Oligoclonality in the Human CD8$^+$ T Cell Repertoire in Normal Subjects and Monozygotic Twins: Implications for Studies of Infectious and Autoimmune Diseases

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ABSTRACT

Background: We have previously demonstrated CD8$^+$ T cell clonal dominance using a PCR assay for the CDR3 length of T cell receptors belonging to a limited number of TCRBV segments/families. In this study, we have modified this approach in order to analyze more comprehensively the frequency of oligoclonality in the CD8$^+$ T cell subset in 25 known TCRBV segments/families. In order to assess the relative roles of genes and environment in the shaping of a clonally restricted CD8$^+$ T cell repertoire, we have analyzed clonal dominance in the CD8$^+$ T cell population of monozygotic twins, related siblings, and adoptees.

Materials and Methods: Oligoclonality was assessed in the CD8$^+$ T cell subsets using a multiplex PCR approach to assay for CDR3 length variation across 25 different TCRBV segments/families. Specific criteria for oligoclonality were established, and confirmed by direct sequence analysis of the PCR products. This assay was used to investigate the CD8$^+$ T cell repertoire of 56 normal subjects, as well as six sets of monozygotic (MZ) twins.

Results: Seventy-two percent of normal subjects (n = 56) had evidence of oligoclonality in the CD8$^+$ T cell subset, using well-defined criteria. Although MZ twins frequently displayed CD8$^+$ T cell clonal dominance, the overall pattern of oligoclonality was very diverse within each twin pair. However, we occasionally observed dominant CD8$^+$ T cell clones that were highly similar in sequence in both members of some twin pairs. Not a single example of such similarity was observed in normal controls or siblings.

Conclusions: Oligoclonality of circulating CD8$^+$ T cells is a characteristic feature of the human immune system; both host genetic factors and environment shape the pattern of oligoclonality in this T cell subset. The high frequency of this phenomenon in normal subjects provides a background with which to evaluate CD8$^+$ T cell oligoclonality in the setting of infection or autoimmune disease. Further phenotypic and functional characterization of these clonally expanded T cells should provide insight into normal immune homeostasis.

INTRODUCTION

The majority of peripheral T cells recognize peptide/MHC complexes by means of a clonotypic α/β T cell receptor (1). The potential repertoire of these clonotypic receptors is very large, in major part due to the somatic diversification which takes place during thymocyte development (2). Therefore, extreme clonality within the peripheral T cell compartment has generally been viewed as due to malignant or premalignant transformation of such cells. However, recent studies have shown that quite restricted clonotypes may arise in the course of some antigen specific responses (3,4) and that even in the absence of malignancy or an obvious antigenic challenge, oligoclonal dominance and/or expansion can occasionally be observed in the peripheral CD8$^+$ T cell compartment (5–8).

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The T cell receptor repertoire has been studied in a wide variety of human diseases including autoimmune (7,9,10), infectious (11), and neoplastic (12) disorders. In some instances, oligoclonality has been found in blood or at sites of inflammation. In order to interpret these studies, it is necessary to characterize the normal T cell receptor repertoire with regard to the frequency of oligoclonality. We have previously demonstrated CD8$^+$ T cell clonal dominance in normal individuals using a polymerase chain reaction (PCR) assay for the CDR3 length of T cell receptors for a limited number of TCRBV segment/families (8). We have now modified this approach in order to analyze more comprehensively the frequency of oligoclonality in the CD8$^+$ T cell population. The results show that oligoclonality is an extremely frequent and characteristic feature of the CD8$^+$ T cell repertoire in normal subjects. Studies of monozygotic twin pairs indicate a large role for environment in the generation of this oligoclonality, a finding consistent with our previous observation that CD8$^+$ clonal dominance is enriched in the memory (CD45RO+) cell compartment (8). Therefore, this feature of the CD8$^+$ T cell repertoire probably reflects previous or ongoing immune recognition events; the ubiquitous nature of CD8$^+$ oligoclonality must be taken into account when interpreting T cell repertoire studies in the setting of human disease.

MATERIALS AND METHODS

Immunomagnetic Separation of CD4$^+$ and CD8$^+$ T Cells

Peripheral blood mononuclear cells (PBMC) were obtained from the peripheral blood of the study subjects, by centrifugation over Ficoll-Hypaque (Lymphoprep; Nyegaard, Oslo, Norway). The cells were washed twice in Dulbecco's modified Eagle's medium (DMEM)-1% FCS and resuspended in DMEM-10% FCS at a concentration of 10 to 20 × 10^6 cells/ml. Positive selection for CD8$^+$ cells was carried out by incubating the PBMC with anti-CD8 immunomagnetic beads (DYNAL, Great Neck, NY, U.S.A.) for 30 min at 4°C on a rotating shaker, as recommended by the manufacturer. The unbound cells were then incubated with anti-CD4 immunomagnetic beads for selection of CD4$^+$ T cells. The cells bound to the beads were placed directly into RNAzol (Biotecx, Houston, TX, U.S.A.) for isolation of total RNA.

