Clonal Biases of Peripheral CD8 T Cell Repertoire Directly Reflect Local Inflammation in Polymyositis

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Clonal Biases of Peripheral CD8 T Cell Repertoire Directly Reflect Local Inflammation in Polymyositis

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Polymyositis (PM) is a chronic autoimmune disease that damages systemic striated muscles. Histopathology of the affected muscles is characterized by infiltration of inflammatory cells, interstitial fibrosis, and necrosis of the muscle fibers. It is commonly observed that CD8 T cells carrying αβ TCR heterodimers dominate at the sites of the muscle injury and that some of them surround and invade the nonnecrotic muscle fibers (1). The infiltrating CD8 T cells express activation markers such as CD45RO and ICAM-1, whereas the invaded myocytes overexpress HLA class I molecules on their cell surface (2–4). In addition, these CD8 T cells express cytotoxic effector molecules including perforin and granzyme B (5, 6). Furthermore, it has been found that the perforin molecules in the CD8 T cells are oriented toward the target muscle fibers (7). Thus, the tissue injury in PM appears to be mediated by infiltrating cytotoxic CD8 T cells.

Using biopsy specimens of the muscles, PCR analysis of TCR transcripts and immunohistochemical analysis with anti-TCRV mAbs were conducted to show that the T cells in the muscles are diverse but, compared with the peripheral lymphocytes, use a restricted set of V genes (8, 9). Subsequent nucleotide sequence analyses of the PCR-amplified TCR transcripts have shown that the infiltrating T cells contained clonally expanded populations. These results suggest that Ag-specific immune responses operate in the muscles affected by PM (10, 11). However, the pathological roles of the individual clones are obscure because clonally expanded T cells in the site of autoimmune inflammation could be either autoaggressive or regulatory (12).

Unavailability of large pieces of the PM-affected muscles for research studies has hampered identification and isolation of myocytotoxic T cell clones. Past studies on the T cells in PM have relied on the analysis of cDNA derived from the muscles en bloc. Inevitably, the two chains of the TCR heterodimers used by the expanded clones have never been identified, nor have the effector molecules expressed by these cells been characterized. The only exception is a variant type of PM, where CD4+CD8− T cells with TCR γδ heterodimers dominated at the sites of inflammation (13). Although molecular analysis has revealed nuclear acid sequences of both chains of the heterodimers, it has not led to determination of the responsible autoantigen (14). Thus, autoantigens targeted by the aberrant immune reaction in PM have yet to be identified.

It is known that CD8 T cells, unlike CD4 T cells, often expand clonally in the peripheral blood. This clonal expansion of CD8 T cells is commonly observed in healthy individuals, and is more frequent in the elderly than in the young (15–18). Phenotypically, the expanded clones are confined in a CD28+CD57− subset, which is known to be a group of cytotoxic effector T cells (17, 19–24). Indeed, the CD8 T cells with this phenotype are common in individuals with previous exposure to viruses, and have responded well to in vitro stimulation with the viral Ags to which the hosts had been exposed (21, 24, 25).

Recently, tetrameric MHC/peptide complexes were developed to quantitate Ag-specific T cells with flow cytometry (26). This technique revealed that peripheral CD8 T cells expanded massively during the acute phase of viral infection and that expansion persisted during the chronic phase (26–29). The expansion continued even after the viruses were cleared from the hosts (27). Similarly, clonal expansion of CD8 T cells specific to tumor Ags was found in peripheral blood from tumor-carrying patients (30). Thus, when CD8 T lymphocytes are stimulated by Ags, they tend to expand massively in the peripheral blood and to persist as large populations in an Ag-independent manner. In this regard, several reports have shown that memory CD8 T cells could survive or...
proliferate without specific MHC/Ag complexes that triggered them (31–33). Accordingly, it has been conceived that the clonal expansion of peripheral CD8 T cells represents footprints of the past and/or persistent viral infection and anti-tumor immunity.

