Oligoclonality of CD8$^+$ T Cells in Breast Cancer Patients

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ABSTRACT

Substantial evidence has suggested that T cells play an important role in antitumor immunity. T cells with cytotoxic activity against tumors have been isolated from in vitro culture of tumor-infiltrated lymphocytes of cancer patients. In addition, clonal expansions of T cells have been identified in lesions of tumors by using a PCR-based CDR3 analysis of T cell receptors (TCR). Since the CDR3 region of the T cell receptor directly interacts with the antigen–MHC complex and is thus highly polymorphic, a dominant CDR3 length in a particular TCR Vβ population will indicate the clonal expansion of a specific T cell clone. Utilizing this technique, we have analyzed the T cell repertoire in lymph nodes (LNs) and peripheral blood of 20 breast cancer patients. Our results show that in most cases, peripheral blood mononuclear cells (PBMCs) and LN express dominant CD8$^+$ T cell clones in different Vβ gene families, and the number of dominant clones is higher in PBMC than in the LN. Furthermore, in 7 out of 16 patients' lymph nodes, there is a dominant Vβ18 T cell clonal expansion in the CD8$^+$ T cell subset. The frequency of an oligoclonal expansion of Vβ18 CD8$^+$ T cells in non-breast cancer lymph nodes is 1 out of 9, but no obvious motif in the CDR3 region of Vβ18 TCR can be identified. The prevalence of the clonal dominance found in breast cancer is discussed in the context of a possible tumor-related antigen stimulation.

INTRODUCTION

A large body of evidence indicates that T cells play an important part in antitumor immunity (1). Tumor-specific T cells have been found in tumor-bearing patients, and many human solid tumors are infiltrated by T cells (2,3). In some cases, the presence of tumor-infiltrating lymphocytes (TILs) has been considered to be a favorable prognostic indicator, as T cell infiltration of the tumor is felt to reflect the patient's ability to develop an immune response against the tumor (4,5). The development of tumor immunity suggests that new antigen determinants may emerge during the transformation from normal to malignant cells, and that these new non-self antigens can be detected by T cells (6–13). Indeed, T cells isolated from tumor sites express activation markers indicating in vivo activation by antigens (14–20). Furthermore, the antitumor activity of these T cells can be demonstrated by their ability to proliferate, secrete cytokines, or induce cytolysis of target cells when they encounter tumor cells in vitro (21–25).

Each T cell is characterized by the expression of a unique T cell receptor (TCR), which is composed of an α- and a β-chain (26–28). PCR analysis indicates that in some, but not all tumors, there is a dominant usage of a particular V gene by TILs, suggesting that they may be elicited by a common antigen (17,18,29). The hypervariable CDR3 region of the TCR, containing the V(D)J junction, is thought to carry the fine specificity of antigen recognition (30). The length of the CDR3 region varies from 6 to 14 amino acids. In general, there is a Gaussian distribution of CDR3 length among TCR using a particular V gene family. Upon antigen stimulation, a dominant CDR3 length may emerge, which represents the

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TABLE 1. Sequence of primers used

Primers	Sequence (5′–3′)	
First-step PCR		*Nested primers*
BV1	CAACAGTTCCCTGACTTGCAC	CTCAGCTTTGTATTTCTGTG
BV2	TCAACCATGCAAGCCTGACCT	CAGCAGCCTCTACATCTGCA
BV3	TCTAGAGAGAAGAAGGAGCGC	GACATCTATGTACCTCTGTG
BV4	CATATGAGAGTGGATTTGTCATT	CAGCAGCATATATCTCTGCA
BV5	CCTAACTATAGCTCTGAGCTG	CTCGGCCCTCTATCTCTGTG
BV6	AGGCCTGAGGGATCCGTCTC	CTC(G or C or A)GCC(G or A)TGTATCTCTGTG
BV7	CTGAATGCCCCAACAGCTCTC	CTC(G or A)GCCCTGTATCTCTGCG
BV8	TACTTTAACAACAACGTTCCG	CTCAGCTGTGTACTTCTGTG
BV9	AAATCTCCAGACAAAGCTCAC	CTCTGCTGTGTATTTCTGTG
BV10	CAAAAACTCATCCTGTACCTT	CACAGCACTGTATTTCTGTG
BV11	ACAGTCTCCAGAATAAGGACG	TACCTCTCAGTACCTCTGTG
BV12	GACAAAGGAGAAGTCTCAGAT	GACATCTGTGTACTTCTGTG
BV13.1	GACCAAGGAGAAGTCCCCAAT	GACATCTGTGTACTTCTGTG
BV13.2	GTTGGTGAGGGTACAACTGCC	GACATCTGTGTACTTCTGTG
BV14	TCTCGAAAAGAGAAGAGGAAT	GACCTCTCTGTACTTCTGTG
BV15	GTCTCTCGACAGGCACAGGCT	GACAGCTCTTTACTTCTGTG
BV16	GAGTCTAAACAGGATGAGTCC	TTCTGGAGTTTATTTCTGTG
BV17	CACAGATAGTAAATGACTTTCAG	GACAGCTTTCTATCTCTGTG
BV18	GAGTCAGGAATGCCAAAGGAA	TTCGGCAG(G or C)TTATTTCTGTG
BV19	CCCCAAGAACGCACCCTGC	CACGGCACTGTATCTCTGCG
BV20	TCTGAGGTGCCCCAGAATCTC	CTCTGGCTTCTATCTCTGTG
CB-14	CTCAGCTCCACGTG	
CB-R	CTTCTGATGGCTCAAACAC	
BJ primer used for sequencing		
BJ1S1	AACTGTGAGTCT GGTGCCTT	
BJ1S2	ACGGTTAACCTG GTCCCCGA	
BJ1S3	CTCTACAACAGT GAGCCAAC	
BJ1S4	GACAGAGAGCTG GGTTCCAC	
BJ1S5	TGGAGAGTCGAG TCCCATCA	
BJ1S6	TGAGCCTGGTCC CATTCCCA	
BJ2S1	CCTCTAGCACGG TGAGCCGT	
BJ2S2	TACGGTCAGCCT AGAGCCTT	
BJ2S3	CTGTCAGCCGGG TGCCTGGG	
BJ2S4	CTGAGAGCCGGG TCCCGGCG	
BJ2S5	CCTCGAGCACCA GGAGCCGC	
BJ2S6	CCTGCTGCCGGC CCCGAAAG	
BJ2S7	TGACCGTGAGCC TGGTGCCC	

BV: TCR Vβ; BJ: TCR Jβ; CB: TCR Cβ.
CB-14 was used for first-strand cDNA synthesis, and CB-R was used for sequencing.