Multiplex PCR Assay for CDR3 Length of TCRBV Chains

Total RNA (1–5 μg) from the selected T cell populations was used for the first strand cDNA synthesis using a TCRB chain C region primer, CB-14 (see Fig. 1 and Table 1). The cDNA synthesis was performed with M-MLV reverse transcriptase in buffer supplied by the manufacturer (Gibco-BRL, Gaithersburg, MD, U.S.A.) at 42°C, for 1 hr, in a total volume of 120 μl.

Specific combinations of 2 or 3 Vβ (BV)-specific forward primers (20 pmoles each) were used for each multiplex PCR reaction. Each reaction also contained 20 pmoles of a reverse primer specific for the C region (CB-R), out of which 3 pmoles were end labeled with ^{32}P using T4 kinase (GIBCO-BRL). The BV primer combinations were selected on the basis of the location of each V specific primer with respect to the end of the V region. The different reaction sets used were as follows: A: TCRBV1,18,23; B: BV2,4,8; C: BV3,13S1; D: BV5S2,5S1; E: BV6,20; F: BV7,22; G: BV9,16; H: BV11,12; I: BV15,13S2; J: BV14,17; K: BV19,24; and L: BV10,21. Figure 1 represents the strategy used in a multiplex PCR assay using a combination of upstream TCRBV1, 18, and 23 specific primers and a common radiolabeled CB-R primer.

A total of 10 μl of the cDNA was used for each PCR reaction. Conditions for the PCR on the cycle sequencer (model 9600; Perkin-Elmer Corp., Norwalk, CT, U.S.A.) were as follows: denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec and extension at 72°C for 1 min. PCR buffer conditions were Tris HCL 10 mM pH 8.3, $MgCl_2$ 2 mM, 50 mM KCL, with 20 pmoles of each primer in a 100-μl reaction volume. After 35 cycles, an additional extension at 72°C for 10 min was carried out. Five microliters of the amplified products were loaded on a standard 6% polyacrylamide sequencing gel. Bands (spaced three base pairs apart) were visualized after overnight exposure to Kodak AR film. The radioactive bands were also analyzed using a phosphorimager (model PhosphorImmager SF; Molecular Dynamics, Sunnyvale, CA, U.S.A.).

Direct Sequencing of the PCR Amplified Products

In selected samples, the cDNA was reamplified using a single TCRBV segment primer and an unlabeled CB-R/BJ-R primer. The amplified product was used as a substrate for DNA sequencing using

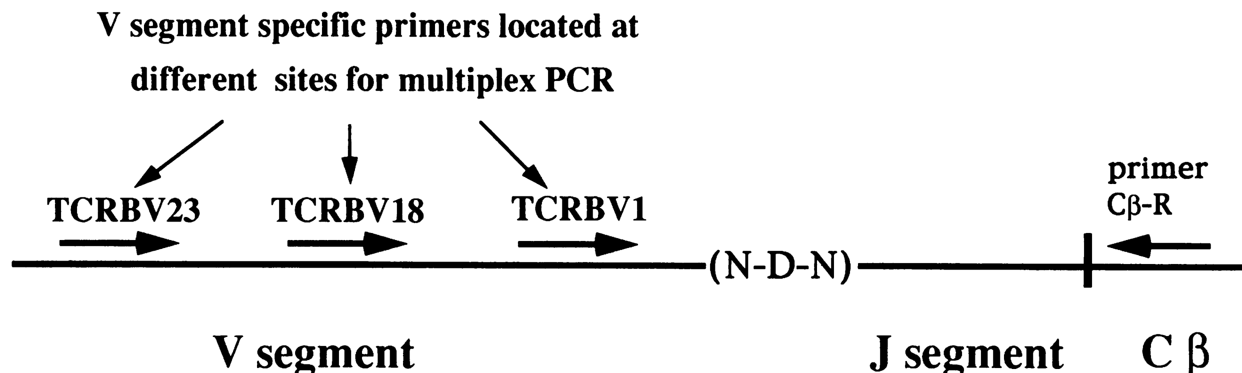

FIG. 1. The representative map of the placement of three different TCRBV upstream primers along with a common radiolabeled CB-R primer in a multiplex PCR reaction

the CB-R/BJ-R primer, following purification over Magic Prep DNA purification columns (Promega, Madison, WI, U.S.A.). Direct sequencing of the PCR products was carried out with fluorescent dideoxy terminators and analyzed on an Applied Biosystems Model 373A Automated Sequenator (Foster City, CA, U.S.A.). The sequences were further confirmed using specific BJ-R primers (Table 1) for sequencing the same PCR products.