As was reviewed earlier, PM seems to be mediated by cytotoxic CD8 T cells. The target striated muscles occur throughout the body. This made us assume that the autologous CD8 T cells damaging the muscles might be expanded clonally in the peripheral blood from the patients. In the present report, we show that the clonal expansion of CD8 T cells was indeed more frequent in patients with PM than in age-matched controls. The CD8 T cell expansion in the PM patients persisted over time like that of virus Ag-specific CD8 T cells. Analysis of the TCR transcripts in the muscle biopsy specimens showed that some of the expanded clones were present in the inflamed muscles. These results show that organ-specific autoimmunity directly biases repertoire of T cells in systemic circulation, and argue that the expanded cells are candidates of autologous T cells damaging the muscles. Using a flow cytometer, peripheral lymphocytes were readily isolated at a single-cell level. Subsequent sensitive RT-PCR techniques enabled us to show that these cells indeed express cytotoxic effector molecules. Thus, our findings should provide a direct clue to studying T cells involved in the immunopathology of PM and probably other CD8 T cell-mediated organ-specific autoimmune diseases.

Materials and Methods

**Samples from PM patients and normal donors**

PBMC were isolated from seven normal donors (N1–N7) and eight patients (P1–P8) (34). Age of the patients ranged from 35 to 64 years old (mean 46), and the controls were age-matched (mean 48). No patients had major complications except for patient P2, who had bronchitis obturating the lung cavity. The muscle biopsy samples, and were converted to cDNA. In- cluding from patients P1 and P2 during diagnostic biopsy. All procedures in the present study were approved by the ethics committee of the Tokyo Medical and Dental University. PBMC were also collected from five children who had already shown to have no detectable clonal expansion in their peripheral T cell pool (35). PBMC were stained with CD4 (SFC1214D11; Beckman Coulter, Hialeah, FL) and CD8 (SFC1218D3; Beckman Coulter) Abs, and 300,000–800,000 CD8− or CD8+positive cells were collected by an EPICS ELITE cell sorter (Beckman Coulter). To collect individual CD8 T cells carrying TCR Vβ2 or Vβ17, the cells were stained with the CD8 Ab together with anti-Vβ2 (MPB2D5; Beckman Coulter) or Vβ17 (E17.5F3.15.3; Beckman Coulter) Ab, and sorted by the autoclone function of the same cell sorter.

**Polymerase chain reaction**

Total RNA were prepared from the sorted single positive cells and serial cryosections of the muscle specimens, and were converted to cDNA. To collect individual CD8 T cells carrying TCR Vβ2 or Vβ17, the cells were stained with the CD8 Ab together with anti-Vβ2 (MPB2D5; Beckman Coulter) or Vβ17 (E17.5F3.15.3; Beckman Coulter) Ab, and sorted by the autoclone function of the same cell sorter.

**Polymerase chain reaction**

Total RNA were prepared from the sorted single positive cells and serial cryosections of the muscle specimens, and were converted to cDNA. Individual cells, which were sorted into microwells, were lysed in 8 μl of lysis buffer together with 50 JY cells (a gift from Dr. Miyuki Azuma, Tokyo Medical and Dental University) (36). First-strand cDNA were directly synthesized in the same wells (36).

The cDNA of the CD4 and CD8 cells were subjected to 20 cycles of primer-directed amplified TCR CDR3 (37). The products were amplified with nested primers to determine TCRBV gene repertoire (37). For TCR CDR3 length spectratyping (CLS) (3), the anchored PCR products were amplified using a panel of sense oligonucleotide primers specific to different BV subset genes, and a fluoresceinated BC-specific antisense primer (Cβ) (37). The amplification reaction consisted of 25 cycles of 1 min at 94°C, 1 min at 60°C, and 1 min at 72°C, with final extension at 72°C for 10 min.

