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Published In/Presented At

Roullet, M., Gheith, S. F., Mauger, J., Junkins-Hopkins, J. M., & Choi, J. K. (2009). Percentage of {gamma}{delta} T cells in panniculitis by paraffin immunohistochemical analysis. *American Journal Of Clinical Pathology*, *131*(6), 820-826. doi:10.1309/AJCPMG37MXKYPUBE

Percentage of γδ T Cells in Panniculitis by Paraffin Immunohistochemical Analysis

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Key Words: T cells; Panniculitis; Immunohistochemistry; Lymphoma

DOI: 10.1309/AJCPMG37MXKYPUBE

Abstract

Cutaneous T-cell lymphomas with panniculitis-like histologic features have different clinical courses depending on whether they are composed of αβ T cells or γδ T cells, necessitating their distinction for proper prognostication. However, unlike αβ T cells, γδ T cells cannot be reliably detected in formalin-fixed, paraffin-embedded sections. We demonstrated that a commercially available antibody can detect γδ T cells and examined 2 cases of flow cytometry–proven γδ T-cell lymphomas and 15 control cases of nonneoplastic panniculitis. In both lymphomas, the atypical lymphocytes were γδ T cells, whereas the reactive lymphocytes were αβ T cells. In contrast, nonneoplastic panniculitis had predominantly αβ T cells with many fewer and individually scattered γδ T cells. The detection of γδ T cells in paraffin sections provides a powerful new tool to characterize T cells in lymphomas and inflammation.

Most mature T cells express T-cell receptor (TCR) αβ and one of the TCR-associated molecules, CD4 or CD8, whereas few T cells (1%-5% of total T cells) express TCR γδ that are mostly negative for CD4 and CD8 expression.[1] γδ T cells have features of innate and adaptive immunity and may serve critical roles in bridging these types of responses, providing antimicrobial and antitumor immunosurveillance.[2,3] Similar to normal mature T cells, most cases of mature T-cell neoplasms express TCR αβ, whereas only a few cases express TCR γδ.[4]

One specific subentity of mature T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma (SPTCL) in the original World Health Organization (WHO) classification, is a malignancy of mature αβ T cells or γδ T cells.[4] SPTCL αβ is usually confined to the subcutaneous tissue and has a CD4–/CD8+/CD56–/βF1+ phenotype, whereas SPTCL γδ frequently involves the dermis and/or epidermis, sometimes with ulceration, and demonstrates a CD4–/CD8–/βF1– phenotype with frequent coexpression of CD56.[5] An associated hemophagocytic syndrome can be seen in either entity. Although uncommon in SPTCL αβ, hemophagocytic syndrome confers a significantly worse prognosis.[5] SPTCL γδ has a poor prognosis regardless of the presence or absence of a hemophagocytic syndrome.[5] Given the different immunophenotypes and clinical courses, the original SPTCL is now restricted to cases with malignant T cells expressing TCR αβ, whereas cases expressing TCR γδ are now classified as primary cutaneous γδ T-cell lymphoma.[6,7] Thus, T-cell lymphomas with a panniculitis-like pattern require characterization of the TCR subtype for proper subclassification in the latest WHO–European Organization for Research and Treatment of Cancer and the fourth edition of the WHO classifications.[6,7]

Although αβ T cells can be detected in formalin-fixed, paraffin-embedded (FFPE) sections using antibodies directed against the βF1 epitope, no equivalent antibody can reliably detect γδ T cells in FFPE sections,[8] thus requiring their detection by flow cytometric or frozen tissue immunohistochemical analysis. However, skin biopsy specimens often have insufficient cells for flow cytometric analysis, and good frozen sections of subcutaneous adipose tissue of the skin biopsies are inherently extremely difficult to achieve. For these reasons, primary cutaneous γδ T-cell lymphomas are often presumed based on the presence of βF1–/CD3+ cytotoxic T cells. A reliable, reproducible and practical method for the detection of γδ TCR in FFPE sections would be useful in confirming the diagnosis of primary cutaneous γδ T-cell lymphoma, would aid in understanding the biology of certain diseases, and would aid in subclassification of T-cell lymphomas.[9,10] In this study, we characterized a commercially available antibody that can detect γδ T cells in FFPE sections.

