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Restricted V Gene Usage in T-cell Lymphomas as Detected by Anti–T-cell Receptor Variable Region Reagents

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Monoclonal antibodies reactive with variable regions of the human T-cell receptor (TCR) may be used to detect populations of T lymphocytes with restricted V gene usage. The authors have studied a series of 44 T-cell lymphomas with a panel of seven reagents reactive with four different TCR β-chain variable region families. The authors found that, with this limited panel, restricted V gene usage of the neoplastic cells could be demonstrated in 29% of TCR-positive lymphomas. Whereas this is somewhat higher than expected, no preferential use of specific families could be demonstrated. An additional, unexpected finding was that most large cell T-cell lymphomas did not express TCR despite the presence of cytoplasmic CD3. The findings indicate that with a somewhat expanded panel it should be feasible to demonstrate restricted V gene usage as an indicator of clonality in a majority of T-cell lymphomas in a manner similar to the application of anti-immunoglobulin subclass antibodies in the diagnosis of clonal B cell proliferations. (Am J Pathol 1991, 138:1479–1484)

Non-Hodgkin lymphomas can be regarded as monoclonal proliferations of T or B lymphocytes. For diagnostic purposes, kappa or lambda light chain restriction has been accepted as an indicator of monoclonality in B-cell lymphomas. Final proof of clonality can be obtained by immunoglobulin gene analysis.[1] Similarly clonality of T-cell proliferations can be shown by T-cell receptor gene analysis.[2] No simple immunologic method has been available to demonstrate clonality of T-cell lymphomas. In recent years, a number of antibodies have been developed that are reactive with variable regions of the human T-cell antigen receptor (TCR).[3–10] The TCR consists of two chains, which are closely associated with the CD3 protein complex. CD3 probably plays an important role in the signal transduction from the TCR to the cytoplasm.[11] Two main types of TCR have been identified: the classical TCRαβ and the alternative TCRγδ. T-cell antigen receptor αβ and TCRγδ can be identified by antibodies against the common framework, such as antibody βF1[12] and anti-TCRδ1 or TCRγ.[13] The variable regions of the TCR allow for an almost unlimited number of specificities. Based on similarities at the nucleic acid level, the Vβ pool of TCR gene segments has been grouped into at least 20 and the Vα pool into at least 19 'families.'[14,15] Antibodies reactive with specific V region families can be used to demonstrate restricted TCR variable region usage in autoimmune and other immune-mediated diseases.[16–19] Recently it was reported that a majority (10 of 16) of cases of cutaneous T-cell lymphoma reacted with the MX11 monoclonal antibody that recognizes Vβ8.[20] This was highly surprising, as based on the normal distribution of Vβ8-positive cells, only 3% to 5% of lymphomas were expected to react. Here we report the results of testing a series of 44 T-cell lymphomas with a panel of seven monoclonal antibodies specific for four different Vβ families as well as with CD3 and βF1 antibody.

Materials and Methods

Frozen tissue blocks of 44 cases of T-cell lymphoma that had been submitted for immunophenotyping to the surface marker laboratory of the Cross Cancer Institute from April 1987 until January 1990 were used for this study.

All cases were studied with antibodies against CD3 (OKT3) as well as against the common framework of the β-chain (βF1) and the δ-chain of the TCR (TCRδ1). In addition, the following antibodies against variable regions of the TCR were obtained from T Cell Sciences; 1C1 (Vβ5.2 + 5.3), W112 (Vβ5.3), 16G8 (Vβ8), S511 (Vβ12), OT145 (Vβ6.7), and LC4 (Vβ5.1).[3–8] Antibody 5REX9H5 (Vβ8) was a gift by Dr. Ellis Reinherz, Dana Farber Can-

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cer Institute, Boston, Massachusetts[9,10] (Table 1). Each of these antibodies recognizes between 0% and 5% of peripheral blood lymphocytes.

Five-micron–thick frozen sections of the lymphomas were stained with 1:10 dilutions of the monoclonal antibodies and a second step consisting of peroxidase conjugated goat anti-mouse immunoglobulin (Ig) antibody. Peroxidase activity was demonstrated by aminoethylcarbazole and H_2O_2.

A mixture of the antibodies reactive with variable regions of the β-chain of TCR was used to evaluate the total percentage of lymphocytes recognized by these reagents in peripheral blood of five normal controls by flow cytometry (FACScan).