TCR clonotype-specific PCR for T cells in the muscle tissues and sorted peripheral lymphocytes was performed with one of the sense primers specific to TCRBV CDR3 sequences of the clonally expanded T cells (P1/1s: GCC AGC AGC GTA GAC C, P1/2s: TCT GCA GTG CCG CCG, P1/6s: CAG CAG CTT GGC GCC GGG, P1/6ss: GTG CTA GCA GCC CAG ACC C, P1/16s: TGC CAG CAG CCA GCT GTA TG, P1/17s: GCC AGT AGT AGT ACG GGG AG, P1/24s: GCC ACC AGA GAT GGG G, P2/6S1As: TGT GTC CCC ATG GAG GCT, P2/6S1Bs: GCC AGC AGC TCC CTC CAG, P2/6S5s: CCA GCA AGG GCT TCG A, P2/125s: CAT CAG CAA AAT CGG ACA AGG, P2/15s: CAC CAG TGA TTA TGG GGG AGG, P2/16s: GCC AGC AGA GGG TTC G, P2/16ss: GTG CCA CTT CTA CGA CCT T A, and P2/65s: GCA GCC TCA TAG GGG GA AA) and a TCRBV-specific antisense primer (Cβ: CCT ATG GGG CTA CTC GTG). The reaction consisted of 40 cycles of 30 s at 94°C, and 90 s at 50°C or 59°C, followed by final extension at 72°C for 5 min.

**TCR clonotype-specific PCR for the individual cells was performed in two steps. Sense primers specific to BV subset genes used by the expanded T cell clones and BC-specific antisense primers were ampliﬁed in parallel (TG1-3, TGG TGG GAG ATC TCT GCT TC) were used in primary PCR, which consisted of 30 cycles of 45 s at 94°C, 45 s at 53°C, and 60 s at 72°C, followed by final extension at 72°C for 5 min. One-fifth of the products was amplified with the corresponding clonotype-specific primers described above and a nested BC-specific antisense primer (Cβ) (37). The reaction consisted of 30 cycles of 45 s at 94°C, 45 s at 59°C, and 60 s at 72°C, followed by final extension at 72°C for 5 min.

Perforin mRNA were PCR-amplified first with a perforin-specific sense primer (Ptos: TGG AGC TGA CTC GTA AGC CC) and an antisense primer (Ptos: GCC GTG GAT GCC TAT GTT GAC). The reaction consisted of 45 cycles of 45 s at 94°C, 45 s at 59°C, and 60 s at 72°C, followed by final extension at 72°C for 5 min. One part per 5000 of the products was subjected to the nested PCR using a sense primer (Ptos: TGG ATG CTG TGG CCG GC) and an antisense primer (Ptos: CAG TCA GGC AGT CCT CCA CC). The reaction consisted of 45 cycles of 45 s at 94°C, 45 s at 61°C, and 60 s at 72°C, followed by final extension at 72°C for 5 min.

**TCR CLS**

The fluoresceinated PCR products representing TCRBV cDNA of the individual TCRBV subsets were fractionated on denaturing 7% polyacrylamide gel in a Hitachi SQ-5500 sequencer (Hitachi Electronics Engineering, Tokyo, Japan). The data were analyzed with the associated software to display histograms. Relative percentage of the TCRBV transcripts of a given length to total TCRBV transcripts in the BV subsets was calculated by dividing the fluorescence intensity of the corresponding peaks by the sum of the intensity of all peaks.

**Standard profiles of TCRBV CLS**

Although TCR CLS profiles of T cell populations without clonal biases display roughly Gaussian-like distribution, we realized that the typical profiles of the individual TCRBV subsets had subtle differences (J. Nishio, M. Suzuki, N. Miyasaka, and H. Kohsaka, manuscript in preparation). This urged us to establish a standard profile for each BV subset by analyzing peripheral CD4 T cells from five children. Our previous studies showed that they had no detectable signs of clonal T cell expansion (35). The average distribution profiles derived from these donors displayed symmetrical patterns and were used as standard profiles.

