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Research Article

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Analysis of T Cell Receptor Repertoire of Muscle-infiltrating T Lymphocytes in Polymyositis

Restricted Vα/β Rearrangements May Indicate Antigen-driven Selection

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Abstract

Polymyositis is an inflammatory myopathy characterized by mononuclear cell infiltration of muscle tissue. Myocytotoxic T lymphocytes have been recognized in the infiltrates, but the muscle antigen, target of the immune attack, has not been identified. Molecular characterization of the variable regions of T cell receptors (TCRs) on the infiltrating lymphocytes can be expected to provide insights into the pathogenic process. The Vα/β TCR repertoire was investigated by RNA-PCR in muscle biopsies from 15 polymyositis patients and 16 controls (6 Duchenne muscular dystrophy and 10 with no inflammatory or dystrophic myopathy). A variety of rearranged variable TCR genes was found in polymyositis, Vα1, Vα5, Vβ1, and Vβ15 being the most common (present in 60–100% of patients). In Duchenne muscular dystrophy patients TCR Vα or β rearrangements were found although no restriction was observed; no rearrangements were found in muscles from the other controls. Sequence analysis revealed the presence of the Jβ2.1 region in 90% of the Vβ15 clones studied, no random N additions in the diversity region, and a common motif within the CDR3 region. These results suggest that selection of muscle-infiltrating T lymphocytes is antigen driven in polymyositis. (*J. Clin. Invest.* 91:2880–2886.) Key words: polymyositis • Duchenne muscular dystrophy • T cell receptor • T lymphocyte • polymerase chain reaction

Introduction

Polymyositis (PM)[1] is an inflammatory myopathy in which intense mononuclear cellular infiltration leads to the degeneration of the muscular fibers (1–3). The cellular infiltrate has been extensively characterized by immunocytochemistry and mainly consists of T lymphocytes, macrophages, and, to a lesser extent, B cells and natural killer cells (4, 5). The T cell component is predominantly $CD8^+$ lymphocytes with cytotoxic activity (6–8). Increased expression of MHC class I and II in muscle (9–11) and of lymphokines (IL-1α, IL-2, IFN-γ) in serum (12, 13) have also been reported. These findings point to immune activation towards an as yet unknown muscle antigen. The vast majority of peripheral blood $CD4^+$ and $CD8^+$ T lymphocytes express α/β T cell receptors (TCR), but the TCR repertoire of PM-specific T cell infiltrate is not known. Recently, a subset of γ/δ T lymphocytes was demonstrated to be infiltrating the muscle of one PM patient (14) and the molecular characteristics of their TCR described (15). We report here on the characterization of the cellular infiltrates from muscles of 15 PM patients, including α/β TCR usage at the site of muscle fiber degeneration, since this information would provide indications on T cell clonality in the site of lesion.

Methods

Muscle samples obtained by needle biopsy from 15 PM patients (referred to as 1–15), were frozen and stored in liquid nitrogen. PM diagnosis was on the basis of clinical signs of weakness, elevated serum creatine kinase, and myopathic changes in the electromyogram (16). Dermatomyositis was ruled out by lack of skin rash. No symptoms or signs of associated neoplasm, collagen, or vascular diseases were present. For control purposes, muscle samples from 10 subjects without inflammatory myopathy or muscular dystrophy, and from 6 Duchenne muscular dystrophy (DMD) patients were also analyzed. Portions of the same muscle biopsy were used for both immunocytochemical and molecular analyses.

Immunocytochemistry. Cellular infiltrates were characterized by immunocytochemistry on acetone-fixed 4–6-μm thick cryosections of muscle biopsies. The following Abs were used: anti-CD3 (Dako, Copenhagen, Denmark), anti-CD4, anti-CD8, anti-CD19, anti-CD14 (Becton Dickinson, Mountain View, CA), and anti-α/β TCR (Identi T® βF1, β-chain specific; T Cell Sciences, Inc., Cambridge, MA). All Abs were mouse mAbs, except anti-CD3 which was an affinity-purified rabbit polyclonal Ab. The mouse mAbs were detected using rhodamine-conjugated goat anti-mouse IgG; the rabbit Ab was detected using biotinylated goat anti-rabbit IgG and by fluorescein-isothiocyanate-labeled streptavidin. From sections stained with peroxidase-conjugated secondary Abs, mAb-positive cell frequencies were counted and statistical analysis was performed as described elsewhere (5).

TCR repertoire analysis. Analysis of the TCR repertoire used the RNA-PCR procedure (17). The common reverse primers specific for the α and β C regions, and family-specific V region forward primers (Vα 1–18, and Vβ 1–20) were used (18). Total RNA was extracted by

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1. *Abbreviations used in this paper:* DMD, Duchenne muscular dystrophy; EB, ethidium bromide; PM, polymyositis; TCR, T cell receptor.