In samples where direct sequencing was not possible, the PCR amplified products were cloned using the TA cloning system (Novagen, Madison, WI, U.S.A.). Plasmid DNA was extracted from the resulting recombinant clones using the Magic Miniprep DNA purification system (Promega, Madison, WI, U.S.A.). The plasmid DNA was sequenced as described above using the T7 promoter primer.

Human Subjects

Forty-six normal unrelated individuals with a mean age of 32.3 years, ranging from 14 to 57 years, participated in this study. In addition, six pairs of monozygotic twins were identified and interviewed in person by one of us (PKG). All twins were monozygotic by self-report, and were highly similar in appearance. Previous studies have found false positive self-reporting for monozygosity to be extremely uncommon (13). Four twin sets were HLA typed and found to be identical. Three twin sets were analyzed by PCR amplification of the androgen receptor locus (14), which contains a highly polymorphic triplet repeat, and were found to be identical. The ages and medical status of the twins are shown in Table 2. Twin sets 6 and 1 were discordant for SLE and RA, respectively. Twin 6M is a 29-year-old female and has a history of arthritis and lupus nephritis. She was on medrol 16 mg q.o.d. and Immuran 100 mg q.d. at the time of the study. Twin 1An is a 51-year-old female and has a 12-year history of mild seropositive polyarthritis consistent with rheumatoid arthritis. She had minimal disease activity and was on hydroxychloroquine at the time of the study.

Four families of healthy related siblings with two to five members in each, with ages ranging between 18 and 40 years participated in this study. A pair of healthy adoptees of ages 19 and 20 years who were raised together for 19 years were also included in this study.

RESULTS

Multiplex PCR Assay for Oligoclonality of the TCRBV Chain

We initially used a multiplex PCR approach to assess comprehensively for oligoclonality of 26 TCRBV segments/families. By using a combination of 2 or 3 V segment primers per reaction tube along with radioactive CB-R primer, CDR3 length variation of the 26 different BV segments could be analyzed in 12 reactions. The radioactive PCR products were separated through a standard sequencing gel, and the lengths of the products differed from each other depending on the location of the BV specific primer. Figure 2 shows the autoradiograph of a sequencing gel through which the multiplex PCR products of $CD4^+$ and $CD8^+$ T cell cDNA of a normal individual were separated. In a PCR reaction wherein a combination of 3 BV specificprimers were used (reaction set A containing BV1, BV18, and BV23—see Fig. 1 and Table 1),

TABLE 1. Sequence of the primers used

Primer	Sequence	Position	Reaction Set
BV1	5′-CAACAGTTCCCTGACTTGCAC-3′	84	A
BV2	5′-TCAACCATGCAAGCCTGACCT-3′	86	B
BV3	5′-TCTAGAGAGAAGAAGGAGCGC-3′	86	C
BV4	5′-CATATGAGAGTGGATTTGTCATT-3′	122	B
BV5S1	5′-TTCAGTGAGACACAGAGAAAC-3′	135	D
BV5S2	5′-CCTAACTATAGCTCTGAGCTG-3′	75	D
BV6	5′-AGGCCTGAGGGATCCGTCTC-3′	81	E
BV7	5′-CTGAATGCCCCAACAGCTCTC-3′	86	F
BV8	5′-TACTTTAACAACAACGTTCCG-3′	144	B
BV9	5′-AAATCTCCAGACAAAGCTCAC-3′	84	G
BV10	5′-CAAAAACTCATCCTGTACCTT-3′	76	L
BV11	5′-ACAGTCTCCAGAATAAGGACG-3′	90	H
BV12	5′-GACAAAGGAGAAGTCTCAGAT-3′	117	H
BV13S1	5′-GACCAAGGAGAAGTCCCCAAT-3′	117	C
BV13S2	5′-GTTGGTGAGGGTACAACTGCC-3′	135	I
BV14	5′-TCTCGAAAAGAGAAGAGGAAT-3′	84	J
BV15	5′-GTCTCTCGACAGGCACAGGCT-3′	87	I
BV16	5′-GAGTCTAAACAGGATGAGTCC-3′	132	G
BV17	5′-CACAGATAGTAAATGACTTTCAG-3′	137	J
BV18	5′-GAGTCAGGAATGCCAAAGGAA-3′	117	A
BV19	5′-CCCCAAGAACGCACCCTGC-3′	79	K
BV20	5′-TCTGAGGTGCCCCAGAATCTC-3′	111	E
BV21	5′-GATATGAGAATGAGGAAGCAG-3′	143	L
BV22	5′-CAGAGAAGTCTGAAATATTCGA-′	122	F
BV23	5′-TCATTTCGTTTTATGAAAAGATGC-3′	146	A
BV24	5′-AAAGATTTTAACAATGAAGCAGAC-3′	129	K
CB-14	5′-CTCAGCTCCACGTG-3′		
CB-R	5′-CTTCTGATGGCTCAAACAC-3′		
BJ1S1	5′-AACTGTGAGTCTGGTGCCTT-3′		
BJ1S2	5′-ACGGTTAACCTGGTCCCCGA-3′		
BJ1S3	5′-CTCTACAACAGTGAGCCAAC-3′		
BJ1S4	5′-GACAGAGAGCTGGGTTCCAC-3′		
BJ1S5	5′-TGGAGAGTCGAGTCCCATCA-3′		
BJ1S6	5′-TGAGCCTGGTCCCATTCCCA-3′		
BJ2S1	5′-CCTCTAGCACGGTGAGCCGT-3′		
BJ2S2	5′-TACGGTCAGCCTAGAGCCTT-3′		
BJ2S3	5′-CTGTCAGCCGGGTGCCTGGG-3′		
BJ2S4	5′-CTGAGAGCCGGGTCCCGGCG-3′		
BJ2S5	5′-CCTCGAGCACCAGGAGCCGC-3′		
BJ2S6	5′-CCTGCTGCCGGCCCCGAAAG-3′		
BJ2S7	5′-TGACCGTGAGCCTGGTGCCC-3′		