It has been shown that expansion of a specific clone makes a peak of the corresponding CDR3 length stand out of the standard distribution. Arbitrarily, we set up criteria to identify such biased profiles; we considered TCRBV subsets to contain clonal expansion when the CLS profiles implied that a specific clone occupies >50% of the subsets. This is when the frequency of a dominant peak (%) is >50% plus half of a standard peak of the corresponding CDR3 length (%): d% > 50% + s/2%. This formula was derived from the fact that quantity of the dominant peak should be Q × s/100 + ΔQ, and also (Q + ΔQ) × d/100, where Q and ΔQ stand for total original population and increase owing to the expansion of a specific clone, respectively. When d and s fulfill the formula, the relative frequency of the expanded clone in the BV subset should be greater than 50%.
and BV12S2 subsets; BV13S1/4/6 and BV13S2/3/5 subsets). When estimating the frequencies of these subsets, the BV family gene usage measured with PCR-ELISA was divided in proportion to the fluorescence intensity of the PCR products generated with the corresponding primers.

**Nucleotide sequence determination of TCRB transcripts**

TCRB transcripts of BV subsets that displayed the biased profiles were PCR-amplified with a BV-specific sense primer and the C5c primer. The products were subcloned to generate BV subset-specific TCRB cDNA plasmid libraries. Ten to 20 plasmid clones of the TCRB CDR3 length of the dominant clonotypes were selected and subjected to sequence determination by the Hitachi SQ-5500 sequencer.

**Results**

**Frequency of clonal expansion of peripheral CD4 and CD8 T cells from PM patients and normal donors**

Peripheral CD4 and CD8 T cells from eight patients with active PM and seven age-matched normal donors were examined for clonal expansion with CLS of the TCRB transcripts. The TCR CLS technique has been applied, in many reports, to the analysis of T cell clonality (38). We sorted CD4 and CD8 T cell populations from the peripheral blood of patients and normal donors, and extracted their RNA, which were converted to cDNA. The cDNA were PCR-amplified with a fluoresceinated antisense TCRBC-specific primer and a panel of sense TCRBV-specific primers that were used previously for PCR-ELISA to analyze TCRBV repertoire (37). The products were fractionated on denaturing polyacrylamide gel. The gel was then analyzed by an automatic gel reader and associated software to generate TCR CLS profiles of individual BV subsets.

The results revealed that the spectratyping profiles of most BV subsets in CD4 T cells displayed Gaussian-like distribution. In contrast, the spectratyping profiles of the CD8 T cells often contained prominent peaks that bias Gaussian-like distribution. This is consistent with the previous observation that clonal expansion of CD8 T cells, but not that of CD4 T cells, is common in the peripheral blood. To assess the bias of each profile, we defined standard profiles for individual BV subsets by analyzing peripheral CD4 T lymphocytes from children who had no detectable clonal expansion in their peripheral blood (35). Based on these standard profiles, we set up criteria for “biased” profiles indicating that the TCRBV transcripts of an expanded T cell clone account for more than half of total TCR transcripts in the corresponding BV subset. The criteria were used to enumerate BV subsets that should contain clonally expanded T cells. Representative TCR CLS profiles of peripheral CD4 and CD8 T cells from a normal donor (N1) and those from a patient (P1) are shown in Fig. 1. The CD8 T cells from the patients had more biased profiles than those from the normal donors. Fig. 2 summarizes the number of BV subsets that showed the biased profiles. No more than one subset of CD4 T cells from the normal donors fulfilled the criteria (the mean number ± SD of the subsets: 0.1 ± 0.4). A similar number of the BV

![Figure 1](http://www.jimmunol.org/Downloaded_from_http://www.jimmunol.org by guest on May 11, 2017)
A subset in CD4 T cells from the patients had the biased profiles (0.7 ± 0.5). The difference was not statistically significant (Fig. 2A). In contrast, many profiles of CD8 T cells were biased. The numbers of the biased subsets in CD8 T cells from the normal donors and from the patients were 2.0 ± 1.4 and 6.4 ± 2.1 (mean ± SD), respectively. The difference was statistically significant (p < 0.001, Fig. 2B). Thus, clonal expansions of CD8 T cells were more frequent in peripheral blood from the patients than from the normal donors.