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the single step guanidinium-thiocyanate-phenol-chloroform method (19) on 10–60 mg of frozen muscle. cDNA for PCR amplification was prepared using reverse transcriptase according to standard protocol (17). The resulting cDNA mixture was used directly in PCR amplification with the addition of 50 pmol of forward and reverse primers, 1× PCR buffer and 1 U *Taq* polymerase (Perkin-Elmer Corp., Norwalk, CT). 30 cycles of the following profile were performed on automated DNA thermocycler: 95°C denaturation for 1 min, 55°C annealing for 1 min, 72°C extension for 1 min. Aliquots of the amplified products were electrophoresed on 4% NuSieve-Agarose (FMC Corp., Rockland, ME) (3:1, wt/wt) gels stained with ethidium bromide (EB). cDNA from a mixed lymphocyte reaction of healthy individuals was used to amplify all Vα and Vβ families and served as a positive control.

"Mini" Southern blot and internal probing. Amplified products were electrophoresed on a mini-gel apparatus (Bio-Rad Laboratories, Richmond, CA) in 2% NuSieve-Agarose (FMC Corp.) (3:1, wt/wt) gel stained with EB. Gels were soaked in a denaturing solution (0.5 M NaOH, 1.5 M NaCl) for 30 min, then in a neutralizing solution (0.5 M Tris-HCl, 1.5 M NaCl, pH 7.0) for 30 min, with shaking at room temperature. The gels were blotted onto Genescreen Plus nylon filters (DuPont Instruments, Wilmington, DE) for 2 h. The DNA was covalently bound to the nylon membranes by ultraviolet cross-linking (550 μJ) using a Stratalinker apparatus (Stratagene Inc., La Jolla, CA). Membranes were boiled in 1% SDS for 5 min, briefly dried, and prehybridized in 5× sodium chloride-sodium phosphate-ethylenediaminetetraacetate (SSPE)/5× Denhardt's solution/100 μg/ml salmon sperm DNA/0.1% SDS at 42°C for 10 min. C region internal horseradish peroxidase (HRP)-labeled probe (Cα 5′ HRP-CAGAACCCTGACCCTGCCGTGTAC-3′ and Cβ 5′ HRP-AGCGACCTCGGTTGGGAACAC-3′) was added and hybridized for 1 h at 42°C. After hybridization, filters were washed once with 1× SSPE + 1% SDS for 15 min, then in 0.1× SSPE + 1% SDS for 5 min, and lastly in PBS for 10 min, at room temperature. The amplified TCR products were detected with the enhanced chemiluminescence gene detection kit (Amersham International, Amersham, UK), which uses chemiluminescence to reveal hybridized products; developing procedures were performed according to manufacturer's instructions, and HYPERfilm-enhanced chemiluminescence detection films (Amersham International, Amersham, UK) were developed after 2–10 min.

Unique restriction site analysis. Vα1, Vα5, Vβ1, and Vβ15 were also checked for the appropriate restriction pattern by unique site restriction analysis. The PCR products were extracted from minigels and purified using Geneclean II kit (BIO 101, Inc., Vista, CA). They were used as templates for PCR reamplification in which forward and reverse primers were contained within the V sequence region of the corresponding α or β TCR families (see Fig. 4 legend for primers and PCR profile). Aliquots of the reamplified products were digested to reveal the presence of unique restriction sites. The presence of expected size bands was observed on 4–6% of EB-stained agarose gels (see Fig. 4 for restriction endonucleases used and size of bands in each TCR PCR product).

TCR Vα and Vβ assignment in PM patients. Specific Vα and Vβ TCR rearrangements in any individual were considered detected only when the band observed in the EB gel was confirmed on the mini-blot.

Sequence analysis. Vα1, Vα5, Vβ1, and Vβ15 amplification products from the mixed lymphocyte reaction were blunt-end cloned into the SmaI site of pGEM 7ZF(−) (Promega Corp., Madison, WI) and transformed into JM 109 *Escherichia coli* strain (Promega Corp.); a total of eight randomly chosen colonies were sequenced by the dideoxy chain-termination method (20), using the Deaza G/A ^{T7}Sequencing kit (Pharmacia, Uppsala, Sweden). cDNA of two PM patients (1 and 5) was amplified by PCR using Vβ 15 and Cβ primers containing EcoRI and HindIII restriction sites, respectively. The PCR product was purified from 0.6% low melting agarose gel by phenol-chloroform extraction and digested sequentially with EcoRI and HindIII (Boehringer Mannheim GmbH, Mannheim, Germany). After purification by Centricon 100 (Amicon, Beverly, MA), the product was cloned into EcoRI-HindIII digested pGEM 7ZF(−) and transformed into JM 109 *E. coli* strain. Plasmid DNA samples were prepared from white colonies and analyzed by digestion with DraII, which cleaves a unique restriction site contained in the Cβ region but not present in the plasmid. Positive colonies were sequenced by the dideoxy chain-termination method with the Deaza G/A ^{T7}Sequencing kit. Nucleotide sequences were analyzed using Gene Works 2.2 software (IntelliGenetics, Mountain View, CA). The amino acid sequences were aligned to the sequence of the clone ATL 21, known to be Vβ15 (21).