expansion of a specific antigen-reactive T cell clone (31). Therefore, examining the CDR3 length is not only another means of analyzing T cell repertoire diversity but it can also provide information on the clonality of T cells (32,33). By using this technique, several investigators have

TABLE 2. Description of Breast Cancer Patients

Patients	Age	Primary tumor	Positive nodes	HLA haplotype			Specimen
				A	B	C	
S.G.	39	Noninvasive, ductal	0				LN, PBL
D.C.	47	Microinvasive, ductal	0				LN, PBL
E.H.	61	Microinvasive, ductal	0				LN, PBL
B.F.	45	Microinvasive, ductal	0				LN, PBL
G.L.	60	Microinvasive, lobular	0				PBL
D.M.	63	Invasive, ductal	0	2,3	18,53	4,7	LN, PBL
M.T.	76	Invasive, ductal	0	26,30	18,38	5.8	LN, PBL
C.D.	56	Invasive, medullary	0	2,11	45,50	6,7	LN
M.B.	56	Invasive, lobular	0	2,26	44,50	6,7	LN
I.L.	66	Invasive, ductal	0				LN
D.D.	40	Invasive, lobular	0	1,25	8,37	7,—	LN
V.V.	55	Invasive, ductal, lobular	0				LN
J.W.	59	Invasive, ductal	0				LN
J.K.	65	Invasive, ductal	0				LN
M.Z.	58	Invasive, cystosarcoma phylloides	0				PBL
P.V.	40	Invasive, medullary	2/23	24,30	44,52	4,7	LN, PBL
H.E.	72	Invasive, ductal	8/19	2,24	39,57	6,7	LN
H.H.	69	Invasive, lobular	23/39	2,23	44,50	5,6	LN
I.D.	70	Invasive, ductal	6/19				PBL
J.P.	49	Invasive, ductal	22/22				PBL

shown clonal expansion in TILs of melanoma, glioma, and other solid tumors (34–37).

The majority of breast cancer–specific cytotoxic T lymphocytes (CTLs) that have been characterized were generated by in vitro stimulation with allogenic tumor cells or tumor-antigenic peptides and were expanded in the presence of interleukin 2 (IL-2). They can be either $CD4^+$ or $CD8^+$ T cells with cytotoxic activity against tumor cell lines (38–40). In general, T cells isolated from patients' axillary lymph nodes have been used as a source of tumor-infiltrating lymphocytes as these lymph nodes directly drain the tumor and so would be expected to be enriched for specific breast cancer–reactive lymphocytes. In vitro oligoclonal expansion of these propagated CTLs may sometimes be found. However, these cells may reflect an artifact, such as random outgrowth in culture (41–43). Therefore, it is important to determine whether a tumor-specific local response exists in vivo. Furthermore, it should be advantageous to analyze the repertoire of TILs at a resolution permitting the detection of potential tumor-specific clonal expansions in breast cancer patients. Therefore, we decided to perform CDR3 length analysis to examine potential tumor-specific clonal expansion of T cells in breast cancer patients' blood and LNs.

MATERIALS AND METHODS

Lymph Node and Blood Preparation

Blood was drawn from breast cancer patients prior to their surgical procedure. After surgery, lymph nodes were obtained from pathologic specimens of surgical dissections from the same patients. Lymph nodes were selected and sterilely sectioned into two equal parts. One-half of the lymph node was used for immediate histologic analysis. At the same time, the other half of the lymph node sample was placed in a conical tube with sterile RPMI 1640 medium. This sam-

ple was then passed through a wire mesh to obtain a single-cell suspension. The typical yield of lymphocytes for this procedure was between 5 and 10 million cells. Controls consisted of LNs of non-breast cancer patients that were obtained from autopsy samples. Blood (30–50 ml) was obtained from healthy female volunteers whose ages ranged from 40 to 80.

T Cell Isolation and RNA Preparation

Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood and mononuclear cells were isolated from a single-cell suspension of lymph nodes by Ficoll-Hypaque centrifugation. $CD4^+$ or $CD8^+$ T cells were then subjected to positive selection by using anti-CD4- or anti-CD8-coated magnetic beads (Dynal, Great Neck, NY). RNA was extracted directly from bead-bound cells by using RNAzol according to the manufacturer's instructions (Biotecx, Houston, TX).

CDR3 Length Analysis

Total cellular RNA was extracted from 1×10^5 T cells and reverse transcribed into cDNA with a TCR Cβ anti-sense primer. Each Vβ-specific DNA fragment was generated from a portion of first-strand cDNA reaction mixture, using polymerase chain reaction (PCR) technique with the same Cβ anti-sense primer and a Vβ-specific sense primer (Table 1). The PCR reaction consisted of 35 cycles of 30 sec at 94°C, 30 sec at 55°C, and 1 min at 72°C and an additional extension cycle at 72°C for 10 min. One to two microliters of this first PCR reaction product was then reamplified with a nested Vβ-specific sense primer and a ^{32}P-labeled Cβ anti-sense primer for another 15 thermal cycles (33). The second PCR product was separated on a 6% acrylamide sequencing gel and visualized by overnight exposure to Kodak AR film. The radioactivity of each DNA fragment was analyzed on a Phosphorimager (Molecular Dynamics, CA). The dominant band was defined as that containing more than 50% of the combined radioactivity of all bands in that particular Vβ family.

CDR3 Region Sequencing

After CDR3 analysis, the dominant band of the Vβ18 gene family was cut out of the acrylamide gel, extracted in H_2O, and purified using the PCR Prep DNA purification kit, according to the manufacturer's instructions (Promega). Purified DNA fragments were sequenced using fluorescent dideoxy nucleotides and a Cβ reverse primer on an Applied Biosystem Model 373A Automatic Sequencer. If ambiguous sequences were found, additional sequencing was carried out using a Jβ-specific reverse primer (44).

In some cases, readable sequences could not be obtained from direct sequencing. We then determined the sequence by bacterial cloning. Vβ18 TCR DNA fragments were generated by PCR technique with a Vβ18-specific forward primer and a Cβ reverse primer and ligated into a pAMP1 vector according to the manufacturer's instructions (Gibco/BRL). Ligated plasmids were transformed into DH5-competent cells, positive clones were selected, and those clones containing correct inserts were amplified and plasmid DNAs were isolated and purified by using Minipreps DNA purification kit (Promega). Purified plasmid DNA was sequenced using the automatic sequencer.