The CD8 TCRBV subsets of the patients that displayed the biased spectratype profiles are listed in Table I. No particular BV subsets contained clonally expanded populations.

Nucleotide sequence analysis of TCRB transcripts in BV subsets with biased profiles

The cDNA of CD8 T cells from patients P1, P2, P3, and P4 were used to generate BV-specific TCRB cDNA plasmid libraries. Sequence analyses of 10–20 clones of the same TCRB CDR3 length as the peaks revealed that 50–100% had an identical nucleotide sequence (Table II). An exception was the BV6S1 subset of P2, where two different TCR sequences of the same CDR3 length dominated with the ratio of 58:42%. Because no other clones appeared in the subsets, both should have expanded. These results demonstrated that the BV subsets with the biased profiles based on our criteria actually contained expanded T cell clones. They also showed the TCRB junctional sequences derived even from the same patients were diverse, and that no conserved CDR3 motifs were shared by the clones (Table II).

Persistence of the expanded CD8 T cell clones

To estimate the frequencies of the expanded clones in the total CD8 T lymphocyte pool, frequencies of the BV gene usage were multiplied by calculated frequencies of the particular clones in the BV subsets. The BV gene frequencies were quantitated by the PCR-ELISA technique (37). The deduced frequencies of the clones at the initial analyses ranged from 0.05 to 19%. To study further whether the expanded CD8 T cell clones persist for a long term, we examined the expanded clones from patient P3 6 and 12 mo after the initial examination. Also, the expanded clones from patient P5 6 mo later were examined. The myositis had been ameliorated by corticosteroid administration at the time of these follow-up analyses. We found that all clones that had been expanded at the initial analysis still occupied considerably large parts of the peripheral CD8 lymphocyte pool although they tended to shrink during the long interval (Fig. 3).

The expanded TCR clonotypes in the inflamed muscles

To investigate pathological relevance of the expanded T cell clones, the inflamed muscles from patients P1 and P2 were examined for presence of the expanded clones. First, serial cryostat sections were prepared from biopsy specimens and stained with H&E. The tissues from the two patients displayed marked infiltration of mononuclear cells, but contained no large vessels or extravascular erythrocytes suggesting hemorrhage (data not shown). Unstained sections were used for isolation of RNA, which were converted to cDNA. Based on the TCRB CDR3 nucleic acid sequences of the expanded clones, sense oligonucleotide primers specific to TCR CDR3 sequences of clones 1–7 from P1 and clones 1–8 from P2 were synthesized. One of these primers together with an antisense BC-specific primers (Cbd) were used for TCRB clonotype-specific PCR to detect the expanded clones. The same PCR was performed to amplify the TCRB clonotypes in peripheral blood CD8 T cells from patient P1 and in peripheral lymphocytes from patient P2. Quantities of the template cDNA were standardized with respect to the amount of the products generated by PCR amplification of the TCRBC gene.

After the PCR amplification, the products were fractionated with agarose gel electrophoresis (Fig. 4). Fifteen clonotype-specific PCR of peripheral lymphocyte cDNA generated 14 distinct bands of expected sizes. The amplification for clone 8 in the peripheral lymphocytes from patient P2 did not yield visible products. However, when the same PCR was performed as nested PCR after preamplifying the cDNA with BV23- and BC-specific primers, it generated significant amounts of the appropriate products (data not shown). The requirement of such a sensitive PCR technique to detect this clone is consistent with the fact that it was the least prevalent clone (~0.05% in the total CD8 pool).

When the muscle tissues were used for the sources of cDNA, the clonotype-specific PCR generated only three products from P1 and four products from P2. Of note, the PCR for clone 8 of P2 generated a visible band from the muscle cDNA. Also, the clonotype-specific PCR that generated intensive bands from peripheral blood cDNA did not necessarily do the same from cDNA of the muscles. Thus, the clonally expanded T cells in the inflamed muscles did not simply represent those in the peripheral blood, but appeared to be infiltrating preferentially in the muscles.