Materials and Methods

Case Material

With institutional review board approval, 2 cases of flow cytometry–proven γδ T-cell lymphomas and 15 cases of nonneoplastic panniculitis were identified.

Histologic Studies

Tissue samples were fixed in formalin before being submitted for routine processing and paraffin embedding. H&E was used to stain 4-μm sections.

Immunohistochemical Analysis

Tissue sections, 4 μm thick, were deparaffinized, and endogenous peroxidase was blocked by treating with 3% hydrogen peroxide in absolute methanol for 30 minutes. Sections were covered with normal horse or goat serum (Vector Laboratories, Burlingame, CA) for 30 minutes, primary antibody for 30 minutes (CD3) or 60 minutes (βF1 and TCRG), biotinylated secondary horse antimouse antibodies (βF1 and TCRG) or goat antirabbit (CD3) antibodies (Vector) at 1:200 for 30 minutes, streptavidin-biotin complex (Vector) for 30 minutes, and diaminobenzidine (DAKO, Carpinteria, CA) for 5 minutes, and counterstained with hematoxylin for 15 seconds.

Antigen retrieval with pepsin was performed at 37°C for 30 minutes (βF1); heat retrieval in EDTA buffer, pH 8, was used for CD3 and TCRG using the Decloaking Chamber (Biocare Medical, Concord, CA). Staining was performed on the DakoCytomation Autostainer (DakoCytomation, Carpinteria, CA) for the following primary antibodies: βF1 (mouse monoclonal, clone 8A3, dilution 1:80; Endogen, Rockford, IL), CD3 (rabbit polyclonal, clone F7.2.38, dilution 1:200, DAKO), and TCRG (mouse monoclonal, clone γ3.20, dilution 1:20, Endogen). TCRD (mouse monoclonal, clone Immu510, Novus Biologicals, Littleton, CO) was also tested.

Flow Cytometric Immunophenotyping

Flow cytometric immunophenotyping was performed on fresh excisional biopsy tissue samples collected in RPMI 1640 culture medium. Specimens were processed routinely, and single-cell suspensions were stained with various 4-fluorochrome-conjugated antibody combinations (fluorescein isothiocyanate, phycoerythrin, peridinin chlorophyll protein, and allophycocyanin) according to the leukemia-lymphoma panel protocol routinely used in the laboratory. The antibodies in the panel included those against leukocyte common antigen CD45, B-cell antigens (CD19, CD20, CD22, and κ and λ light chains), T-cell antigens (CD2, CD3, CD4, CD5, CD7, CD8, TCR pan αβ, TCR pan γδ, TCR-δ1, and TCR-δ2), myeloid antigens (CD11b, CD11c, CD13, CD14, CD15, CD33, CD34, and CD117), and CD10, CD16, CD25, CD36, CD38, CD56, CD103, and HLA-DR (Becton Dickinson Biosciences, San Diego, CA). Testing for terminal deoxynucleotidyl transferase was performed in permeabilized cells. Approximately 10,000 events per tube were acquired on a flow cytometer (dual-laser FACSCalibur, Becton Dickinson Biosciences) and analyzed using the CellQuest computer software program (Becton Dickinson Biosciences).

Results

Two different commercially available antibodies against an epitope on the δ or γ component of the TCR were studied. Both antibodies (clones Immu510 and γ3.20) show specific reactivity to γδ T cells in frozen sections,[11,12] but their reactivities have not been reported for γδ T cells in paraffin-embedded tissue sections. Paraffin immunohistochemical analysis (PIHC) using TCRG, an antibody directed against the γ component, demonstrated strong staining of occasional lymphocytes in control sections of normal lymph nodes and tonsils **Image 1**. The positively staining cells were mostly in the interfollicular region of lymph nodes and tonsils and represented fewer than 5% of the CD3+ T cells, consistent with the expected localization and frequency of γδ T cells in these tissues.[12] In contrast, TCRD, an antibody directed against the δ component, demonstrated no or nonspecific reactivity despite extensive attempts that used 6 different heat, chemical, or enzymatic antigen-retrieval methods; varying antibody concentrations; and varying incubation times (data not shown).