RESULTS

LN and PBMC $CD8^+$ T Cells of Breast Cancer Patients Exhibit Different CDR3 Size Patterns

In the antigen-specific immune response, there may be limited T cell clone(s) generated in response to the eliciting antigen(s), and their proliferation or activation may not be prominent enough to be detected by mRNA analysis or immunofluorescence methods. Recently, however, several laboratories have used CDR3 region analysis of TCR to examine clonal expansion of T cells; previously, this could not be done by semiquantitative RT-PCR techniques (31–37). Although to date, no dominant T cell receptor usage has been observed in breast cancer patients (29), tumor antigens may stimulate an oligoclonal expansion of a subset of T cells. We therefore decided to examine the oligoclonality of both CD4 and CD8 T cells in the lymph nodes and blood of patients with breast cancer.

Sixteen lymph nodes and eleven blood samples from 20 breast cancer patients were analyzed; 7 patients had paired blood and LN samples. One patient had noninvasive tumor and four had microinvasive intraductal type tumor. Fifteen had invasive-type tumors, and five of them had involved nodes (see Table 2). The distribution of CDR3 length of TCRVβ gene families

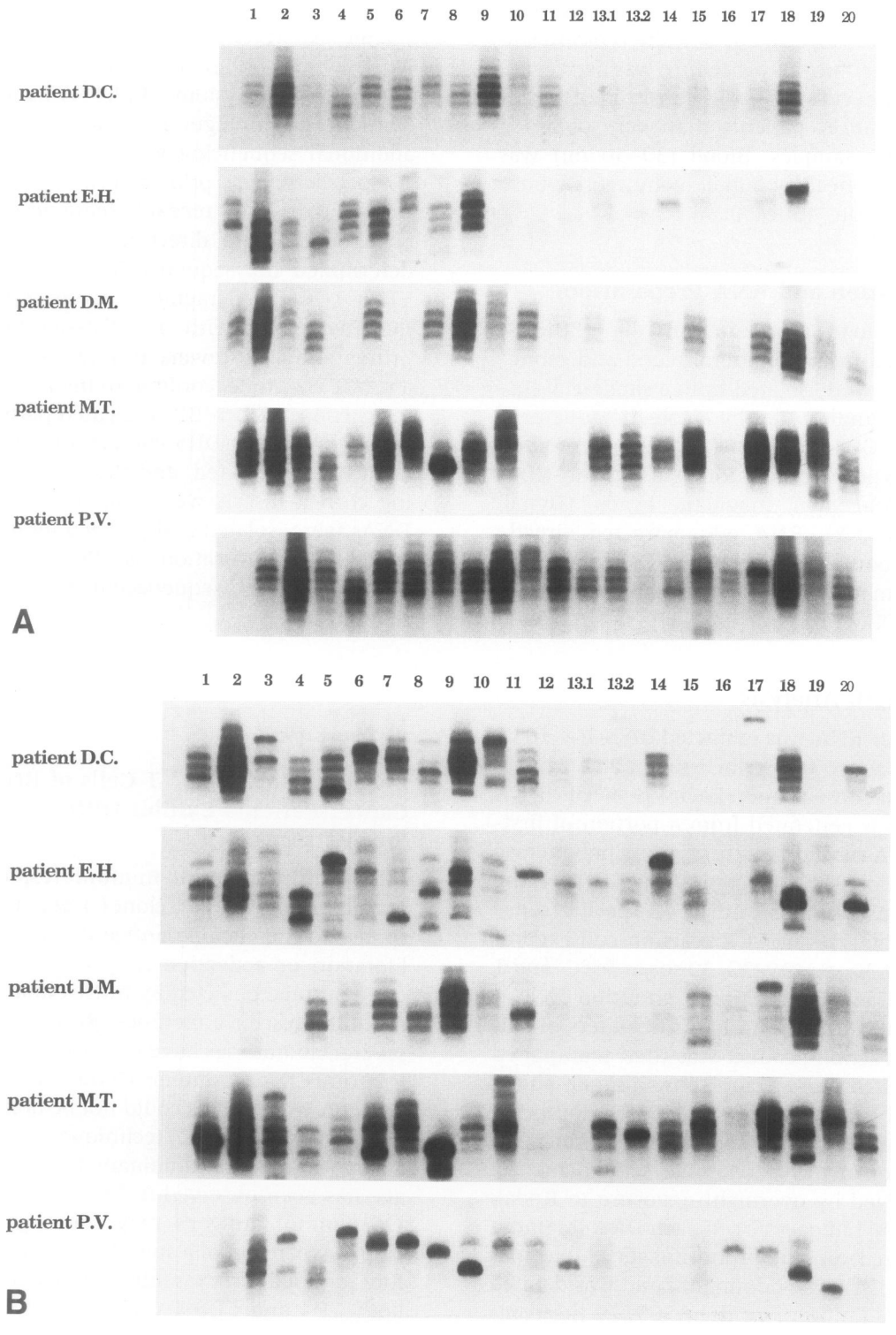

FIG. 1. CDR3 length analysis of T cells isolated from peripheral blood (PB) (A, B) and lymph nodes (LN) (C, D) of breast cancer patients

Mononuclear cells were isolated from PB or LN and $CD4^+$ (A,C) and $CD8^+$ (B,D) cells were positively selected by magnetic beads (see Materials and Methods). CDR3 lengths were analyzed by using a two-step PCR technique with ^{32}P-labeled C, β reverse primer.