Perforin expression by the expanded clones

It has been reported that virus-specific CD8 T cells expanded clonally in the peripheral blood are functionally competent cytotoxic cells, expressing perforin (24, 39). To investigate whether the

Table I. The CD8 TCRBV subsets that displayed the biased TCR CLS profiles

<table>
<thead>
<tr>
<th>Patient</th>
<th>TCRBV Subset</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>BV1, 2, 6S1, 6S6, 16, 17, 24</td>
</tr>
<tr>
<td>P2</td>
<td>BV6S1, 6S5, 12S1, 15, 16, 18, 23</td>
</tr>
<tr>
<td>P3</td>
<td>BV6S2/4, 9</td>
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<td>P4</td>
<td>BV4, 5, 6S2/4, 6S5, 10, 14, 21, 23</td>
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<td>P5</td>
<td>BV1, 6S1, 10, 11, 13S2/3/5, 23</td>
</tr>
<tr>
<td>P6</td>
<td>BV1, 3, 8, 10, 12S2, 20, 21, 22</td>
</tr>
<tr>
<td>P7</td>
<td>BV2, 6S2/4, 6S3, 6S6, 11, 13S1/4/6, 17, 21</td>
</tr>
<tr>
<td>P8</td>
<td>BV1, 8, 11, 15, 16</td>
</tr>
</tbody>
</table>
clonally expanded T cells in the PM patients also express the effector molecules, clones 2 and 6 from P1 were examined for mRNA expression using single-cell RT-PCR. From peripheral lymphocytes of the patient, individual TCRVβ/H9252- or Vβ/H9252-positive CD8 T cells were sorted at a single-cell level. Each cell was lysed and used to synthesize cDNA. The expanded clones were identified by TCRBV-specific PCR followed by clonotype-specific PCR (Fig. 5A). PCR analysis of the same cells revealed that both express perforin messages (Fig. 5B). Thus, it is probable that the expanded cells in the patients, regardless of the localization in the muscle, were differentiated CTL.

Discussion
We have demonstrated for the first time that organ-specific autoimmune disease mediated by CD8 T cells skews peripheral CD8 T cell repertoire significantly. Considering the biological feature of the CD8 T lymphocyte expansion, the expanded cells should be candidates of autoaggressive cells responsible for the tissue injury in PM.

Unlike previous studies, our approach has given us a direct clue to isolating the myocytotoxic T clones from the peripheral blood. The results, derived from isolation of individual candidate T clones from the peripheral blood and subsequent analyses of gene expression, implied that they are functional cytotoxic cells. Using the nested PCR technique, we further succeeded in determining expressed TCRA gene (data not shown). Partly because of limited availability of the large muscle pieces, it has been difficult to study

Table II. TCRB CDR3 amino acid sequences of the expanded CD8 T cell clones from PM patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clone</th>
<th>BV</th>
<th>N-D-N</th>
<th>BJ</th>
<th>Frequency (%)</th>
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<tr>
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<td>VEDL</td>
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<tr>
<td></td>
<td>2</td>
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<td>ICSA</td>
<td>PPPPD</td>
<td>BJ2S7</td>
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<td></td>
<td>3</td>
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<td>LCASS</td>
<td>LAGG</td>
<td>BJ2S3</td>
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<td>RCASS</td>
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<td>BJ2S3</td>
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<td>BV23</td>
<td>FCASS</td>
<td>PEGVH</td>
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</tbody>
</table>

a The sequence analysis allowed us to assign each clone specific BV and BJ genes. The deduced amino acid sequences of C terminals of BV, N-D-N regions, and N terminals of BJ are shown in a single letter format.

b Frequency of the clones with identical TCRB sequences in the total clones sequenced.

c The BV6S1 subset of patient P2 had two dominant TCR sequences.