DOI: 10.1309/AJCPMG37MXKYPUBE

The low frequency of TCRG+ lymphocytes in normal lymph nodes and tonsils strongly suggests that the antibody retained its specificity for γδ T cells, even in fixed paraffin-embedded tissue. To validate this impression, we asked if PIHC using the TCRG antibody would react against 2 cases of peripheral T-cell lymphomas that were proven to exhibit the γδ phenotype by flow cytometry. The first case was a γδ hepatosplenic T-cell lymphoma that involved the spleen, liver, and bone marrow. Flow cytometric analysis of the spleen demonstrated a large discrete population in the lymphocyte gate that was mostly CD3+/αβ TCR–/γδ TCR+ (79% of the lymphocyte gate) **Image 2A**. On H&E-stained sections of the liver, the tumor cells were restricted to the sinusoidal space and consisted of medium-sized lymphocytes with irregular nuclei, dispersed chromatin, and scant cytoplasm **Image 2B**. PIHC demonstrated that the medium-sized atypical lymphocytes were TCRG+ and βF1– **Image 2C** and **Image 2D**. The βF1 highlighted occasional small mature lymphocytes with benign cytologic features that most likely represent reactive αβ T cells.

The second case was a γδ SPTCL. Flow cytometric analysis demonstrated a large, discrete population in the lymphocyte gate that was mostly CD3+/αβ TCR–/γδ TCR+ (66% of the lymphocyte gate, data not shown). On H&E staining, the tumor cells were restricted to the subcutaneous adipose tissue in a panniculitic pattern and consisted of medium-sized to large lymphocytes with irregular nuclei, dispersed chromatin, and scant cytoplasm **Image 3A** and **Image 3B**. PIHC demonstrated that the medium-sized to large atypical lymphocytes were TCRG+ and βF1– that occasionally rimmed the adipocytes **Image 3D**. The βF1 highlighted mostly small mature lymphocytes with benign cytologic features that most likely represent reactive αβ T cells **Image 3C**.

We examined the TCRG staining pattern in 15 cases of nonneoplastic panniculitis. Representative microphotographs are shown in **Image 4**, and the frequencies of T-cell subtypes are summarized in **Table 1**. All cases showed lobular or septal panniculitis composed of small to medium-sized lymphocytes admixed with varying numbers of histiocytes, eosinophils, or neutrophils. The vast majority of lymphocytes were positive for CD3, whereas approximately half of the lymphocytes were positive for βF1. The TCRG was positive in fewer than 10% of the lymphocytes, and the TCRG+ cells were individually scattered with only rare 2-cell aggregates, suggesting that high frequency and clustering of γδ T cells are not typical of nonneoplastic panniculitis.

Discussion

In this study, we identified a commercially available antibody, TCRG (clone γ3.20), that can detect γδ T cells in FFPE sections. This TCRG is directed against the constant region of the human TCR γ chain.[13] We also tested TCRD (clone Immu510), an antibody directed against the constant region of the human TCR δ chain,[14] but were not successful in detecting γδ T cells in FFPE sections, despite extensive efforts. We did not use the previously reported antibody directed against the constant region of the human TCR δ chain (clone TCR δ1, also called 5A6.E9)[15,16] because this antibody, while functional in some cases, is not robust in detecting γδ T cells in FFPE sections (written communication, W. Macon, Mayo Clinic, Rochester, MN, December 2008).

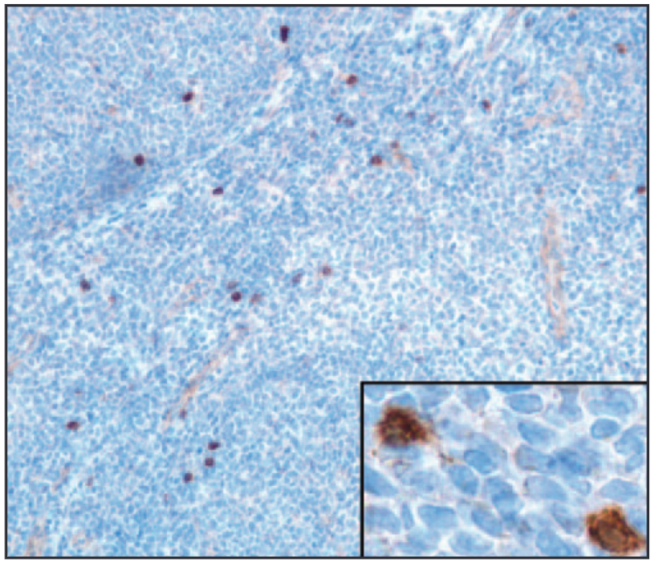

Image 1 Paraffin immunohistochemical analysis using TCRG to detect γδ T cells on normal tonsil section (×100 magnification; inset, ×400).