Results

A total of 44 cases were evaluated. To establish the expression of TCRs, these cases were stained also with CD3 antibody and with βF1 antibody directed against a common framework determinant of the TCR β-chain as well as with TCRδ1 reactive with a common framework determinant of the TCR δ-chain. Forty of the forty-four cases were CD3+ (91%). Thirty-one of the forty-four cases were βF1 positive, indicating expression of the TCR β-chain (70.5%). None of the cases were TCRδ1 reactive. Nine cases showed clonal staining with one of the reagents specific for variable regions. This indicates that a clonal population could be identified in 9 of 31 (29%) of the cases expressing TCRs. Each of these reagents was reported to react with 1% to 5% of peripheral blood lymphocytes. A mixture of the antibodies was reactive with 6.5%, 7.5%, 9.8%, 10.3%, and 13% of peripheral blood lymphocytes in the five normal controls. This reflects approximately 15% of the CD3+ T lymphocytes.

The staining results of the lymphoma subtypes are summarized in Tables 2, 3, 4, and 5. Three of eight lymphoblastic T-cell lymphomas had an immature thymocyte phenotype lacking expression of CD1, CD4, and CD8. All three had cytoplasmic staining for CD3, but in two no staining was obtained with βF1, indicating the absence of TCRs. The remaining five cases all had the common thymocyte immunophenotype with expression of CD1, CD4, and CD8. These cases all had CD3 as well as βF1 positivity. In three of the TCR-positive cases, staining of a majority of cells could be observed with one of the anti-TCR β-chain variable region antibodies (Figure 1). All three expressed TCR of a different 'family'. In these cases as well as in the other cases, scattered cells reacted with the various anti-TCR variable region reagents.

Table 1. *Source of Reagents*

Antibody	Specificity	Source
OKT3	CD3	ATCC
βF1	Framework of β-chain of αβTCR	Dr M. Brenner
TCR δ1	Common epitope δ-chain	T Cell Sciences
1C1	Vβ5.2+5.3	T Cell Sciences
W112	Vβ5.3	T Cell Sciences
16G8	Vβ8	T Cell Sciences
S511	Vβ12	T Cell Sciences
OT145	Vβ6.7	T Cell Sciences
LC4	Vβ5.1	T Cell Sciences
5REX9H5	Vβ8	Dr E. Reinherz

Eight lymphocytic T-cell lymphomas were studied. These included three cases of Sézary's syndrome with lymph node involvement. Five cases, including the three Sézary cases were CD4+, CD8−; one case was CD4−, CD8+; two cases were CD4+, CD8+. All cases expressed CD3 as well as βF1, and two cases, both CD4+, had restricted TCR variable region staining. In these cases as well as in the other cases a minority of small lymphocytes stained with the various other anti-TCR variable region reagents.

The mixed small and large cell lymphomas constituted the largest and also most variable group in terms of morphologic subtypes. It included two cases of Lennert's lymphoma, one AILD (angioimmunoblastic lymphadenopathy with dysproteinemia)-like lymphoma, two T-zone lymphomas, and one so-called plasmacytoid T-cell lymphoma, in addition to 10 regular diffuse mixed small and large cell cases. Thirteen cases were CD4+, CD8−; one case was CD4−, CD8+; and two cases were CD4−, CD8−. All cases were CD3+ and βF1+. In three cases restricted staining with one of the anti-TCR variable region antibodies could be observed. One of these was a case of AILD-like T-cell lymphoma that morphologically had been very difficult to diagnose and previously was shown to be clonal by TCR gene analysis. It could easily be appreciated in the immunostained slides that the cells that stained for Vβ8 were larger than the scattered lymphocytes staining with the other TCR reagents (Figure 2).

The 12 large cell cases included eight CD4+, CD8− cases, one CD4−, CD8+ case, and three CD4−, CD8− cases. Eight cases were CD3+; however only one case was βF1+, indicating the absence of TCRs in the vast majority of large cell lymphomas in this series. The one positive case was a large cell lymphoma of the skin with so-called multilobated nuclei. In this case restricted staining with one of the anti-TCR variable region antibodies also could be observed.

