly extranodal or more rarely nodal sites. The heterogeneity of these cases suggests that non-hepatosplenic  $\gamma\delta$  TCL is not a single entity. It also appears that a high proportion of type II EATL is derived from  $\gamma\delta$  T-cells. However, there is still a subset of T-cell lymphomas with intestinal involvement that exhibits either a  $\gamma\delta$ -TCR or TCR silent phenotype and lacks features of enteropathy. Finally, the recognition of a group of silent TCL's indicates that a lack of TCR $\beta$  expression cannot be used to predict a  $\gamma\delta$  T-cell origin. The addition of genetic or genomic data in the future may help to resolve the apparent diversity in these heterogeneous and aggressive neoplasms.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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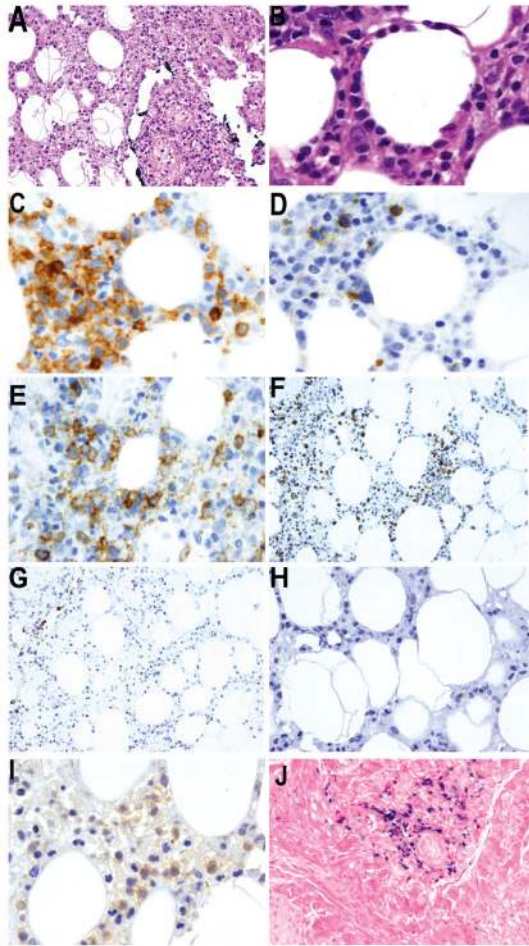
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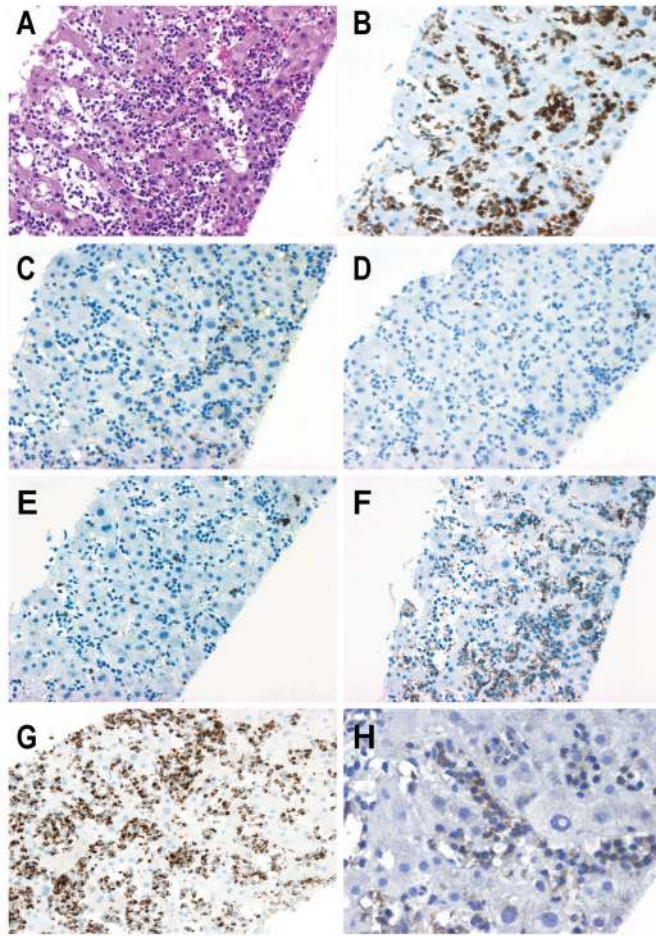