Because primary cutaneous γδ T-cell lymphomas can be difficult to diagnose and are frequently misdiagnosed as panniculitis,[17] we hypothesized that PIHC using TCRG may help distinguish the γδ SPTCL from panniculitis. To test this hypothesis, we examined cases of γδ T-cell lymphoma and cases of reactive panniculitis. Using TCRG, we demonstrated that γδ T cells are infrequent (<10% of CD3+ T cells) in reactive panniculitis. This finding is consistent with the predominance of αβ T cells that has been reported in lupus erythematosus panniculitis.[18] The low percentage of γδ T cells in reactive panniculitis is similar to the percentage (0%-5% of the inflammatory infiltrate) seen in frozen sections of various types of cutaneous inflammatory infiltrates,[19] suggesting that γδ T cells are infrequent in many to all nonneoplastic cutaneous lymphocytic infiltrates. Our studies further demonstrate that the γδ T cells are individually scattered in reactive panniculitis with only rare 2-cell aggregates, suggesting that the presence of small aggregates of γδ T cells is unusual and should raise the concern of a γδ T-cell lymphoma.

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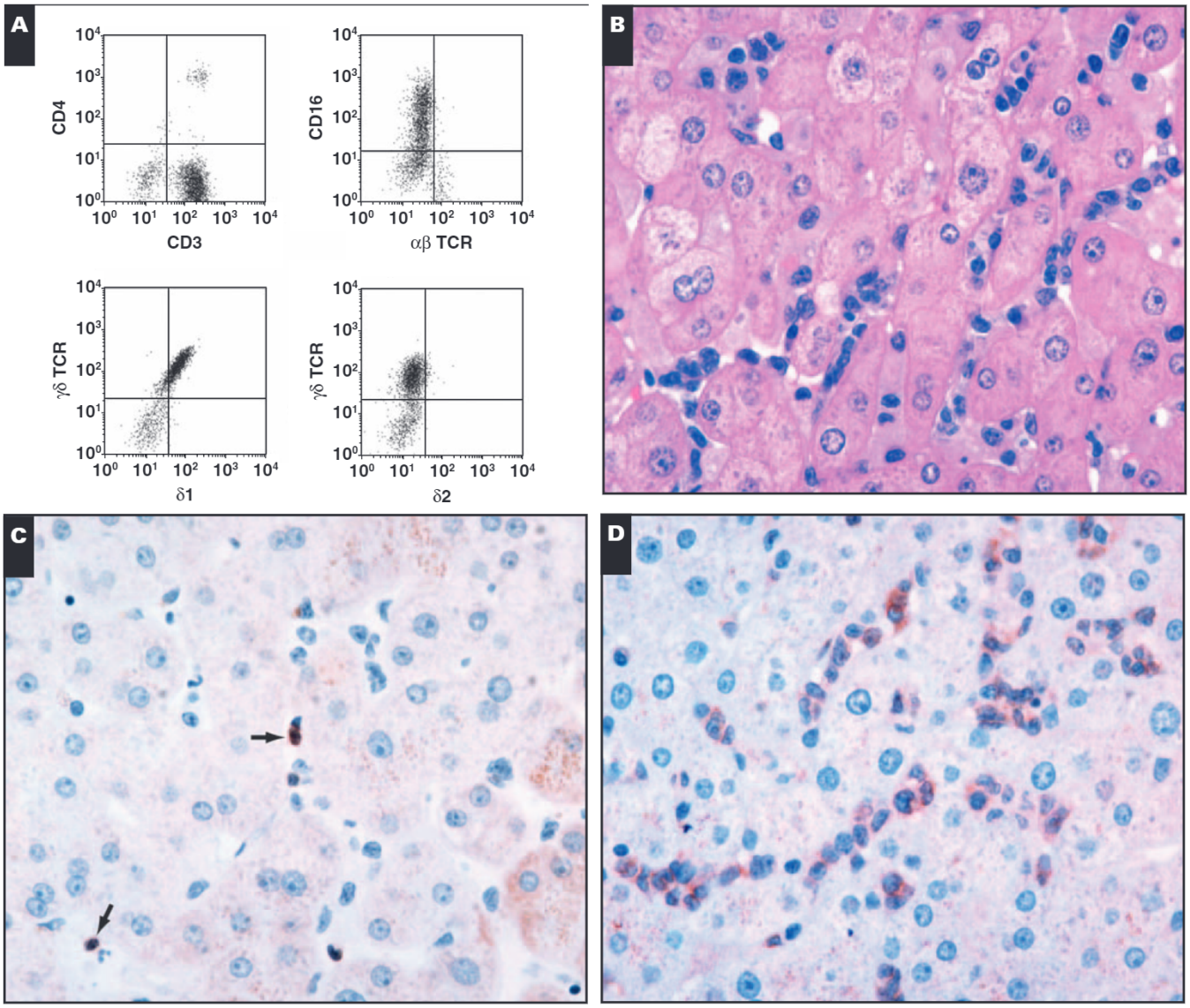