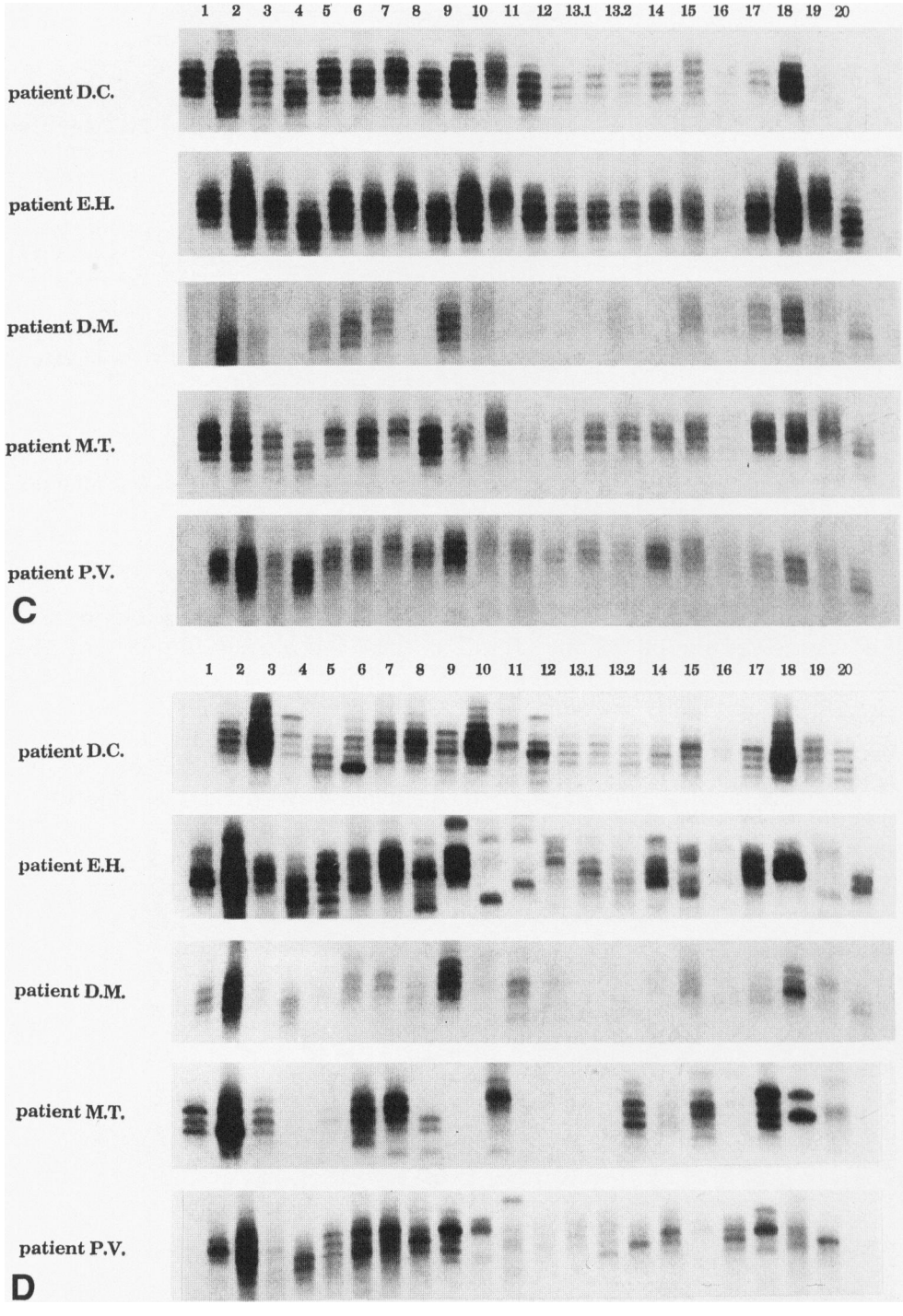

FIG. 1. (Continued)

was visualized by using radioactive Cβ primers, and the radioactivity of each band was then measured and analyzed using a Phosphorimager. Since TCR Vβ19 and 10 genes contain nonfunctional gene segments, we eliminated these Vβs from our analysis (44). Figure 1 shows that in the $CD4^+$ T cell compartment of both lymph node and PBMCs of five breast cancer patients, there is a Gaussian distribution in the CDR3 length of all TCRVβ families, whereas in the $CD8^+$ T cell compartment, a dominant CDR3 size can be seen in many Vβ gene families. Previous studies have demonstrated that most of the dominant bands observed in CDR3 analysis contain a single dominant sequence, suggesting a clonal expansion in that Vβ gene family (33). Comparing the clonal-

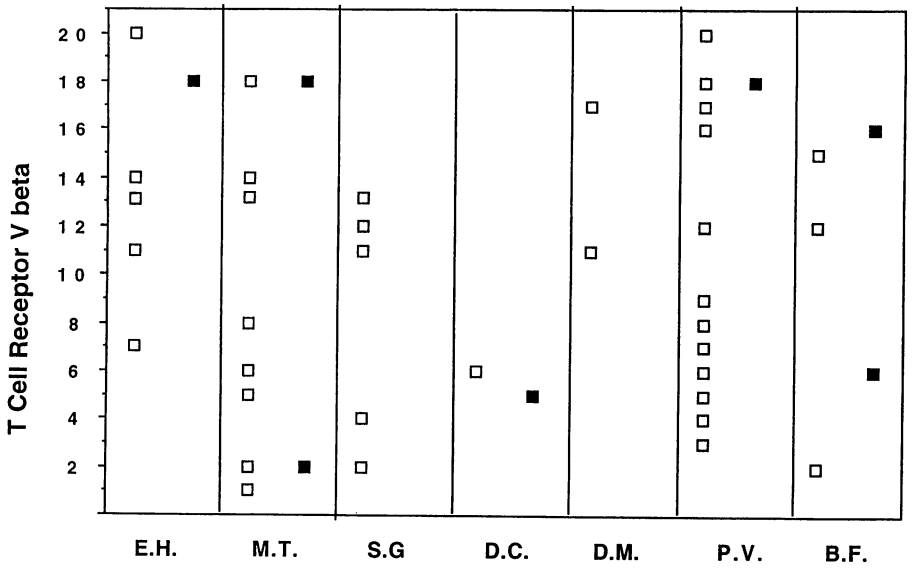

FIG. 2. Presence of dominant CD8$^+$ T cell clone in each TCR Vβ family of seven breast cancer patients: E.H., M.T., S.G., D.C., D.M., P.V., and B.F.

Radioactivity of each band on acrylamide gels (see Fig. 1) was collected and analyzed on a Phosphorimager. The dominant band was determined by the criteria described in Materials and Methods. ■, LN; □, PBMC.

ity in the PBMCs and LN of the same patient, our data show that in most cases, the PBMC and LN express dominant CD8$^+$ T cell clones in different Vβ gene families. In addition, the number of dominant clones is higher in PBMCs than in the LN (Fig. 2).

Although clonal expansion is a common feature of the CD8$^+$ population in normal individuals, the average number of TCR families expressing clonal dominance is approximately 1.7. In breast cancer PBMCs and breast cancer LN, the average numbers are 4.5 and 2.5, respectively. Because the frequency of oligoclonality increases with age (45) and the average age of