Results

The cell infiltrates of PM patients were first characterized immunocytochemically: T lymphocytes ($CD3^+$ cells), macrophages ($CD14^+$ cells) were the major constituents while rare B lymphocytes ($CD19^+$ cells) were found. Fig. 1 exemplifies the immunocytochemical findings from a PM patient: panels *a, c,* and *e* display anti-CD3 (pan T) staining, panels *b, d,* and *f* anti-α/β TCR, anti-CD8 (cytotoxic) and anti-CD4 (helper) staining, respectively. All the $CD3^+$ T lymphocytes observed in PM patients' muscles expressed α/β TCRs on their surface. The relative proportions of CD antigen positive cells in the endomysium, perimysium, and perivascular sites were also determined (Table I). The proportion of $CD8^+$ lymphocytes was significantly greater at endomysial (72%) than perimysial (46%) and perivascular (43%) sites. $CD4^+$ lymphocytes were correspondingly reduced in endomysium (28%), while perimysially and perivascularly they constituted 55 and 58% of the cellular infiltrate, respectively. Furthermore, B lymphocytes were reduced in endomysium (1%) compared to perivascular sites (5%). No significant differences in the proportions of CD3- or CD14-positive cells were observed at different muscle tissue sites.

Immunocytochemical analysis of DMD muscles revealed the presence of mononuclear cell infiltrates, but the CD subsets investigated were distributed relatively uniformly throughout the muscle tissue (data not shown). Cellular infiltrates were not observed in any of the control samples (data not shown).

To characterize the T cells associated with the site of muscle damage in PM, RNA-PCR was used to analyze rearranged genes in the TCRs from PM patients' muscles. The TCR family-specific primers were tested on cDNA from a mixed lymphocyte reaction for control purpose: all Vα and Vβ families were specifically amplified (Fig. 2). Transcripts of the following rearranged α and β TCR genes were obtained from PM patient muscles: Vα1, 2, 3, 5, 7, 12, 13, 14, 16, 17, and Vβ1, 3–8, 11–15, 17, and 20 (Table II). Four TCR V gene rearrangements occurred with high frequency in the muscles of PM patients: Vα1, 11/15 (73.3%); Vα5, 9/15 (60%); Vβ1, 13/15 (86.6%); Vβ15, 15/15 (100%). None of these TCR families were expressed in the controls where only Vα 12 and 14 rearrangements were amplified (see Table II). In DMD muscle biopsies the following TCR families were rearranged: Vα 1–3, 5, 6, 11–13, 16, 17; Vβ 1–10, 12–16, 18, 19, 20.

The Vα-Cα, and Vβ-Cβ amplifications, specific for the various TCR V gene families, migrated on the gels to the predicted molecular weight positions. Fig. 3 shows an example of PM patients PCR amplification and mini Southern blot: Vα1 is at ~ 470 bp, Vα5 at ~ 450 bp, Vβ1 at ~ 230 bp, and Vβ15 at ~ 210 bp. The TCR internal probes correctly recognized TCR but not actin.

The Vα1, Vα5, Vβ1, and Vβ15 PCR products were subsequently reamplified with the corresponding forward primer and an α or β family-specific reverse primer on the 3′ end of the