TABLE 2. Results of direct sequencing of PCR products to establish criteria for oligoclonality by CDR3 length analysis

Counts in Dominant Band	Single Clonal Sequence[a]		Mixed Sequences[b]			
	CB-R (%)	CB-R or BJ-R (%)	1 nt. Position (%)	2 nt. Positions (%)	3 nt. Positions (%)	>3 nt. Positions (%)
≧50% (n = 34)	47	82	3	3	3	9
45–50% (n = 15)	6	67	12	0	12	6

[a]Dominant bands containing ≧50% and 45–50% of the counts within a $CD8^+$ TCR-specific PCR product were sequenced directly with CB-R and/or specific BJ-R primers to establish oligoclonality. The sequence success rate was determined on the basis of cleanly readable dominant sequence with no discrepancies at all.

[b]The percentage of those dominant bands containing ≧50% and 45–50% of the counts in which direct sequencing with CB-R and/or BJ-R primers yielded sequences with discrepancies at 1, 2, 3, or >3 nucleotide (nt.) positions.

three different V-specific PCR products were detected (Fig. 2, Lanes A4 and A8). As seen in this figure, dominant bands of a single CDR3 length were observed only in the $CD8^+$ T cell subset and not in the $CD4^+$ T cell subset (Fig. 2, Lanes C8, D8, F8, I8, and L8).

Criteria for Clonal Dominance by CDR3 Length Analysis

Restriction in CDR3 length within a particular BV segment is visualized as a dominant band depending on its intensity with respect to the rest of the bands within the TCRBV specific PCR product. The radioactive gels were scanned using a phosphorimager and specific criteria were used to define a band as "dominant"–namely, that >50% of the counts within a V segment/family are contained within the dominant band. The clonality of these dominant bands was established by direct sequencing of the PCR product with the CB-R primer. As shown in Table 2, 16/34 instances (47%), in which the counts within the dominant band were 50% or greater, a single readable sequence was obtained using the CB-R primer. Sequencing with a specific BJ-R primer (selected on the basis of the first CB-R primed sequence) yielded a cleanly readable sequence in an additional 35% of cases. Thus, in fully, 82% of the cases in which the 50% criteria for dominance were met, direct sequencing of the PCR product yielded a single dominant sequence. If we reduced the stringency of our criteria for "dominance" to 45–50% of the counts contained in a single band, direct sequencing with the CB-R primer resulted in a clear readable sequence in only 1 out of 15 cases (6%). However, sequencing with a specific BJ-R primer worked in an additional nine cases.

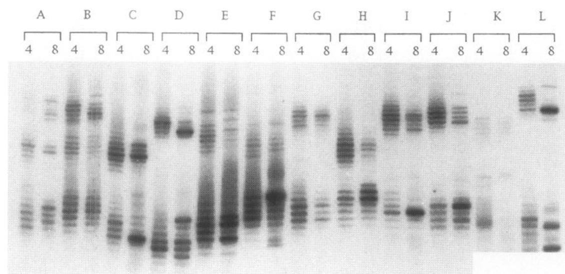

FIG. 2. The results of a multiplex PCR assay for CDR3 length in a normal individual

Peripheral blood $CD4^+$ and $CD8^+$ T cells were analyzed separately in lanes labeled with "4" or "8", respectively. Twelve primer reaction sets A–L were used for the PCR reactions, containing mixtures of TCRBV specific primers as described in Table 1. The TCRBV families in each set appear on the gel from bottom to top. Note that a single dominant band appears in the $CD8^+$ T cells for TCRBV3, 5S1, 7, 15, and 21. In addition, two or more dominant bands are observed in the TCRBV6 and BV10 PCR products, again only in the CD8 population. Specific criteria are used for analyzing these gels, as described in the text.