Image 2 Liver involved by γδ hepatosplenic T-cell lymphoma. **A**, Flow cytometry analysis. δ1 and δ2 represent T-cell receptor (TCR) δ1 and TCR δ2, respectively. **B**, **C**, and **D**, Representative microphotographs of liver sections stained with H&E (**B**, ×400), paraffin immunohistochemical analysis (PIHC) using βF1 for αβ TCR (**C**, ×400; arrows indicate occasional reactive αβ T cells), and PIHC using TCRG for γδ TCR (**D**, ×400).

By using TCRG, we demonstrated the γδ TCR expression in flow cytometry–proven cases of hepatosplenic γδ T-cell lymphoma and cutaneous γδ T-cell lymphoma. Since most skin biopsy specimens are not studied by flow cytometry for γδ TCR expression, PIHC using TCRG may be useful for distinguishing γδ T-cell lymphoma from SPTCL, a lymphoma reserved for the αβ phenotype according to the latest WHO classification.[6] Although hepatosplenic and primary cutaneous γδ T-cell lymphomas are recognized as lymphomas with a poor prognosis, the prognosis of other types of γδ T-cell neoplasms, especially primary nodal γδ T-cell lymphoma, is still being investigated.[1,20] A retrospective identification of such lymphomas would be possible using TCRG.

Although γδ TCR expression was detected in the 17 cases that we examined and in all control lymph nodes and tonsils that were used to initially work up the antibody conditions, the small number of cases of γδ T-cell lymphoma in the present study does not permit a valid estimate of the sensitivity and specificity of the TCR antibody. A larger study is in process to determine these numbers. Nevertheless, our results suggest that PIHC using TCRG can be a useful and