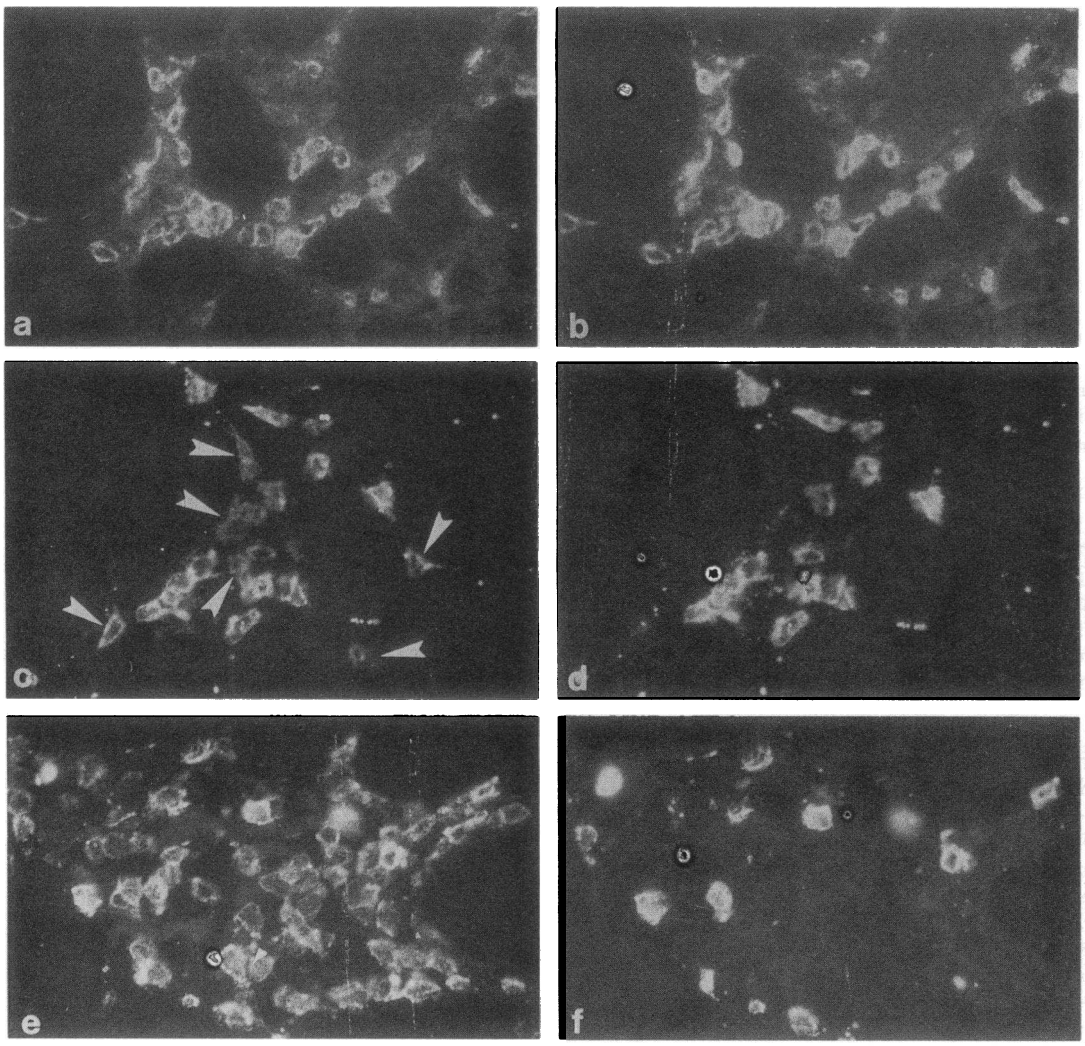

Figure 1. Immunocytochemistry of PM patient muscle. In *a*, *c*, and *e* the CD3 staining is shown and paired to α/β TCR (*b*), CD8 (*d*), and CD4 (*f*) respectively. Arrowheads indicate cells staining with anti-CD3 monoclonal antibody and negative for anti-CD8. All the cells detected by anti-CD3 were also detected by the anti-TCR-β chain antibody, whereas only a few were positive for anti-CD4.

TCR V region. Fig. 4 illustrates samples of these reamplifications from different patients. Unique site restriction analysis demonstrated that the PCR products were as expected: V-Vα1 digested with MboI shows the expected bands at 114 and 180 bp; V-Vα5 digested with MboI shows the expected bands at 88 and 147 bp; V-Vβ1 digested with HinfI shows the expected bands at 27 and 57 bp; V-Vβ15 digested with MaeI shows the expected bands at 39 and 50 bp.

Table I. Analysis of Cellular Infiltrates in Muscle of Polymyositis

Marker	Perimysial cells	Endomysial cells	Perivascular cells
		mean±SD	
CD3	46.7±19.4	57.6±13.3	63.7±9.9
CD8	46.1±11.8	72.0±5.0*	43.1±11.7
CD4	54.7±11.3	28.2±5.5‡	57.5±11.8
CD14	47.1±17.7	41.4±12.6	30.1±8.4
CD19	6.2±6.4	1.1±1.3§	5.5±3.6

Distribution of mononuclear cells in different muscle compartments. All values are expressed as percentage of CD-positive cells, according to reference 5, with minor modifications. Statistical evaluations were performed by a two-tailed *t* test.