In the remaining cases (18%) where the 50% criteria for dominance were met, some ambiguity of sequence was observed at one or more nucleotide positions, as shown in Table 2. Thus, for example, 9% of the sequences in this group had discrepancies at more than three nucleotide positions, although even in these instances an underlying group of related sequences was obviously present rather than a mixed random se-

TABLE 3. MZ twin sets used for study and the corresponding TCRBV segments in which CD8$^+$ oligoclonality was observed

Twin	Member	Age (years)	Status	CD8 Oligoclonality in TCRBV
1	An	51	RA	9, 11, 15, 16
1	As	51	Normal	1, 5S1, 8, 11, 17, 24
2	A	17	Normal	4, 14
2	M	17	Normal	14
3	Da	14	Normal	5S2, 7, 12, 13S2, 16
3	De	14	Normal	5S2
4	F	15	Normal	2, 4
4	V	15	Normal	—
5	J	21	Normal	2[a]
5	K	21	Normal	2, 18
6	M	29	SLE	8, 13S1
6	P	29	Normal	8, 13S2, 19

[a]The dominant band contained <50% counts but clonality was confirmed following sequencing.

quence. A similar situation is observed when a 45–50% criteria is used, although a larger number of sequences with ambiguous nucleotides were present. Therefore, we have preliminarily settled on the 50% criteria as an reflection of oligoclonality; this almost certainly underestimates the frequency of this phenomenon.

Occasionally, two or more apparently "dominant" bands are appreciated on visual inspection of the gel (for example, see Fig. 4, twin 5K). Direct sequencing of such a PCR product results in a mixed sequence. This problem can be resolved by molecular cloning of the PCR products followed by sequencing of the plasmid clones. This situation was infrequent; except as noted, these instances are not included in the data reported below.

Clonality within the CD8 TCR Repertoire of Normal Individuals

The multiplex PCR assay for CDR3 length was used to assess the CD8$^+$ T cell receptor repertoire in 46 unrelated normal individuals. Clonal dominance was observed in all 25 TCRBV segments/families studied, although with varying frequencies as represented in Fig. 3. Overall, a higher frequency (>10% of subjects) of CD8$^+$ T cell clonality involving TCRBV5S1, -8, -10, -14 and -15 was observed while relatively few instances (<2% of subjects) of clonality involving TCRBV3, -4, -9, -13S2, -17, -22, and -23 were detected. The typical pattern of the radioactive bands spaced three base pairs apart following separation through a sequencing gel was not observed in TCRBV19 PCR products. This is consistent with a nonfunctional gene segment (M. A. Robinson, personal communication) and therefore, we eliminated TCRBV19 from our subsequent analysis. Oligoclonality was occasionally observed in CD8$^+$ T cells bearing the TCRBV10 segment. However, the band patterns were often heterogeneous; clear separation of bands 3 bp apart, was not observed in all the individuals tested, perhaps sug-

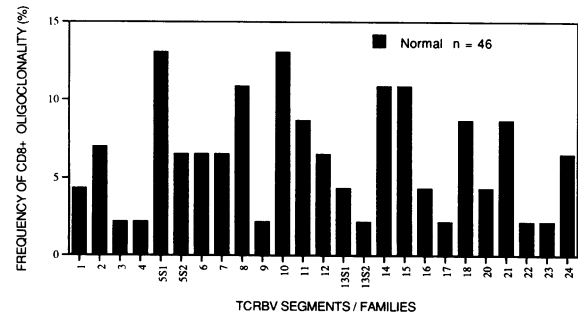

FIG. 3. Bar graph representing the distribution of the frequency of CD8$^+$ T cell clonal dominance across 25 different TCRBV segments/families in normal healthy individuals following multiplex PCR analysis for CDR3 length variation

gesting allelic variation in functional expression of this V segment (M. A. Robinson, personal communication; P. Charmley, personal communication). Seventy-two percent of the normal individuals examined displayed at least one instance of clonal dominance. An average of 1.5 clonal instances per individual was seen in the normal population, with some subjects displaying as high as six instances of oligoclonality while some displayed none. Although some teenagers displayed four to five instances of CD8 clonality, a weak correlation between age and number of clonal instances was observed following Spearman's correlation analysis (95% CI [−0.028, 0.534]). No instance of $CD4^+$ T cell clonal dominance was detected in any of 12 normal individuals.