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**Figure 1. Primary cutaneous  $\gamma\delta$  T-cell lymphoma**

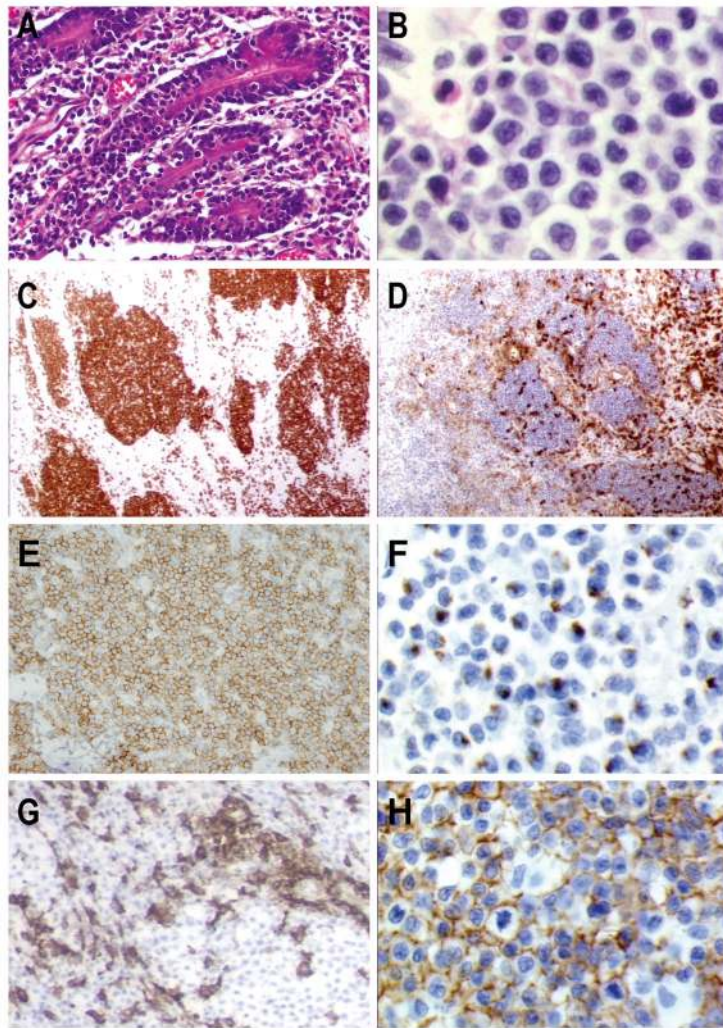
**A)** Subcutaneous involvement with panniculitic like features (H&E X20) **B)** by medium sized atypical lymphocytes with adipocyte rimming (H&E X60) **C)** The atypical lymphocytes are CD3 positive (X60) and **D)** negative for CD5 (X60) **E)** Some tumor cells express CD8 (X60) and **F)** exhibit an activated cytotoxic phenotype with granzyme-B expression (X20) **G)** Neoplastic lymphocytes are negative for CD56 (X20) **H)** Very few reactive lymphocytes express TCR $\beta$ , which is absent in tumor cells (X40) **I)** Homogenous TCR $\delta$  expression is observed in the tumor (X60) **J)** Some perivascular tumor cells are infected by Epstein-Barr virus in the dermis (EBER, X20)



**Figure 2. Hepatosplenic T-cell lymphoma**

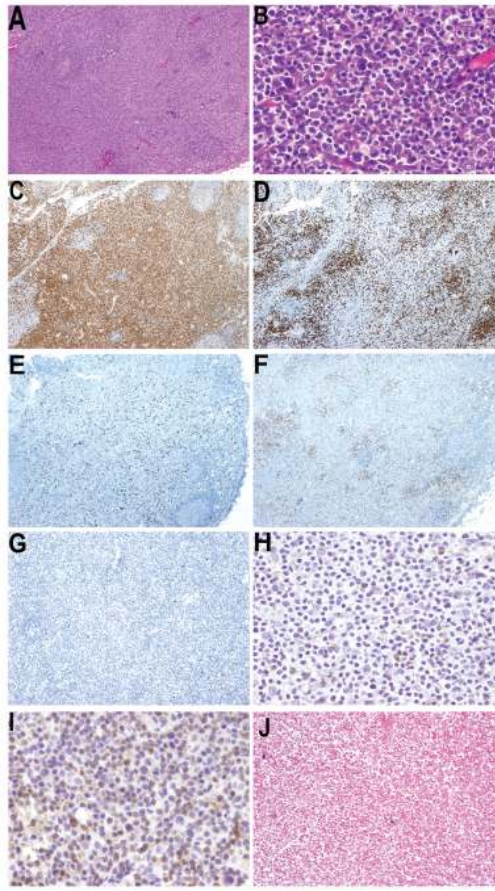
**A)** Diffuse intrasinusoidal liver involvement by monotonous medium sized atypical lymphocytes (H&E X20) that strongly express **B)** CD3 (X20) being negative for **C,D,E)** CD4, CD8 and CD5 (X20). **F)** The atypical cell expresses CD56 (X20) and **G)** TIA-1 (X20). **H)** TCR $\delta$  chain is strongly expressed in the tumor cells (X40) expression by immunohistochemistry (X40).