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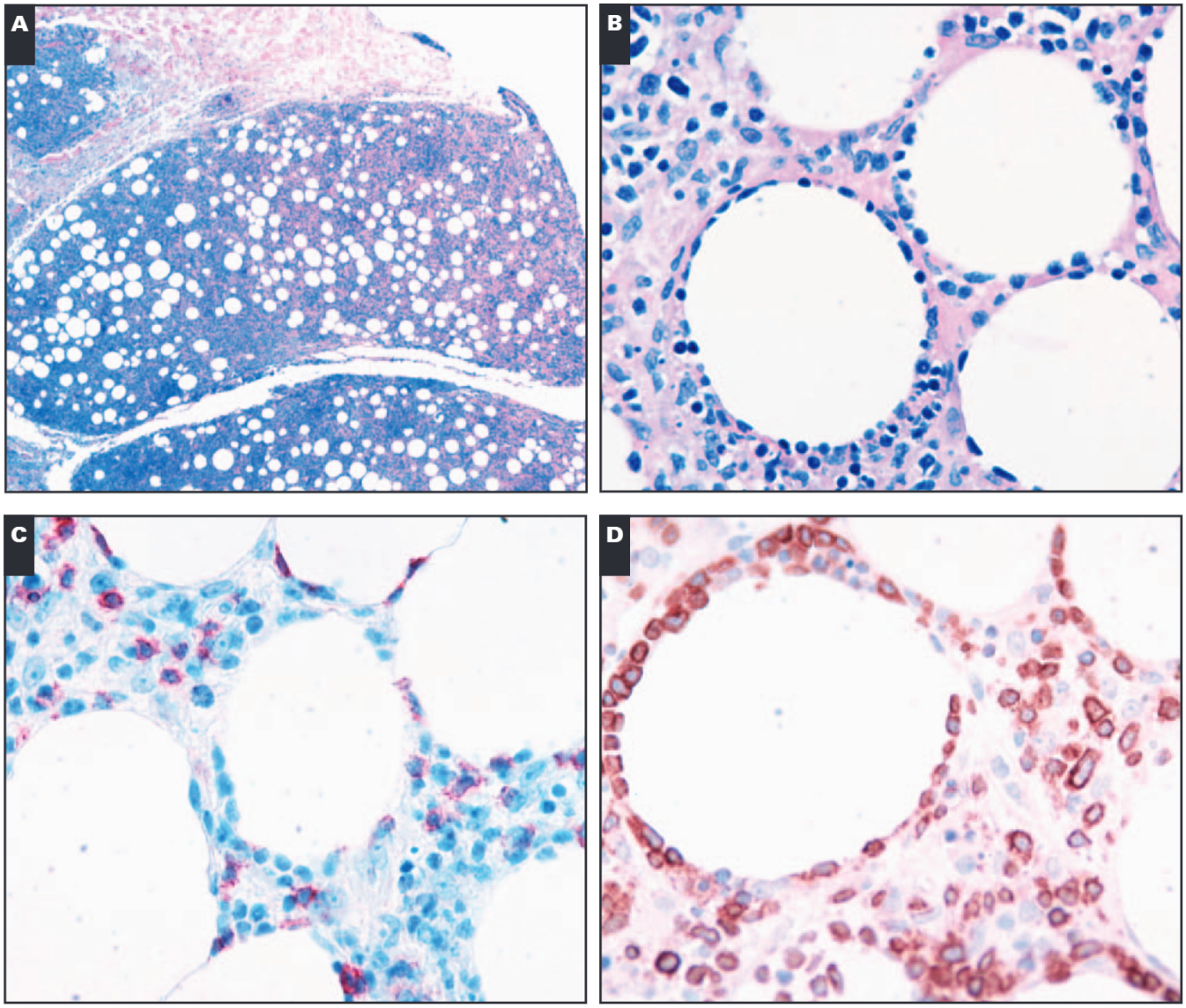

Image 3 Skin involved by γδ subcutaneous panniculitis-like T-cell lymphoma. Representative microphotographs of skin sections stained with H&E (**A**, ×25), H&E (**B**, ×400), βF1 for αβ T-cell receptor (**C**, ×400), and TCRG for γδ TCR (**D**, ×400).

powerful new tool to detect γδ T cells in T-cell infiltrates.

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Acknowledgment: We thank D. Cornfield for critical reading of the manuscript.

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DOI: 10.1309/AJCPMG37MXKYPUBE