MZ Twins Frequently Display Different Patterns of Clonal Dominance in the $CD8^+$ T Cell Population

Using the multiplex PCR assay for CDR3 length, we observed 29 instances of apparent clonal dominance in the $CD8^+$ T cell population of the 12 individuals (six MZ twin pairs) under study. In four twin pairs, the CDR3 length analysis was also performed on $CD4^+$ T cells, but no evidence of oligoclonality was observed. The CDR3 length restrictions within the $CD8^+$ T cell subset involved 18 different TCRBV segments. As shown in Table 3, very different patterns of clonally dominant bands were generally observed in the $CD8^+$ T cells of all six MZ twin sets studied; in 24/29 instances, these dominant bands were present in only one member of a particular twin pair.

Infrequent Occurrence of Similar Dominant Sequences in MZ Twin Pairs

Out of the 29 instances of CDR3 restriction, one instance of dominant bands involving the same V segment/family was observed in both members of five twin pairs (twin sets 1, 2, 3, 5, and 6), as shown in Table 3. In the case of twin sets 1 and 3, the CDR3 lengths of these bands (involving BV11 and BV5S2, respectively) differed within the two twins. However, as shown in Fig. 4, an identical CDR3 length of 10 aa for $BV14^+$ TCRs was observed in both members of twin set 2. In twin set 6, an identical CDR3 length of 10 aa involving BV8 was observed. In twin set 5, a dominant band of CDR3 length of 12 aa was observed in the BV2-specific PCR products from both members with an additional dominant band of CDR3 length of 10 aa in member 5K. Although the counts contained within the 12 aa-dominant band in 5J were <50%, sequence analysis was carried out to detect clonality.

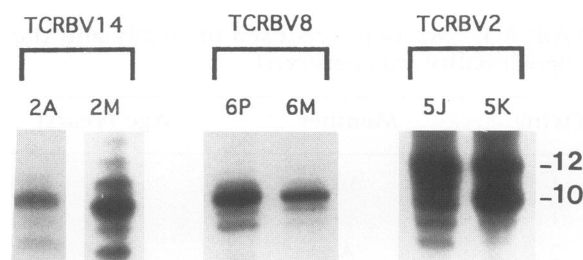

FIG. 4. Comparison of CDR3 length analysis in MZ twin pairs in whom a similar pattern was observed in the $CD8^+$ T cells of both members of the pair

Note that two dominant bands corresponding to CDR3 lengths of 10 and 12 amino acids are seen in twin 5K. The dominant bands seen in both members of twin sets 2 and 6 also correspond to a CDR3 length of 10 amino acids. The CDR3 length is designated according to previously published conventions (15,16).

Sequence Analysis of TCRBV Chains in MZ Twin Pairs

Those TCRBV clones that displayed an identical CDR3 length within the $CD8^+$ T cells of both members of each twin set were further analyzed by sequencing. Direct sequence analysis of the PCR products of twin sets 2 and 6 with the CB-R primer and subsequently the BJ specific primer (BJ2S5 and BJ1S1, respectively) confirmed a single dominant sequence. As shown in Table 4, very similar sequences in the N-D-N region of these receptors were observed. The N-D-N regions of the BV14-specific segment derived from both members of Twin set 2 shared a common nucleotide and amino acid sequence "TTSGK" along with a common BJ2S5 element. Twin set 6 displayed an identical amino acid sequence "FGDM" within the N-D-N region of the BV8 TCR chains in both members. However, differences were observed when the nucleotide sequences of these TCRs were compared between members of each pair.

In the case of twin set 5, direct sequencing of the BV2 PCR products from both members with CB-R primer yielded a mixed sequence probably due to the presence of more than one dominant band within the BV2 PCR product of 5K, and the fact that <50% of the counts were contained in the dominant band of 5J (Fig. 4). Therefore, the BV2

TABLE 4. Nucleotide and amino acid sequences of the CDR3 regions of TCRBV chains which are dominant in both members of twin sets 2, 5, and 6

Twin	92 BV-CASS	96 N-D-N	BJ	CDR3 Lt.
2A	BV14-CASS	T T S G K acg **act agc ggg a**aa	ETQY..2S5	10 aa
2M	BV14-CASS	T T S G K acg **act agc ggg a**aa	ETQY..2S5	10 aa
5K	BV2-CSAR	D P G T L G I gac cca **ggg act** ctg ggt atc	YGYT..1S2	12 aa
5J	BV2-CSAR	D P G T L A gac ccc **ggg acc** tta gct	NYGYT..1S2	12 aa
5J	BV2-CSAR	D P G Q L A I gat ccg **gga cag** ctc gct atc	YGYT..1S2	12 aa
6M	BV8-CASS	F G D M ttt **ggg gat** atg	NTEAF..1S1	10 aa
6P	BV8-CASS	F G D M ttc ggc **gac a**tg	NTEAF..1S1	10 aa

The small letters in bold represent the nucleotide sequences contributed by the germline encoded BD1 and BD2 segments.