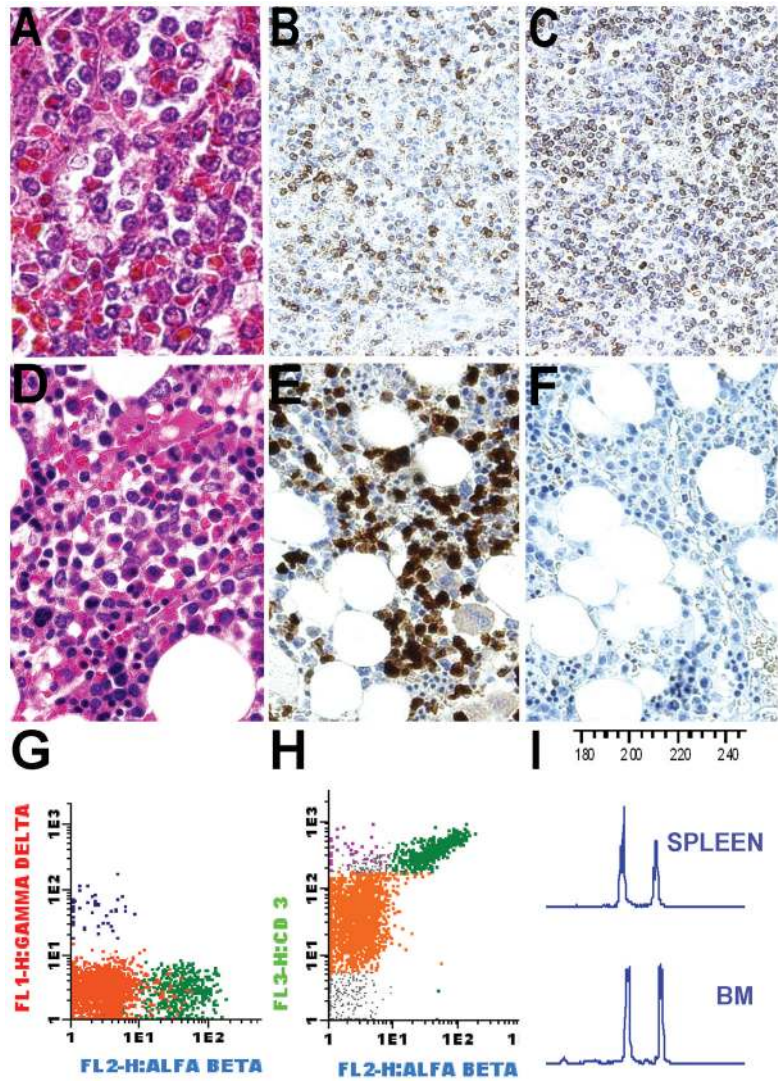
**Figure 3. T-cell lymphoma of  $\gamma\delta$  origin with intestinal involvement. Type II variant of enteropathy-associated T-cell lymphoma**

**A)** The tumor surround mucosal crypts with intraepithelial involvement (H&E X20) **B)** Monotonous lymphoid infiltrate of small medium sized tumor cells (H&E X60) The atypical lymphocytes are **C)** CD8 positive (X10), **D)** CD5 negative (X10) and **E)** CD56 positive (X40) **F)** Neoplastic lymphocytes have an activated cytotoxic phenotype with granzyme-B expression (X60) **G)** Reactive lymphocytes are positive for TCR $\beta$  that is absent in tumor cells (X40) **H)** Homogenous TCR $\delta$  expression is observed in the tumor (X60)