* Endomysial vs perimysial $P = 0.0001$, endomysial vs perivascular $P = 0.0001$;

‡ Endomysial vs perimysial $P = 0.0001$, endomysial vs perivascular $P = 0.0001$;

§ Endomysial vs perivascular $P = 0.003$.

Vβ15 was observed in all the PM patients examined, it was therefore chosen for sequencing. The Vβ15 PCR products, amplified from cDNA with primers containing ad hoc restriction sites, were subcloned. Eight colonies from patient 1 and two from patient 5 were sequenced and compared at both the DNA and aminoacid level (Table III): amino acid substitutions were minor and the clones were highly homologous. The sequences of the variable regions overlapped to the Vβ15$^+$ ATL 21 clone. Moreover, 9 of the 10 clones (90%) had the same Jβ2.1 region. Within the CDR3 portion of Jβ2.1 (see Table III) in the TCR β chain, a consensus motif S-EQF was recognized. In the N-D-N region (first half of the CDR3 region) 4 D (Asp) or 2 E (Glu) were found as first amino acid, followed by 5 L (Leu). These data define the sequence D(or E)L-S-EQF, which is a common motif of the CDR3 region of our Vβ15$^+$ clones.

Discussion

Lymphocyte infiltration of muscle is characteristic of PM and may be intense (1, 4–8); furthermore, activated T cells, mainly of the cytotoxic (CD8$^+$) phenotype (6–8), have been demon-

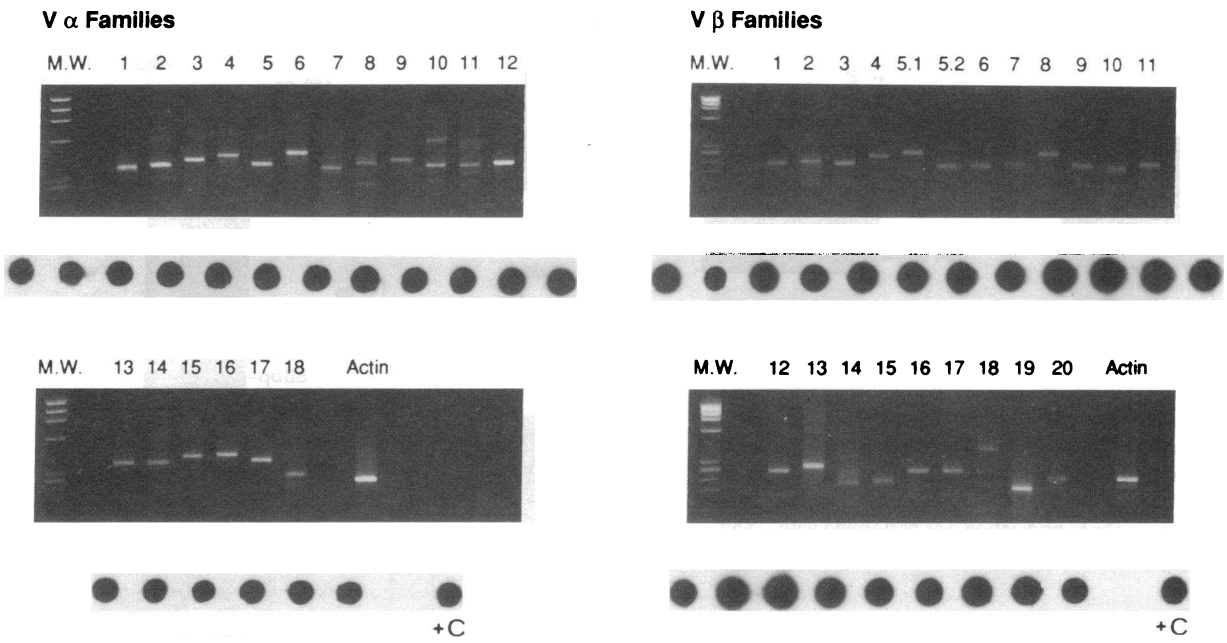

Figure 2. Positive controls for Vα and Vβ TCR families amplifications. The EB-stained 4% agarose gels and the corresponding dot-blots of the PCR products are shown. Dot-blot hybridization was performed with the internal probes and the methods described in the text. The positive control ($^+$C) for TCR α chain is the full length cDNA pGA5, shown to be Vα12; for the β chain we used cDNA from a cytotoxic antimelanoma clone known to be Vβ8 (a gift from Dr. M. L. Sensi, National Cancer Institute, Milan). β-actin is a 234-bp product amplified with the following primers: F 5′-GTGGGCCGCTCTAGGCACCA-3′, R 5′-CGGTTGGCCTTAGGGTTCAGGGGGG-3′.

strated in the muscles of PM patients, and it may be supposed that these cells are intimately involved in PM pathogenesis, either directly by means of perforin/granzyme A (22) or through release of cytotoxic lymphokines (23). The antigen target of autoimmune attack in PM has not been identified. Viruses have suspected to be involved in the immune-mediated mechanisms of PM; however recent findings have shown absence of candidates viral genomes in muscle of patients affected by inflammatory myopathies (24, 25).