PCR products from both members of twin set 5 were molecularly cloned using the TA cloning system. In twin member 5K, three out of three molecular clones containing TCRs with a CDR3 length of 12 aa had the same sequence, "DPGTLGI". This was also observed following direct sequencing of the TCRBV2-specific PCR product with the BJ1S2 primer. In twin member 5J, two out of three clones containing TCRs with a CDR3 length of 12 aa had the same sequence, "DPGTLA", while the third clone contained a very similar TCRBV2 sequence, "DPGQLAI". The "DPGTLA" sequence was confirmed by direct sequencing of the BV2 PCR products using the appropriate BJ sequencing primer (BJ1S2). Thus, the TCR clones from both members of this twin set shared a common sequence of "DPGTL" in the first five residues of the N-D-N region. The codons for this stretch of amino acids were not identical (Table 4).

PCR Analysis with V-J Primer Pairs to Detect Underlying Clonal Dominance in Twin Pairs

Since we used relatively stringent criteria to define clonal dominance, some instances of oligoclonality may have been missed in the analysis of the $CD8^+$ T cell repertoire of the MZ twins. Therefore, in order to detect underlying clonal dominance existing within those TCRBV segments where there is CD8 oligoclonality in one member of the twin pair and not in the other, a PCR amplification was carried out using specific TCRBV-BJ primer combinations. The TCRBV-BJ combinations were selected on the basis of sequence analyses of the dominant clones previously identified in various members of the six twin sets. Out of 10 separate instances tested, a shared dominant TCR was observed in just one case. Figure 5 shows CD8 clonal dominance for TCRBV2 in member 4F but not in member 4V of twin set 4 when their TCRBV2-CB-R PCR products were compared. However, further analysis of CD8 cells using primers TCRBV2 and BJ1S2 revealed dominant TCRs of identical CDR3 lengths in both members of twin set 4. Sequencing of the BV2-BJ1S2 PCR product with BJ1S2 specific primer did not yield a single clear sequence but rather a mixture of sequences with limited heterogeneity at four nucleotide positions involving only two codons (data not shown). Also shown in Fig. 5 is an example of CD8 clonal dominance involving TCRBV17 in member 1As and not in member 1An of twin set 1. This was true when both the TCRBV17-CB-R and TCRBV17-BJ2S1-R primer sets were used.

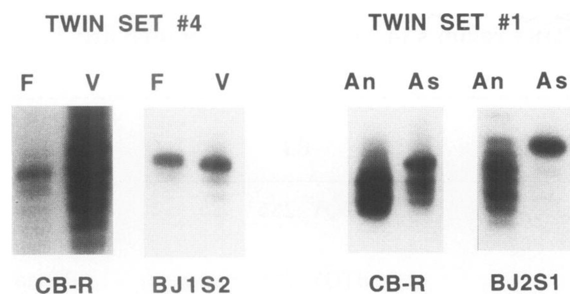

FIG. 5. Comparison of CD8$^+$ T cell clonal dominance in twin set 4 and 1 within the TCRBV2 and 17 segments respectively

Clonal dominance was examined following TCRBV2-CB-R and TCRBV2-BJ1S2 specific PCR amplification in twin set 4 and TCRBV17-CB-R and TCRBV17-BJ2S1 specific PCR in twin set 1.

As stated above, this situation was seen in 9 out of 10 instances where V-J primer combinations were tested on twin pairs.

Siblings Display Diverse Patterns of CD8$^+$ T Cell Oligoclonality

In order to investigate the relative roles of genetic and nongenetic factors in the shaping of the CD8$^+$ T cell repertoire, normal healthy related siblings and adoptees were chosen as a control population with which to compare the results on MZ twins. Related siblings from four families were tested for shared TCRs within their oligoclonal CD8$^+$ T cell populations. Fifteen instances of clonal dominance involving 10 different TCRBV families were observed in these 12 individuals. However, there were no instances of shared clonal dominance observed between any related sibling pair. Oligoclonality was also tested in a pair of unrelated siblings (i.e., adoptees who had lived together for 19 years). Clonal dominance involving TCRBV10 was seen in a single instance in one adopted sibling and was not present in the other adoptee. Therefore, no apparent similarities were observed between the CD8$^+$ T cell receptor repertoires in individuals who have shared similar environments during their childhood.

DISCUSSION

In this study we have established that prominent oligoclonality within the CD8$^+$ T cell population is an extremely common and characteristic feature of the human immune system. Previous reports from this (8) and other laboratories (5,6) have indicated that this phenomenon is occasionally observed in normal individuals. However, we have now comprehensively assessed the presence of oligoclonality in a population of 60 individuals and have shown that the majority of subjects exhibit evidence of oligoclonality within the CD8$^+$ T cell repertoire.