FIGURE 3. Frequencies of the expanded clones at the initial and follow-up analyses. Frequencies of the expanded clones from patient P3 (A) were reanalyzed 6 and 12 mo after the initial analysis, whereas those from patient P5 (B) were re-examined 6 mo after the initial analysis.

FIGURE 4. Clonotype-specific PCR of TCRB cDNA in peripheral blood and muscle tissues. First-strand cDNA derived from the muscle biopsy samples and PBL from patients P1 and P2 were PCR-amplified with a TCRBC-specific primer and one of oligonucleotide primers specific to TCRB CDR3 sequences of the expanded clones. The products were fractionated with agarose gel electrophoresis. The PCR products amplified with BC specific primers (Cβc) were used to standardize the amount of the template cDNA. The numbers of the lanes correspond to those of the expanded clones (Table II).
Expanded clones 2 and 6 were identified from P1 were sorted at a single-cell level and used for cDNA synthesis. A, Regardless of their presence in the peripheral lymphocytes from a few patients with dermatomyositis, we found that approximately half of the expanded clones accounted for systemic expansion of the CD8 T cells specific for target local Ags. The nature of clonal expansion of peripheral CD8 T cells in the periphery has been delineated mainly by investigation of viral Ag-specific CD8 T cells. These studies used MHC/peptide tetramers to quantitate Ag-specific T cells, whereas we used a new method that combined the TCR CLS and PCR-ELISA. The size of the clonally expanded CD8 T cells in PM patients was comparable to that of the virus-specific T cell clones. Our method is useful when Ags recognized by clones of interest are unknown.

Previous studies of the peripheral lymphocytes from PM patients with a PCR assay showed that they were clonally diverse (9–11). This is consistent with our observation that TCR transcripts of all BV subsets were successfully amplified by PCR. Subsequent TCR CLS was required to reveal clonal expansions. This technique turned out to be powerful to detect clonal expansions, especially when used with our criteria. However, it has potential drawbacks. First, it cannot detect two or more clonal expansions of different TCR length within one BV subset because they individually cannot occupy >50%. Second, two clones of the same TCR length will be counted as one. An example of this was in clones 1 and 2 from patient P2 (Table II). These events seem rare and should be equally common among patients and controls. Thus, this technique may have made us underestimate the number of the expanded clones marginally, but should have warranted fair comparison.

The nature of clonal expansion of CD8 T cells in the pathology of rheumatoid arthritis (RA) is frequent (18, 44–46). This is intriguing because CD4 T cells appear to be primarily involved in the pathology of RA. This view is based on the facts that the CD4 T cells infiltrate dominantly in the rheumatoid synovial tissues and that the most dominant genetic factor of the disease is the HLA class II gene, the products of which interact directly with CD4 T cells. Unlike in PM, the expanded CD8 T cell clones in RA do not appear to exert cytotoxic effects on the target organs, i.e., the synovial tissues. Alternatively, the CD8 T cell expansion in RA patients may reflect impaired homeostasis of peripheral T cell diversity and/or curious involvement of CD8 T cells in rheumatoid synovial inflammation (47, 48).

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kinetics in size of the expanded clones did not offer critical information on their function.

The comparative RT-PCR analysis of the peripheral lymphocytes and the muscle biopsy samples from the same patients revealed that some clones were present in the infiltrated muscle tissues. This held true whether the analyzed peripheral lymphocytes were sorted or unsorted for CD8 T cells. Although the analysis was at, most semiquantitative, the results argue that CD8 T cells with specific TCRB clonotypes preferentially infiltrate in the muscles. It is somewhat expected that these T cell clones had diverse TCRB chain structures. We and others have found that the TCR structure of T cells reactive to the identical MHC/peptide combination could be diverse (53, 54). Also, more than one epitope could be involved within a single patient.

We believe that functional analysis of the clonally expanded cells is a key to identifying pathogenic CD8 T cells in PM. Isolation of the candidate myocytotoxic T cells from the peripheral blood of PM patients and establishment of immortalized clones for precise functional analysis are now under way.

Acknowledgments

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