Discussion

Clonality of T-cell proliferations generally can only be demonstrated by TCR gene analysis. The availability of monoclonal antibodies reactive with 'families' of TCR vari-

Table 2. *Immunophenotyping Results in Lymphoblastic Lymphomas*

Case no.	Age (yrs)	Sex	CD1	CD4	CD8	CD3	βF1	TCR V region
287	33	F	–	–	–	+	–	–
514	11	M	–	–	–	+	–	–
7438	18	M	–	–	–	+	+	–
134	20	F	+	+	+	+	+	Vβ5.2
8456	14	M	+	+	+	+	+	Vβ8
3191	12	F	+	+	+	+	+	–
5853	18	M	+	+	+	+	+	Vβ5.1
11931	67	M	+	+	+	+	+	–

able regions suggests the possibility that these might be used to demonstrate restricted TCR V region usage in those cases that do express TCR. Recently it was reported that a majority of cases of mycosis fungoides expressed the Vβ8 variable region family.[20]

Approximately four of a total of at least 20 β-chain families can be identified using monoclonal reagents.[3–10] We performed an analysis of 44 T-cell lymphomas, including all major subtypes, to test 1) whether staining with such reagents is a realistic approach to identify T-cell populations with restricted TCR V region usage as an indication of clonality, and 2) whether preferential use of any of the families, similar to that reported in mycosis fungoides, can be found in other T-cell lymphoma entities.

The results indicate that 29% of the TCR-positive cases could be demonstrated to have staining with antibodies against one of the four β-chain families tested. This percentage is somewhat higher than the approximately 15% of normal peripheral blood T cells that were found to express these β-chain families. If additional reagents against the other families become available, it appears to be a reasonable expectation that virtually all TCR-positive cases can be typed. To reduce the number of slides to be stained, we envision that mixtures of antibodies can be used, resulting in four groups of reagents that in reactive populations would each recognize approximately 25% of the cells, whereas in a monoclonal proliferation a significant shift of these percentages would occur.

Cell populations with restricted V gene usage are not necessarily monoclonal or malignant. For instance, increased populations of Vβ8+ cells have been found in sarcoidosis[17] and Crohn's disease.[18] In sarcoidosis these cells were found to be of polyclonal origin.[19] The demonstration of restricted TCR V regions on a majority of the atypical cells in lymphoid proliferations provides support for a diagnosis of malignant lymphoma just like restricted staining for kappa or lambda light chains on atypical B cells does not prove a monoclonal B-cell population, but is generally taken as evidence for malignancy.

With respect to the second question, ie, whether certain TCR families are preferentially expressed in T-cell lymphoma entities, our results indicate that this is probably not the case. The LC4 reagent was reactive in four of the nine positive cases. LC4 is reactive with TCR region Vβ5.1. Normally less than 5% of peripheral blood lymphocytes is reactive with LC4, and therefore 13% (4 of 31) of the cases is higher than expected. The four positive cases, however, are evenly spread over all four categories.

Antibodies 16G8 and 5REX9H5 both have been reported to react with the Vβ8 family.[10] The finding that the two cases that were positive with 5REX9H5 did not react with 16G8, however, indicates that these two reagents may recognize different members of the Vβ8 family. An alternative, although less likely, explanation could be that 5REX9H5 cross-reacts with another non-TCR antigen. For instance, Posnett et al[18] found reactivity of 5REX9H5 with epithelial cells in Crohn's disease. Similarly the finding that the case that reacted with 1C1 did not react with W112 is consistent with the notion that the first reagent reacts with Vβ5.2 and 5.3, whereas W112 only reacts with Vβ5.3.

When the different subtypes are considered, our results are consistent with the literature indicating that lym-

Table 3. *Immunophenotyping Results in Lymphocytic Lymphomas*

Case no.	Age (yrs)	Sex	CD4	CD8	CD3	βF1	TCR V region
237	82	M	+	–	+	+	Vβ6.7
278	46	M	+	–	+	+	–
10797	78	M	+	–	+	+	Vβ5.1
4290	62	F	+	–	+	+	–
2206	68	M	+	–	+	+	–
4018	58	F	–	+	+	+	–
403	63	F	+	+	+	+	–
3578	39	M	+	+	+	+	–