**Figure 4. Other  $\gamma\delta$  T-cell lymphomas**

**A)** Diffuse lymph node involvement (H&E X4) by **B)** small atypical lymphocytes. **C)** CD3 positive neoplastic cells (X4) that are **D)** CD5 negative (X4). The atypical lymphocytes are double negative for **E)** CD4 (X4) and **F)** CD8 (X4) lacks **G)** CD56 (X4) **H)** TCR $\beta$  is expressed by scattered reactive T-cells (X40) **I)** TCR $\delta$  is expressed in the tumor cells (X40) that are negative for **J)** Epstein-Barr virus (EBER, X10)



**Figure 5. T-cell lymphoma, TCR silent**

**A)** Diffuse red pulp involvement by monotonous medium sized atypical lymphocytes (H&E X60) that strongly express **B)** CD3 (X10) and **C)** TCR $\beta$  (X10) **D)** Interstitial bone marrow involvement (H&E X60) **E)** CD3 staining highlights neoplastic cells (X40) **F)** Neoplastic lymphocytes lack TCR $\beta$  expression by immunohistochemistry (X40) **G,H)** Flow cytometry on bone marrow aspirate confirms a silent phenotype with lack of TCR $\beta$  and TCR $\delta$  expression (green: residual  $\alpha\beta$  T-cells, purple: residual  $\gamma\delta$  T-cells, orange: abnormal T-cells) **I)** TCR gene rearrangement studies demonstrated the same clonal peak pattern in the spleen and bone marrow.



**Table 1**

Clinical features features T-cell lymphoma patients

Case	Diagnosis	Sex/age	Primary site of diagnosis	Secondary involvement	Therapy	Follow up (mo)
1	PCGD-TCL	M/57	Skin		CT	DoD,14
2	PCGD-TCL	F/61	Skin	BM, visceral	CT,RT	DoD,13
3	PCGD-TCL	F/48	Skin		Prednisone	DoD,1
4	PCGD-TCL	M/53	Skin		CT	DoD,16
5	PCGD-TCL	F/63	Subcutaneous tissue		RT,CT,BMT	AIR,25
6	PCGD-TCL	F/49	Skin,	Stomach, lymph node, kidney, adrenal gland, ovary	No treatment <sup>†</sup>	DoD0
7	PCGD-TCL	M/24	Skin, lymph node, BM	Lymph node, liver, PB	CT	AwD,5
8	PCGD-TCL	F/16	Skin		CT	DoD,22
9	HSTL	F/26	Spleen		Splenectomy	
10	HSTL	M/44	Liver, BM		CT	DoD,16
11	HSTL	F/56	Spleen, BM		CT,BMT	AIR,35
12	HSTL	M/62	Spleen, BM		CT, BMT	DoD,15
13	HSTL	F/54	Spleen		Splenectomy	AIR,32
14	HSTL	F/24	Liver		Splenectomy	
15	EATL type II	M/59	Small bowel		CT	DoD,9
16	EATL type II	M/72	Small bowel	Colon	CT	DoD,2
17	TCL, intestinal	M/60	Small bowel		CT	DoD,4
18	TCL, intestinal (TCR silent)	M/49	Small bowel		CT	DoD,34
19	TCL, intestinal (TCR silent)	M/54	Small bowel		CT	AIR,22
20	TCL, intestinal (TCR silent)	M/56	Small bowel		CT	DoD,14
21	Gamma-delta TCL	F/36	Lung	Skin, liver	CT	DoD,8
22	Gamma-delta TCL	F/68	Orbit		Excision	AIR,48
23	Gamma-delta TCL	F/57	Lymph node		CT	AIR,10
24	Gamma-delta TCL	M/29	Tongue		Excision	AIR,15
25	Gamma-delta TCL	M/29	Lymph node		CT	DoD,5
26	TCL, TCR silent	F/49	Spleen, PB		CT,BMT	DoD,12
27	TCL, TCR silent	F/23	Subcutaneous tissue			

Case	Diagnosis	Sex/age	Primary site of diagnosis	Secondary involvement	Therapy	Follow up (mo)
28	TCL, TCR silent	M/22	Subcutaneous tissue			
29	TCL, TCR silent	M/60	Skin	Ethmoidal sinus	CT,RT	AwD,15
30	TCL, TCR silent	M/84	Skin		CT	DoD,2
31	TCL, TCR silent	M/57	Skin			
32	TCL, TCR silent	M/54	Subcutaneous tissue	Submaxillary bone		
33	TCL, TCR silent	M/42	BM, liver and spleen		CT,RT	DoD,11
34	TCL, TCR silent	M/56	Lymph node	Peripheral blood and skin	CT	DoD,63
35	TCL, TCR silent	M/57	Central nervous system		prednisone	DoD,14

CT, chemotherapy; RT, radiotherapy; BMT, bone marrow transplantation; DOD, dead of disease; AIR, alive in remission; AWD, alive with disease.