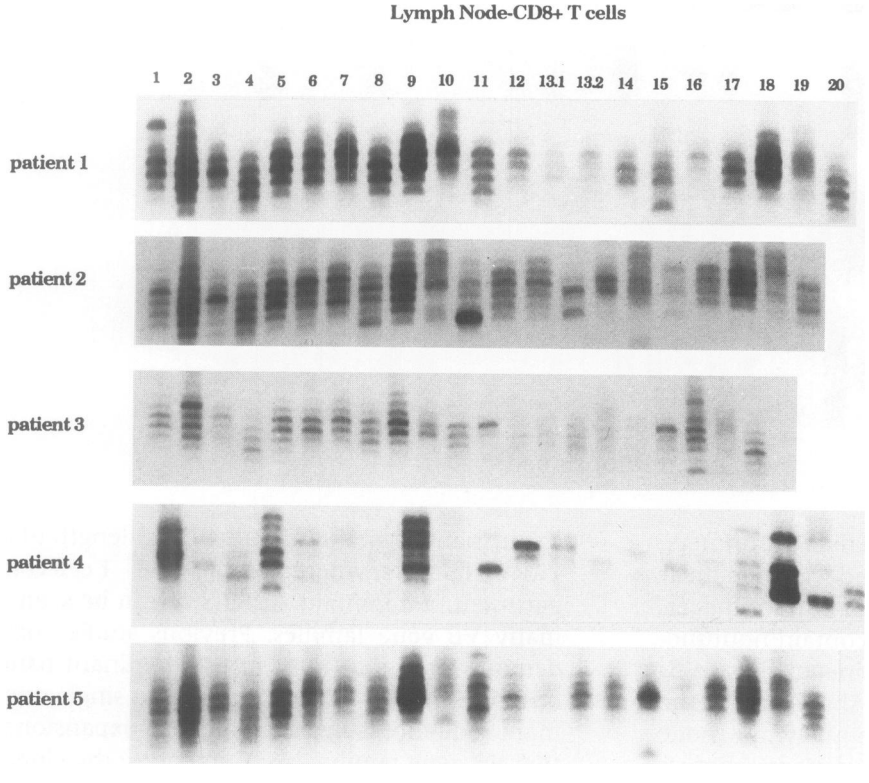

FIG. 3. CDR3 length analysis of CD8$^+$ T cells isolated from LNs of five non-breast cancer patients

The CDR3 length distributions of CD8$^+$ T cells, isolated from autopsy LN samples, were analyzed according to procedures described in Materials and Methods.

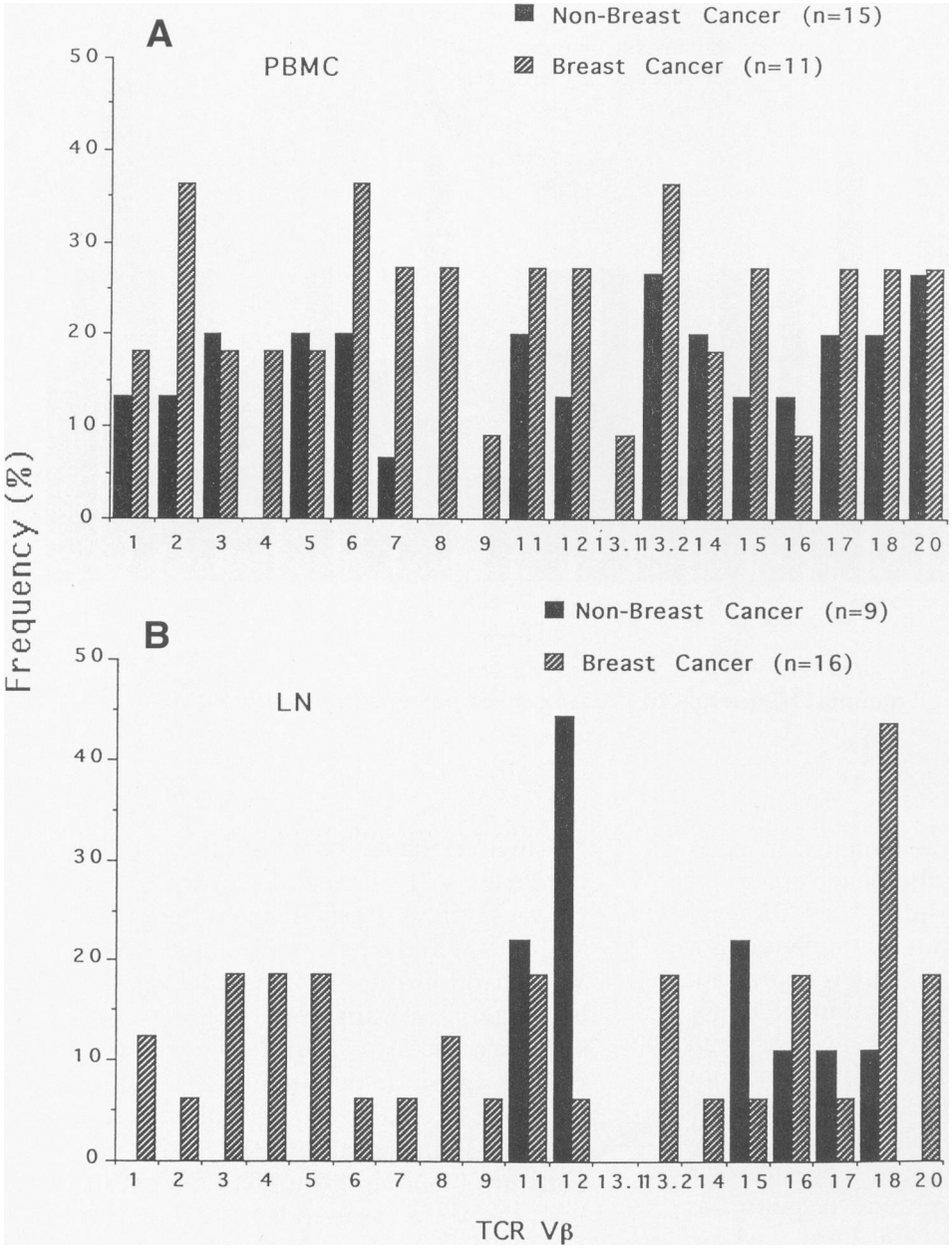

FIG. 4. Comparison of the oligoclonal frequency of $CD8^+$ T cells in breast cancer and non-breast cancer patients

Frequency of oligoclonality is determined by comparing the number of samples containing a dominant band in each Vβ gene family with the total number of samples analyzed. (A) PBMC: blood sample; (B) LN: lymph node sample.

breast cancer patients in our study is 59, we examined the oligoclonality in PBMC $CD8^+$ T cells of 15 age-matched healthy females (ages 40 to 80) and in LN $CD8^+$ T cells of 9 non-breast cancer patients (ages 60 to 80). Dominant CDR3 size distribution can also been seen in LN $CD8^+$ T cells of non-breast cancer patients (Fig. 3) as well as in the PMBC $CD8^+$ T cells of healthy individuals (data not shown). The average number of Vβ gene families expressing clonal expansion for non-breast cancer PBMCs and non-breast cancer LN are 2.7 and 1.2, respectively. Therefore, the number of Vβ gene families containing dominant CDR3 length is slightly increased in $CD8^+$ T cells of breast cancer patients.