Our study has shown the presence of CD3$^+$ (pan T), CD8$^+$ (cytotoxic T cells), CD4$^+$ (helper T cells), and CD14$^+$ (macrophages) cells in the cellular infiltrate of PM muscles. CD19$^+$ (B cell) lymphocytes were also present, but in smaller numbers. These results are similar to those of other authors (4–8). Additionally, we found that the distribution of lymphocyte subsets within the muscle compartments was not homogeneous: the proportion of CD8$^+$ cells in endomysium was significantly greater (P = 0.0001) than in the perimysium or surrounding the perivascular sites; while both CD4$^+$ lymphocytes (P = 0.0001) and CD19$^+$ (P = 0.003) were reduced in the same district (Table I). The differential CD4$^+$ and CD8$^+$ T cell distributions are particularly relevant in view of the uniform distribution of CD3$^+$ lymphocytes in these patients' muscles, and show that cytotoxic T cells are present preferentially in the endomysium of PM muscles.

By staining with anti-CD3 and anti-TCR β chain mAbs on serial sections, we demonstrated that the T lymphocytes in our PM patients' muscles express the α/β TCR. Recently, γ/δ TCR$^+$ T cells were identified in a patient with a unique form of PM and their TCR molecular characteristics were described as well as the putative antigen discussed (14, 15). To our knowledge, however, the molecular characteristics of the α/β TCRs expressed by the muscle infiltrating T cells in PM have not been characterized previously. We studied the repertoire of rearranged TCR V genes in the T cells infiltrating muscles by the RNA-PCR, which allowed us to detect even of poorly repre-

Table II. TCR Vα and β Families Expressed in Polymyositis, DMD, and Control Muscles

Vα Family	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18			
PM patients	11	1	1	—	9	—	1	—	—	—	—	4	3	5	—	2	2	—			
Controls*	—	—	—	—	—	—	—	—	—	—	—	2	—	2	—	—	—	—			
DMD patients	2	1	1	—	2	—	1	—	—	—	1	2	1	—	—	1	1	—			
Vβ Family	**1**	**2**	**3**	**4**	**5.1**	**5.2**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**
PM patients	13	—	1	1	1	1	1	2	2	—	—	1	1	1	2	15	—	1	—	—	2
Controls*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
DMD patients	1	4	3	3	3	3	4	4	4	3	—	2	1	3	3	2	—	1	—	1	1

* Controls: muscles from patients affected by muscular diseases other than inflammatory myopathy and dystrophies.

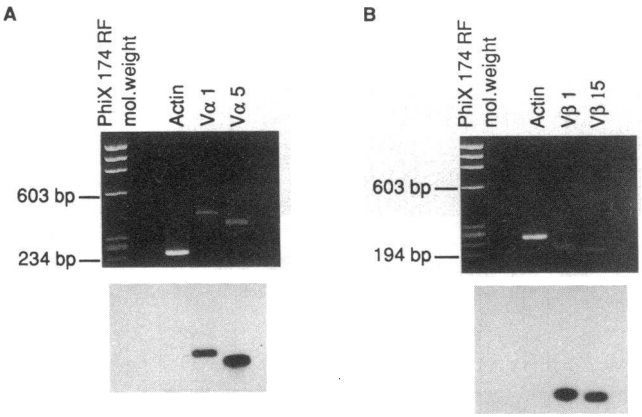

Figure 3. TCR amplifications and Southern blots of PM patients. Vα1 and Vα5 are shown in *A*, Vβ1 and Vβ15 in *B*. The upper panel is an EB-stained 2% NuSieve/Agarose (FMC Corp.) gel used for Southern analysis (*lower panel*); the hybridization was performed with α and β TCR internal probes and revealed by chemiluminescence.