Table 4. *Immunophenotyping Results in Mixed Small and Large-cell Lymphomas*

Case no.	Age (yrs)	Sex	CD4	CD8	CD3	βF1	TCR V region
112	50	F	+	−	+	+	Vβ8
52	54	F	+	−	+	+	−
69	24	M	+	−	+	+	−
236	90	F	+	−	+	+	Vβ12
272	63	F	+	−	+	+	−
294	79	M	+	−	+	+	Vβ5.1
495	73	M	+	−	+	+	−
158	42	F	+	−	+	+	−
398	66	M	+	−	+	+	−
578	74	F	+	−	+	+	−
1407	49	F	+	−	+	+	−
12354	39	M	+	−	+	+	−
2980	89	M	+	−	+	+	−
406	23	M	−	+	+	+	−
385	75	M	−	−	+	+	−
571	58	M	−	−	+	+	−

phoblastic T-cell lymphomas with immature thymocyte phenotype (CD1−, CD4−, CD8−) do not express TCRs in the presence of cytoplasmic CD3, whereas those with common thymocyte phenotype (CD1+, CD4+, CD8+) do express TCRs in combination with membrane CD3.[21,22] One of the cases with immature phenotype, however, did react with βF1 antibody, and this may reflect a transitional stage.

Once the TCR subtype is established on the primary tumor, this may be a useful adjunct in the search for residual or recurrent tumor in bone marrow, peripheral blood, or cerebrospinal fluid. The major limitation would be the presence of a small normal T-cell population with the same TCR subtype.

The lymphocytic lymphomas and the mixed small and large cell lymphomas all expressed TCRs. Mixed small and large cell lymphomas are the most important group in terms of differential diagnosis, as these can be difficult to distinguish from mixed-cellularity Hodgkin's disease, reactive lymphadenopathy, and T-cell–rich B-cell lymphomas. Our one case of AILD-like T-cell lymphoma clearly illustrates that staining with these reagents can demonstrate restricted V gene usage of the T cells. We also tested reactive lymph nodes and a series of 40 cases of Hodgkin's disease of all different subtypes and have found no cases with a restricted population of T cells with this panel of antibodies. Also no positive Reed–Sternberg cells were found.

The large cell T-cell lymphomas in this series had remarkably consistent results. Eight of the cases (67%) were CD3+, but only one case (8%) was βF1+, indicating that the vast majority of large cell T-cell lymphomas do not express TCRs, in contrast to the lymphocytic and mixed small and large cell T-cell lymphomas. It is not possible to identify whether the CD3 staining was membranous or cytoplasmic by immunohistology on frozen tissue sections. Because it is generally accepted that the TCR-CD3 complex must assemble intracellularly before expression on the cell surface,[23] it appears that the CD3 staining reflects cytoplasmic CD3. The one TCR-positive case was an example of multilobated lymphoma of the skin of T-cell origin.

CD3-positive, TCRβ-negative cases in fact might express TCRγδ. The immunophenotype of most TCR γ/δ

Table 5. *Immunophenotyping Results in Large-Cell Lymphomas*

Case no.	Age (yrs)	Sex	CD4	CD8	CD3	βF1	TCR V region
389	60	M	+	−	+	+	Vβ5.1
197	60	M	+	−	−	−	−
246	40	F	−	−	+	−	−
49	65	F	−	−	+	−	−
66	61	M	+	−	+	nd	−
78	30	F	+	−	+	−	−
6089	45	M	−	−	−	−	−
5893	65	M	+	−	+	−	−
7925	24	M	+	−	−	−	−
56	50	F	−	+	−	−	−
5537	47	M	+	−	+	−	−
4553	55	M	+	−	+	−	−

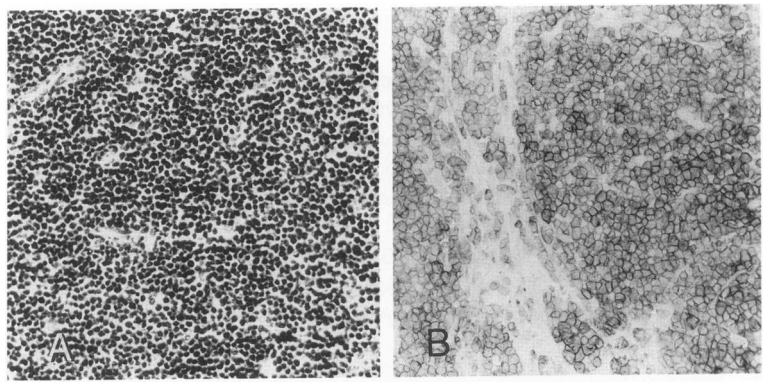

Figure 1. *Lymphoblastic T-cell lymphoma. Paraffin section stained with H&E* **(A)** *and frozen section stained with antibody reactive with TCR variable region Vβ5* **(B)**. *Note that virtually all cells are staining in this lymphoblastic lymphoma.*