The Presence of Dominant $CD8^+$ T Cell Clones in Breast Cancer Patients' LN

The distributions of the oligoclonal frequency of TCR Vβ gene families among the $CD8^+$ T cells in PBMCs of breast cancer and non-breast cancer patients are similar (Fig. 4A). However, the distributions in LNs are quite different in breast

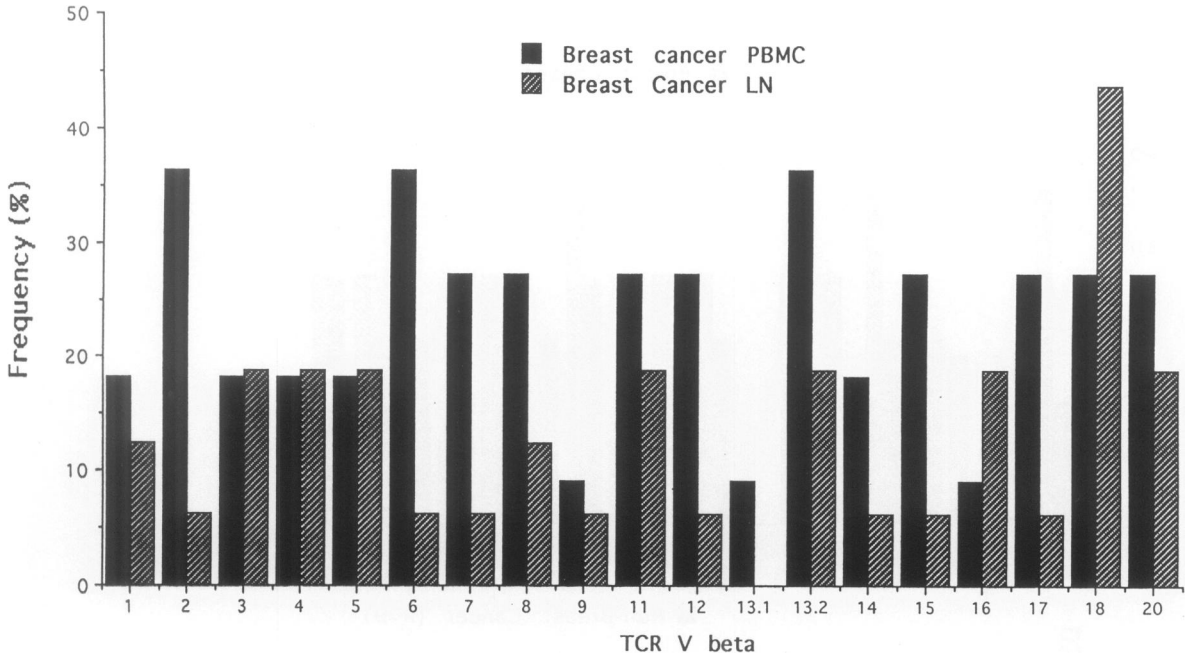

FIG. 5. Comparison of the oligoclonal frequency in breast cancer patients' LNs and PBMCs

cancer and non-breast cancer patients. In non-breast cancer patients' LNs, the oligoclonality of $CD8^+$ T cells is clustered in just a few Vβ gene families (Fig. 4B), and in 4 out of 9 individuals a dominant clone in the Vβ12 family is expressed. On the other hand, the distribution of oligoclonality in breast cancer patients' LNs is spread out in many more Vβ gene families, and in 7 out of 16 patients a dominant clone in the Vβ18 family is expressed. The prevalence of $CD8^+$ oligoclonality in Vβ18 suggests the existence of a breast cancer LN–specific immune response, as Vβ18 is the only TCR Vβ gene family whose frequency of clonality is increased more in LNs than in PBMCs of breast cancer patients (Fig. 5).

Sequence Analysis of Vβ18 $CD8^+$ T Cells of Breast Cancer Patients

To determine whether all dominant Vβ18 clones of breast cancer patients share a common sequence, DNA fragments were eluted out of the dominant bands and sequenced directly (Fig. 6). By using this method we obtained a single readable Vβ18 sequence from five Vβ18 bands of five LN samples (H.H., V.V., M.T., H.E., and J.W.), and two out of three dominant Vβ18 bands from three PBMC samples (M.T. and I.D.; see Table 3). No single readable sequence can be derived from dominant Vβ18 bands of patient P.V. and H.E. However, through sequencing the 10 aa–length Vβ18 band in the LN sample and the 9 aa–length Vβ18 band in the PBMC sample of patient P.V. by bacterial cloning, we obtained a dominant sequence that was found in over 50% of the clones. Again, no dominant Vβ18 sequence was found in LN $CD8^+$ T cells of patient H.E. In patient M.T., the same Vβ18 clone appeared in both her blood and the LN, whereas in patient P.V., the Vβ18 clone in her LN is quite different from that in her peripheral blood. All together, six Vβ18 sequences were obtained from seven LNs of breast cancer patients, three of which use Jβ1.1. To determine whether Jβ1.1 is preferentially used in combination with the Vβ18 gene, we sequenced the Vβ18 TCR from LN $CD8^+$ T cells of one non-breast cancer patient and two breast cancer patients who do not express dominant Vβ18 clones. Figure 7 shows that there is no obvious bias of Jβ1.1 usage by $CD8^+$ Vβ18 cells of patients without dominant Vβ18 clones. The CDR3 sequence of the dominant Vβ18 clone isolated from one non-breast cancer LN does not use Jβ1.1. Whether the increased Jβ1.1 usage by Vβ18 clones in breast cancer patients is specific requires further studies with additional samples.