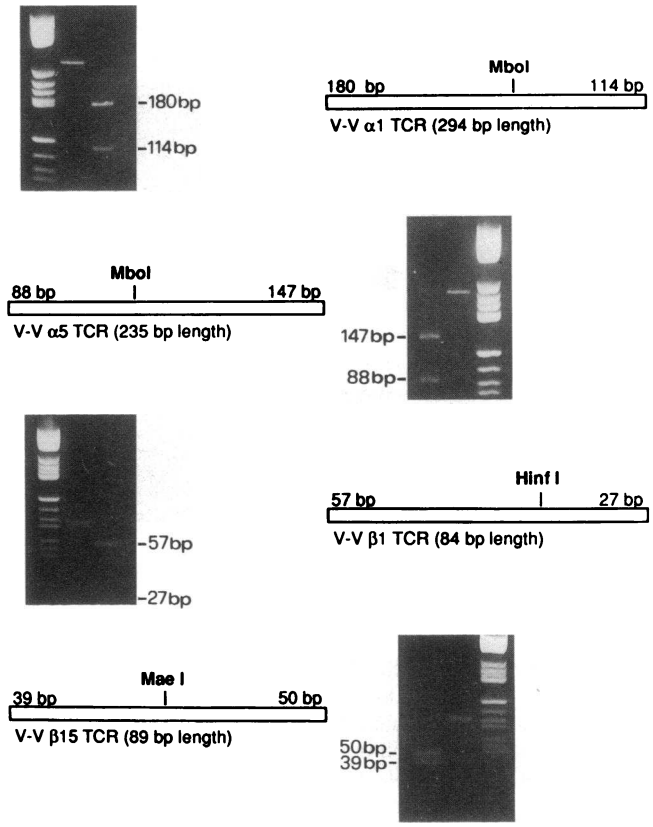

Figure 4. Unique restriction site analysis of V-V amplifications. The EB agarose gels with the uncut PCR amplification products and their digested fragments are shown; each gel is paired with the graphic map of the V-V restriction site analysis showing the size of the PCR amplification product and the relative digested fragments. Forward primers for Vα1, α5, Vβ1, and β15 are described in reference 18; reverse primers for Vα1: 5′-ACAGAAGTACTCAGCTGTGTC-3′, for Vα5: 5′-GGTAGCTGAGTCTGCAGGCTGGGA-3′, for Vβ1:5′-GCTGGCACAGAAATACAAAGC-3′, for Vβ15:5′-CTGGTGGCACAGAAGTAAAGA-3′. Profile amplification:95°C denaturation for 1 min, 55°C annealing for 1 min, 72°Cextension for 1 min; 30 cycles.

sented TCR mRNAs. We verified that this procedure was able to amplify known Vα and Vβ families from a mixed lymphocyte reactions (Fig. 2). The primers used in the PCR reactions have been used to specifically amplify TCR rearrangements from antigen-specific T cell clones (18). Using these primers, we detected specific TCR rearrangements in the muscle of PM and DMD patients, but not in patients with no dystrophy or inflammatory myopathies. The complete analysis of the TCR repertoire could be achieved with amounts as small as 10 mg of frozen muscle. Because the RNA-PCR technique is so sensitive in detecting TCR rearrangements, it could be used as a diagnostic help in PM cases where lymphocyte infiltration is not observed on routine histochemical or immunocytochemical analysis.

The TCR specificity of the PCR products was confirmed by hybridization with internal Cα and Cβ probes on Southern blot, reamplification with V-V primers followed by unique site restriction analysis, and sequencing the PCR products. This analysis revealed a wide spectrum of TCR V gene rearrangements expressed on the infiltrating T cells of both PM and DMD patients (Table II). It is noteworthy to emphasize that in PM Vα1 is present in 73.3% of the patients examined, Vα5 in 60%, Vβ1 in 86.6%, and Vβ15 in all the patients. Differences in TCR frequencies between PM patients and controls (DMD and others) favor the supposition that these rearranged TCRs reflect T cell specificity of the cytotoxic damage in PM lesion.

T cells may home to inflammation sites as a result of superantigen activation or conventional antigen stimulation. These two possibilities may be distinguished at the molecular level: the presence of random D and J segments on the TCR indicates selection of superantigens (26), whereas the presence of a conserved sequence at the V-D-J junctions suggests that conventional antigen selection of the T lymphocytes is taking place (27).

To better understand T cell clonality at the inflammation sites we subcloned and sequenced the TCR Vβ15 transcripts, since these were present in all our PM patients. Analysis of 10 colonies from two patients showed complete amino acid homology at the Vβ15 region. Remarkably, rearrangements between V and J regions were skewed towards a preferential usage (9 out of 10) of Jβ2.1. In the N-D-N region the D(or E)L motif was identified in 50% of the colonies examined. The CDR3 region contains TCR residues that are responsible for antigen binding, which confer T cell specificity. According to recent works (28, 29) the CDR3 region may reside between amino acids 93-106 on ATL21 (see Table III). Comparison of the CDR3 region sequences of our colonies indicates the presence of the common motif: D(or E)L-S-EQF. No random D or J segments were observed and our data favor an antigen selection of the T cells within the sites of inflammation. Our data could not show whether the relevant TCR V families were ascribable to $CD8^+$ or $CD4^+$ T lymphocytes preferentially, because RNA was extracted from the homogenate of whole muscle. Analysis of the TCR characteristics of $CD8^+$ or $CD4^+$ T cell clones derived from PM muscles would resolve this problem.