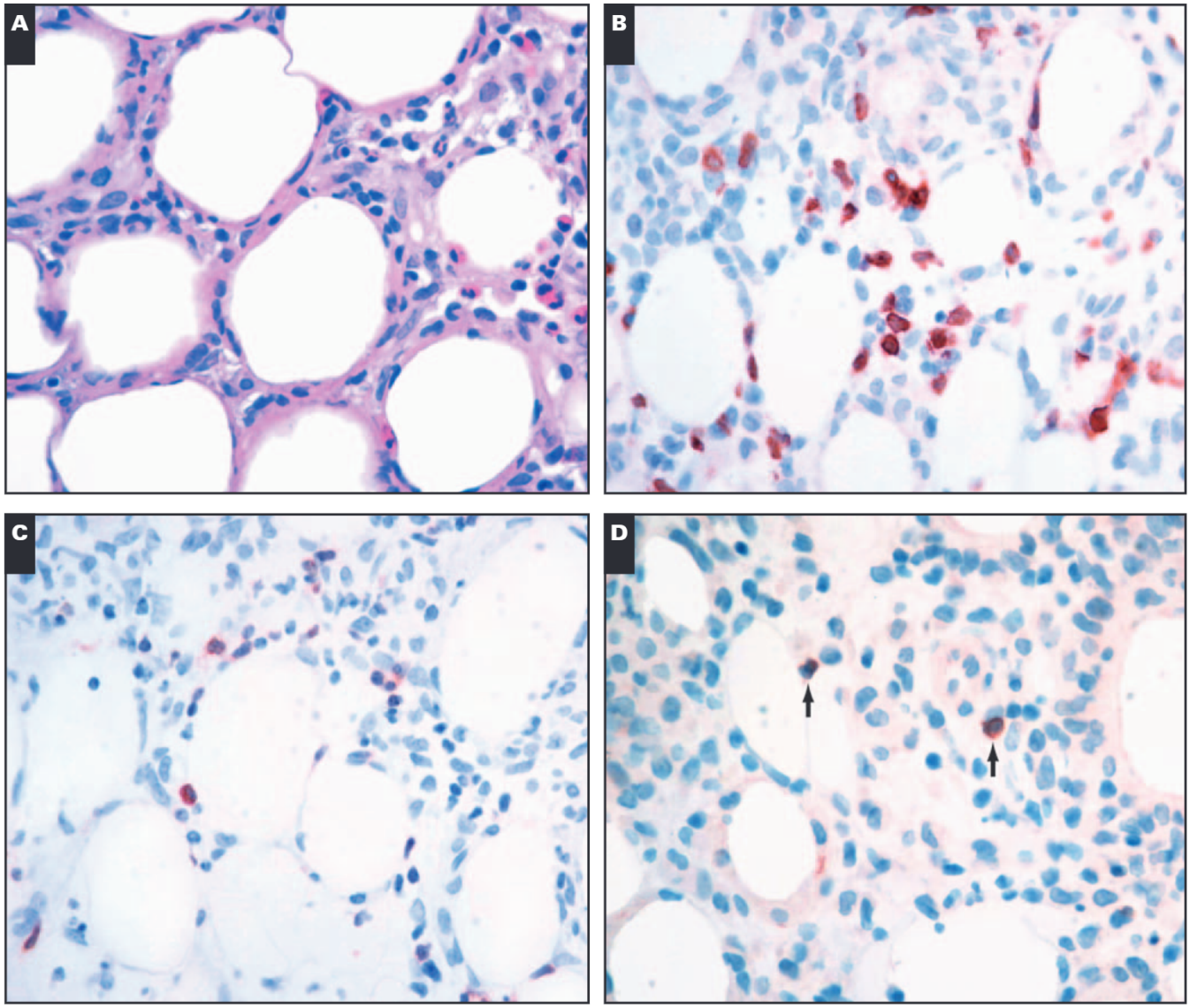

Image 4 Skin involved by nonneoplastic panniculitis. Representative microphotographs of skin sections stained with H&E (**A**), CD3 (**B**), βF1 (**C**), and TCRG (**D**; arrows indicate occasional γδ T cells) (**A**-**D**, ×400).

Table 1
Frequencies of T-Cell Subtypes

Case No.	Histologic Findings	TCRG*	βF1*
1	Minimal, focal lymphocytes	<5	60-70
2	Mostly histiocytes; only occasional lymphocytes	<5	>90
3	Histiocytes and lymphocytes	0	50
4	Granulomatoid, lymphoid aggregates	5-10	50
5	Septal and lobular lymphocytes and histiocytes	<5	50-75
6	Lobular lymphocytes and eosinophils	<5	>90
7	Focal septal lymphohistiocytic	<5	50
8	Extensive lobular lymphohistiocytic	<5	50
9	Focal minimal lobular panniculitis, mixed	0	0
10	Mostly histiocytes; only occasional lymphocytes	<10	<10
11	Mostly histiocytes; only occasional lymphocytes	<5	>90
12	Focal minimal locular panniculitis, mixed	0	0
13	Focal minimal lobular, mostly neutrophils	0	>90
14	Focal lobular eosinophilic	<5	50
15	Dermatitis; mostly lymphocytes and histiocytes	<10	50

* Percentage of lymphocytes with positive staining.

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