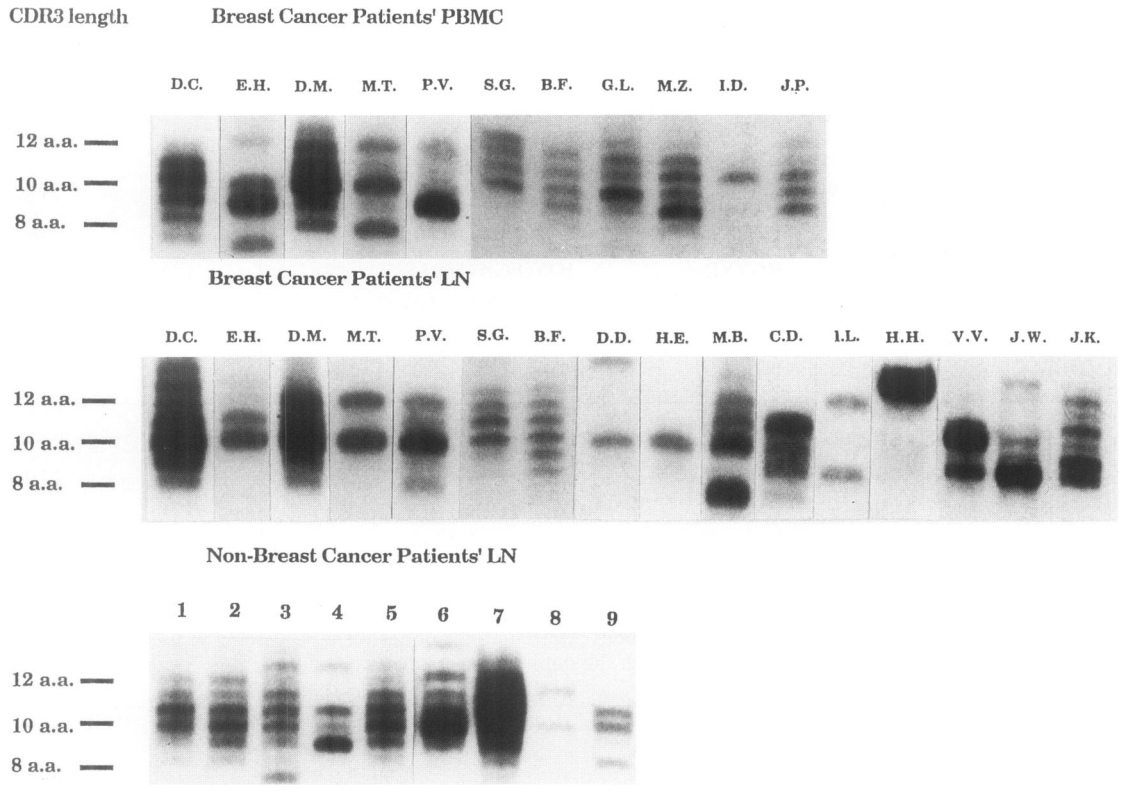

FIG. 6. CDR3 length distribution in Vβ18 $CD8^+$ T cells of breast cancer and non-breast cancer patients
$CD8^+$ T cells were isolated either from lymph nodes (LN) or peripheral blood mononuclear cells (PBMC).

Furthermore, examining CDR3-region sequences of all TCR Vβ18 clones of breast cancer patients did not reveal any identifiable motif.

It is possible that all breast cancer patients expressing a dominant Vβ18 clone share the same HLA type, or they have a similar type of cancer. We therefore correlated HLA typing of eight breast cancer patients with the pathological findings of their tumors. In 4 patients who have dominant Vβ18 clones in their LN T cells, different HLA gene products were expressed (Table 4). Furthermore, all patients possessing Vβ18 clones in their LNs had an invasive tumor, and three had tumor-infiltrating LNs.

DISCUSSION

Oligoclonality of $CD8^+$ T cells has been observed in PBMCs of healthy individuals, and its frequency is increased in the aged population, in patients with autoimmune diseases, and in tumor-infiltrating lymphocytes (34–37,44). Furthermore, viral infection or active immunization can induce the transient appearance of clonal expansion of $CD8^+$ T cells (46). Although clonal expansion of $CD8^+$ T cells has been documented by many investigators, the function of these clones has not been elucidated. Gregersen's group has shown that the clonally expanded $CD8^+$ cells are, in general, $CD57^+CD28^-$ T cells (32). CD57 was first identified on natural killer (NK) cells, but it is also present on subsets of T cells (47). The function of CD57 is not known. This subpopulation expressing activation markers and a shorter telomeric length may represent chronically activated cells (32,48). Our report is the first to analyze and compare the oligoclonality of $CD8^+$ T cells in the LNs and PBMCs of breast cancer patients and age-matched non-breast cancer controls. Our data show that the frequency of clonality is slightly higher in breast cancer patients, and there is an increased inci-

TABLE 3. TCR Vβ18 sequences of CD8$^+$ T cells of breast cancer patients

Patient (LN)	Vβ18	NDN	J	C	CDR3 length
H.H.	CASS	SRTSGGQG	DTQY FGPGTRLTVL (2.3)	ED	13 aa[a]
V.V.	CASS	PPGLSD	TEAF FGQGTRLTVV (1.1)	ED	11 aa[a]
M.T.	CASS	PSLGRD	EQY FGPGTRLTVT (2.7)	ED	10 aa[a]
P.V.	CASSP	PLGF	TEAF FGQGTRLTVV (1.1)	ED	10 aa[b]
H.E.	CASSP	RGYYD	IQY FGAGTRLSVL (2.4)	ED	10 aa[a]
J.W.	CASS	VGEG	TEAF FGQGTRLTVT (1.1)	ED	9 aa[a]
Patient (PBMC)	**Vβ18**	**NDN**	**J**	**C**	**CDR3 length**
I.D.	CASSP	WPSG	TQY FGPGTRLTVL (2.3)	ED	11 aa[a]
M.T.	CASS	PSLGRD	EQY FGPGTRLTVT (2.7)	ED	10 aa[a]
P.V.	CASSP	PAGT	EQF FGPGTRLTVL (2.1)	ED	9 aa[b]

[a]Sequence obtained by directly sequencing DNA fragments eluted from those domiant CDR3 bands on acrylamide gel.
[b]Sequence obtained by bacterial cloning method. cDNA fragment for each TCR Vβ gene family was synthesized and cloned according to the procedures described in Materials and Methods. More than 20 clones were picked and sequenced. The sequence obtained from more than 50% of all clones was designated the dominant sequence.

dence of oligoclonal expansion of Vβ18 CD8$^+$ T cells in breast cancer patients' LNs.

Comparing PBMC samples from breast cancer patients and controls indicates that the number of TCR Vβ families expressing a dominant clone in breast cancer patients is slightly higher than that in non-breast cancer females, but there is no obvious prevalence of oligoclonal expansion in any Vβ gene family. Although the distribution pattern of clonality among different TCR Vβ families is similar between the two groups, there is a profound difference in the frequency of the Vβ18 gene family. Three out of 11 breast cancer patients, but none of the 16 control subjects, showed a dominant CD8$^+$ T cell clone in the Vβ18 family. However, Monteiro et al. have previously analyzed 46 healthy controls with an average age of 32 (ages 14 to 57) and reported that the frequency of oligoclonality in Vβ18 was approximately 15% (44). Therefore, further analyses with additional age-matched female samples are required to determine whether the