In order to study T cell populations for the presence of oligoclonal dominance, we have modified previously described assays for the CDR3 length of T cell receptor β chain (8,17,18) to allow for the analysis of multiple TCRBV families within a single PCR reaction. In this manner, we were able to assess the presence of oligoclonality in 26 TCRBV segments/families using only 12 PCR reactions. In addition, we have developed criteria for identifying oligoclonality based upon direct sequence analysis of these PCR products. As shown in Table 2, we have found that when a band of one length contains >50% of the counts within a given V segment amplification group, then direct sequencing of the PCR product almost always (82% of the time) reveals a single dominant sequence. If a less stringent criterion is used for clonal dominance (45–50% of the counts in the dominant band), the success rate of finding an unambiguous dominant sequence using CB-R as the sequencing primer is substantially lower (Table 2). Thus we have settled on the 50% value as a reasonable starting point for estimating the frequency of clonal dominance, although it clearly underestimates the phenomenon overall.

Having established preliminary criteria for detecting oligoclonality within T cell populations, we determined the frequency of this phenomenon in a group of unrelated normal subjects (n = 46). When CD4$^+$ T cells were examined, not a single instance of clonal dominance was detected in 12 individuals, consistent with our previous observations (8). In contrast, 72% (33/46) of subjects had evidence of clonal dominance involving at least one V segment family in the CD8$^+$ T cell subset. The distribution of oligoclonality among the different V segment families is shown in Fig. 3, and reveals that this phenomenon can involve all the V segments/families under study. On average, 1.5 instances of clonal dominance per individual are found in the CD8$^+$ T cells of the normal population. A weak correlation was observed between the age of the subjects and the number of CD8$^+$ T cell clones detected, although subjects in their teens also displayed four to five CD8 clones per individual.

In our previous, more limited analysis (8),

we observed the appearance of clonally dominant CD8$^+$ T cells after immunization and established that these cells are markedly enriched in the CD45RO$^+$ subset. Thus, although there appears to be a considerable influence of environment on the appearance of these cells, we wondered whether host genetic factors might also control the specificity of these clones. We therefore compared the oligoclonality of the CD8$^+$ T cell repertoire in six sets of monozygotic twins. Most of the twin pairs exhibited very diverse patterns of clonality in the CD8$^+$ compartment. Furthermore, reanalysis of some twin pairs with selected V-J combinations to search for any "hidden" similarities resulted in the detection of just one shared clonality out of 10 instances.

However, in three different MZ twins, we found highly similar or identical TCRB receptors in both members of the twin pair (see Fig. 4 and Table 4). In twin pair 6, identical amino acid sequences were encoded by different nucleotide sequences, strongly indicating a role for selection in the generation of these clonally dominant receptors. In order to control for the possible effects of a shared environment in generating this phenomenon, we searched for evidence of shared patterns of oligoclonality among the siblings of four families and in one pair of genetically unrelated adoptees raised together from the first few months of life. We did not observe any instance of similar clonality among these 14 subjects, nor among any of our normal control population. Although the number of control observations is small, the data suggest that host genetic factors do play a role in the patterns of oligoclonality we have observed in the CD8$^+$ T cell population. A larger study of unrelated adoptees raised together from birth will be required to definitively establish this fact.

Two of the twin pairs we studied were discordant for an autoimmune disease. Although there were differences in the patterns of CD8$^+$ oligoclonality in these twin pairs, the high frequency of differences among normal MZ twins emphasizes the difficulty of interpreting such data. Indeed, recent studies on antigen-specific T cell responses in normal MZ twins suggest a highly variable pattern of immune responses can occur in such individuals (19). Thus, it is risky to ascribe T cell repertoire differences in twins as necessarily being due to the presence of disease in one member of the twin pair.

These results indicated that there exists a large amount of diversity in the patterns of CD8$^+$ T cell oligoclonality, with complex genetic and environmental contributions to this phenomenon. The function of these oligoclonal cell populations is obviously of some interest. We have recently found that, in normal individuals, oligoclonal expansion of these cells is frequently enriched within the CD57$^+$ subset of CD8$^+$ T cells (20), a population which appears to have regulatory properties (21). Interestingly, recent work by Behar et al. indicates that in patients with rheumatoid arthritis, clonally expanded CD8$^+$ T cell populations can exhibit autologous reactivity (22). In the setting of rheumatoid arthritis, CD8$^+$ oligoclonality may preferentially involve particular V segment families, including TCRAV12 (7) and TCRBV3 (Ref. 23 and R. Hingorani et al., submitted). These findings suggest that further characterization of this cell population may provide insight into normal immune homeostasis and contribute to an understanding of variations in disease activity in patients with autoimmune diseases.

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