The TCR repertoire observed in DMD muscles revealed a wide range of α/β TCR families, but no dominant TCR Vα or Vβ rearrangements were found. The presence of T lymphocytes in the context of muscle degeneration in DMD can be explained as nonspecific cell recruitment consequent on the inflammatory reactions accompanying muscle cell destruction. In PM a specific pathogenic antigen may trigger the T-

Table III. Alignment of TCR-Vβ15 Amino Acid Sequences from Two PM Patients

	V region	(N)D(N) 93 ↓	J region CDR3 106 ↓	C region
Vβ15 (ATL21)	SVSRQAQAKFSLSLESAIPNQTALYFCATS	DPGQ	SNQPQHFGDGTRLSIL (Jβ 1.5)	EDLNN
Consensus		DL		
		E	S EQF	
Patient n.1 col 1	SVSRQAQAKFSLSLESAIPNQTALYFCATS	ELGF	EQFFGPGTRLTVL (Jβ2.1)	EDLKN
col 2	SVSRQAQAKFSLSLESAIPNQTALYFCATS	DLD	SY EQFFGPGTRLTVL (Jβ2.1)	EDLKN
col 3	SVSRQAQAKFSLSLESAIPNQTALYFCATS	DLWD	EQFFGPGTRLTVL (Jβ2.1)	EDLKN
col 4	SVSRQAQAKFSLSLGSAIPNQTALYFCATS	EGQES	SYNEQFFGPGTRLTVL (Jβ2.1)	EDLKN
col 5	SVSRQAQAKFSLSLESAIPNQTALYFCATS	DLN	SYGQQFFGPGTRLTVL (Jβ2.1)	EDLKN
col 6	SVSRQAQAKFSLSLESAIPNQTALYFCATS	GTSS	EQFFGPGTRLTVL (Jβ2.1)	EDLKN
col 7	ALYFCATN	GTCS	EQFFGPGTRLTVL (Jβ2.1)	EDLKN
col 8	SVSRQAQAKFSLSLDSAIPNQTALYFCATT	DSFGMV	SYNEQFFGPGTRLTVL (Jβ2.1)	EDLKN
Patient n.5 col 1	SVSRQAQAKFSLSLESAIPNQTALYFCATS	VLD	SRNEQFFGPGTRLTVL (Jβ2.1)	EDLKN
col 2	SVSRQAQAKFSLSLESAIPNQTALYFCAVS	QGQG	RDTQYFGPGTRLTVL (Jβ2.3)	EDLKN

Deduced amino acid sequences of the Vβ15-D-J regions. Underlined characters identify residues in the CDR3 involved in the common motif. ATL21 aa sequence has been used to identify V-D-J boundaries and CDR3.

mediated immune reaction and this might not be the case in DMD. Moreover, expression of MHC antigens have been reported on muscle cells after IFN-γ treatment (30, 31) and it has been demonstrated that myoblasts might act as antigen presenting cells for antigen-specific T lymphocytes (32).

Over the last few years, the molecular analysis of T cell involvement in the animal model of autoimmunity such as experimental allergic encephalomyelitis has provided evidence for restricted TCR usage in the pathogenesis of the disease (33, 34). These observations led to the use of anti-TCR mAbs and vaccination with synthetic peptides of the CDR2 and CDR3 TCR regions; both proved beneficial in experimental allergic encephalomyelitis (33, 35). Limited TCR gene rearrangements have been found in lymphocytes infiltrating brains of multiple sclerosis patients (36), synovial tissues of rheumatoid arthritis patients (37), liver of primary biliary cirrhosis (38), and in the thyroid of thyroiditis patients (39). In multiple sclerosis plaques a dominant CDR3 sequence identical with that of TCR clones specific for myelin basic protein has been found (40). Probing the molecular characteristics of TCRs in combination with T cell function studies might lead to the identification of more selective immunosuppressive treatments in patients with these diseases (33, 41, 42). Currently nonspecific therapies like steroids or cytotoxic drugs (methotrexate) are given to treat PM. In particular the fact that Vα1, Vα5, Vβ1, and Vβ15 are expressed in a restricted manner in PM patients may be relevant for the development of new specific immunotherapies in this disease.

Our results show that the TCR repertoire of the T cells infiltrating muscle in PM is restricted. Finding nonrandomly rearranged V-D-J regions of the TCR may indicate that these lymphocytes have undergone positive muscle antigen selection. PM should be added to the list of diseases whose pathogenesis involves a restricted group of T cells.

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