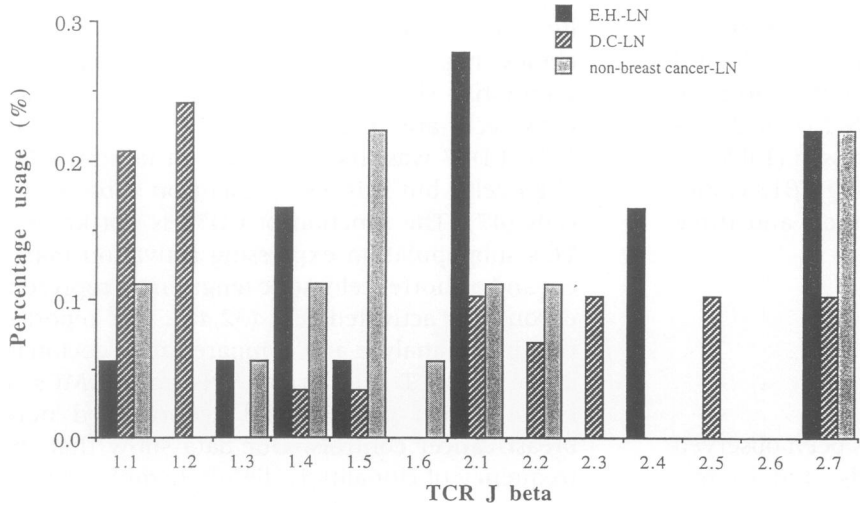

FIG. 7. Jβ usage by Vβ18 CD8$^+$ T cells isolated from LNs of one non-breast cancer patient and two breast cancer patients who do not have dominant Vβ18 clones

Sequence data were obtained from Table 3.

TABLE 4. TCR Vβ18 clonality

Patients		Positive nodes	PBMC	Jβ	CDR3 length (aa)	LN	Jβ	CDR3 length (aa)
S.G.	Noninvasive	0	−			−		
D.C.	Microinvasive	0	−			−		
E.H.	Microinvasive	0	−			−		
B.F.	Microinvasive	0	−			−		
G.L.	Microinvasive	0	−			ND		
D.M.	Invasive	0	−			−		
M.B.	Invasive	0	ND			−		
C.D.	Invasive	0	ND			−		
J.K.	Invasive	0	ND			−		
M.Z.	Invasive	0	−			ND		
J.P.	Invasive	22/22	−			ND		
M.T.	Invasive	0	+	2.7	10			
I.L.	Invasive	0	ND			−		
D.D.	Invasive	0	ND			−		
V.V.	Invasive	0	ND			+	1.1	11
J.W.	Invasive	0	ND			+	1.1	9
I.D.	Invasive	6/19	+	2.3	11	ND		
P.V.	Invasive	2/23	+	2.1	9	+	1.1	10
H.E.	Invasive	8/19	ND			+	2.4	10
H.H.	Invasive	23/39	ND			+	2.3	13

oligoclonal expansion in Vβ18 $CD8^+$ T cells in breast cancer patients' PBMCs is significant.

Analyzing the T cell oligoclonality in LNs of normal controls has generated two interesting observations. First, oligoclonality is clustered in a few Vβ families with a noticeable increase in the Vβ12 families. This biased distribution may be a result of a small sample pool ($n = 9$), and it may disappear upon increasing the sample size. However, it is possible that clonal expansion of Vβ12 $CD8^+$ T cells is a result of chronic stimulation by LN-specific antigen(s). Since oligoclonality in LNs of healthy controls has not been previously examined and no information is available, we need to analyze additional samples to determine whether restricted oligoclonality in $CD8^+$ T cells is an LN-specific phenomenon. Another interesting finding is that the CDR3 length distribution among Vβ gene families is quite different between the LN and PBMCs, even in the same patient (Fig. 2). This difference may be due to different homing properties among T cells, or to an active local immune response occurring at the LN which attracts a different subset of T cells. Furthermore, PBMCs have a higher frequency of oligoclonality than do LNs. It is possible that T cells in PBMC are coming from different tissues and lymphoid organs, and thus the T cell clonality displayed in PBMCs may represent a collective repertoire. However, studies on melanoma or glioma patients have shown that the oligoclonality of T cells at lesion sites is increased, compared with that in their PBMCs. TILs examined in these studies may have been obtained from late-stage tumors, and hence they may have been chronically stimulated by increasing numbers of tumor antigens, which may eventually lead to the generation of multiple clonal expansions. It is also plausible to speculate that breast cancer tumors possess fewer or a more restricted set of antigenic determinants than do other types of tumors. Nonetheless, to identify T cells important for or related to a specific local immune response, it is necessary to analyze T cells at the site of inflammation or at tumor infiltration sites.

In breast cancer LNs, the average number of TCR families displaying dominant $CD8^+$ clones is

slightly higher than that in non-breast cancer patients. Furthermore, there is an increased frequency of oligoclonality in the Vβ18 gene family whereas the frequency in the Vβ12 family is decreased, compared with that in non-breast cancer patients LNs. This phenomenon may reflect the existence of a new antigenic stimulation(s) in breast cancer patients' LNs that preferentially activates Vβ18 T cells. The other piece of evidence suggesting that Vβ18 T cells may play a role in breast cancer in a specific immune response is our finding that Vβ18 is the only Vβ gene family with an increased frequency of clonality in the LN compared with the PBMC of breast cancer patients.

Because T cells recognize peptides presented together with MHC molecules, the same peptide–MHC complex is likely to activate T cells expressing restricted TCR Vβ gene products. Depending on the antigens, some responses can also be polyclonal. In the case of breast cancer, 6 out of 7 patients who have clonal expansion of $CD8^+$ Vβ18 T cells in their PBMCs or LNs express different HLA haplotypes. A dominant Vβ usage by T cells among individuals expressing different MHC haplotypes is commonly seen in superantigen-mediated activation. However, superantigen stimulation generally leads to a polyclonal activation of T cells expressing a specific Vβ gene. Therefore, it is unlikely that the clonal expansion of Vβ18 T cells is a result of conventional superantigen stimulation. The prevalence of clonal expansion of Vβ18 T cells in breast cancer may resemble a previous study performed by Boitel et al., who demonstrated that the Tetanus Toxoid (TT)–specific $CD4^+$ T cells isolated from TT-immunized individuals preferentially express Vβ2 gene, regardless of the HLA type (49). It is possible that genomic-encoded Vβ18 gene sequences in the CDR3 region interact with a common structural determinant formed by the antigen and different HLA molecules. On the other hand, the CDR3-region sequence of all our Vβ18 T cell clones is quite heterogenous. This finding suggests that the junctional region may either interact with a polymorphic determinant on the HLA–peptide complex, or it may not play a major role in the recognition of HLA–peptide complex and therefore, may not be structurally conserved. Another possibility is that a common motif is formed by a combination sequence derived from CDR3 regions of both Vβ and Vα chains. Presently, we are analyzing the Vα gene of these dominant Vβ18 clones to determine whether there is a biased Vα usage.

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