<sup>†</sup> Diagnosed at autopsy

**Table 2**

Phenotypical and genotypical features T-cell lymphoma patients

Case #	Diagnosis	$\gamma\delta$ -TCR	$\alpha\beta$ -TCR	PCR TCR	CD3	CD4	CD5	CD7	CD8	CD56	TIA-1	Gr-B	EBV
1	PCGD-TCL	+	-	germline	+	-	-	-	-	+	+	+	-
2	PCGD-TCL	+	-	clonal	+	-	-	-	-	-	-	+	-
3	PCGD-TCL	+	-	clonal	+	-	-	-	-	+	+	+	-
4	PCGD-TCL	+	-	clonal	+	-	-	-	-	-	+	+	-
5	PCGD-TCL	+	-	clonal	+	-	-	-	+	-	+	-	-
6	PCGD-TCL	+	-	clonal	+	-	-	-	-	+	+	+	+
7	PCGD-TCL	+	-	nd	+	-	-	-	+	-	-	+	+
8	PCGD-TCL	+	-	clonal	+	-	+	+	-	-	+	+	-
9	HSTL	+	-	clonal	+	-	-	-	-	+	+	-	-
10	HSTL	+	-	clonal	+	-	-	-	-	+	+	-	-
11	HSTL	+	-	clonal	+	-	-	+	-	+	+	+	-
12	HSTL	+	-	clonal	+	-	-	+	-	+	+	-	-
13	HSTL	+	-	clonal	+	-	-	+	-	+	+	-	-
14	HSTL	+	-	nd	+	-	-	-	-	+	+	+	-
15	EATL type II	+	-	clonal	+	-	-	+	+	+	+	+	-
16	EATL type II	+	-	clonal	+	-	-	+	+	+	+	-	-
17	TCL, intestinal	+	-	clonal	+	-	-	+	+	+	+	-	-
18	TCL, intestinal (TCR silent)	-	-	clonal	+	-	-	+	+	+	-	+	-
19	TCL, intestinal (TCR silent)	-	-	clonal	+	-	-	+	+	+	+	-	-
20	TCL, intestinal (TCR silent)	-	-	clonal	+	-	-	+	+	+	+	-	-
21	Gamma-delta TCL	+	-	clonal	+	-	-	-	-	+	+	-	-
22	Gamma-delta TCL	+	-	clonal	+	-	-	+	+	-	+	+	-
23	Gamma-delta TCL	+	-	clonal	+	-	-	-	-	-	+	+	+
24	Gamma-delta TCL	+	-	clonal	+	-	-	-	+	-	+	-	-
25	Gamma-delta TCL	+	-	clonal	+	-	+	+	-	-	-	+	-
26	TCL, TCR silent	-	-	clonal	+	-	-	-	-	+	+	-	-
27	TCL, TCR silent	-	-	clonal	+	-	-	-	+	-	+	+	-

Case #	Diagnosis	$\gamma\delta$ -TCR	$\alpha\beta$ -TCR	PCR/TCR	CD3	CD4	CD5	CD7	CD8	CD56	TIA-1	Gr-B	EBV
28	TCL, TCR silent	-	-	clonal									-
29	TCL, TCR silent	-	-	clonal	+		-			+	+	+	-
30	TCL, TCR silent	-	-	germline	+	-	-	-	+		+		-
31	TCL, TCR silent	-	-	clonal	+	-			-				
32	TCL, TCR silent	-	-	clonal	+	+	+	-	-	-	+		-
33	TCL, TCR silent	-	-	clonal	+	-	-	+	-	-	+	-	-
34	TCL, TCR silent	-	-	germline	+	+	+	+	-	-	+	+	-
35	TCL, TCR silent	-	-	clonal	+	-	-	+	+	-	+		-