cells is CD4−, CD8−, or CD4−, CD8+,[12] whereas most of the TCRβ-negative lymphomas in our study were CD4+. To definitely exclude the presence of γ/δ receptors, we have tested these cases with an antibody reactive with a common epitope of the δ-chain of the γ/δ TCR (T Cell Sciences, Cambridge, MA). All lymphomas were found to be negative, with staining only of very few scattered small lymphocytes. As an alternative, the lack of TCR expression may be consistent with the finding that large cell T-cell lymphomas tend to have incomplete or otherwise aberrant immunophenotypes.[24] Loss of TCRs may be associated with loss of regulatory control mechanisms on the proliferating cell population.

The absence of TCR expression in most large cell T-cell lymphomas prevents the application of this method to demonstrate a population with restricted V gene usage. The large cell morphology and the absence of TCR expression in itself are sufficient criteria for the diagnosis of lymphoma in these cases, however.

In conclusion, antibodies directed against TCR β-chain V regions can be used to identify restricted TCR V region usage in TCR-positive T-cell non-Hodgkin lymphomas. With a panel of seven antibodies recognizing four different TCR Vβ region families, cell populations with restricted V region usage could be detected in 29% of TCR-positive lymphomas, supporting the idea that with a higher number of reagents the majority of TCR-positive lymphomas could be analyzed.

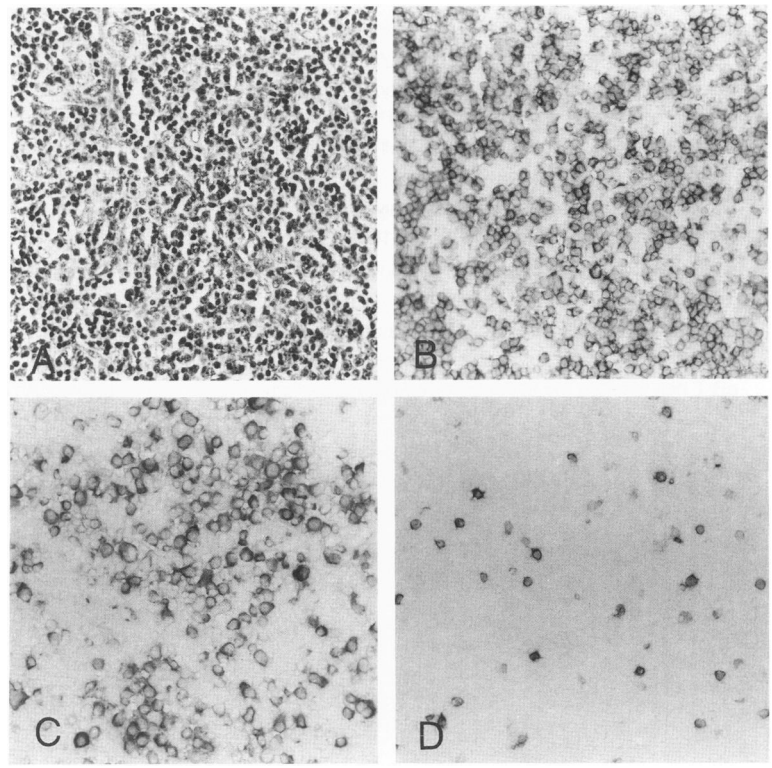

Figure 2. *AILA-like T-cell lymphoma. Paraffin section stained with H&E* **(A)**. *Frozen sections stained with βF1* **(B)**, *5REX9H5 (Vβ8)* **(C)**, *and 16G8 (another Vβ8 reagent)* **(D)**. *Note positive staining of larger lymphocytes with 5REX9H5 and staining of few scattered small lymphocytes with the other reagent.*

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