Highly Restricted Human T Cell Repertoire in Peripheral Blood and Tissue-infiltrating Lymphocytes in Omenn's Syndrome

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Abstract

Omenn's syndrome is an inherited human combined immunodeficiency condition characterized by the presence of a large population of activated and tissue-infiltrating T cells. Analysis of the TCRB repertoire revealed a highly restricted TCRBV usage in three patients. More strikingly, T cell clones from the three patients expressed TCRB chains with VDJ junction similarities, suggesting a common antigenic specificity. Analysis of the TCRA repertoire in one patient also revealed a restricted TCRAV usage. Finally, analysis of the TCRBV repertoire of tissue-infiltrating T cells in one patient suggested nonrandom tissue migration. These results suggest that the oligoclonal expansion of T cells observed in Omenn's syndrome could be the consequence of autoimmune proliferation generated by a profound defect in lymphocyte development. (*J. Clin. Invest.* 1998. 102:312–321.) Key words: combined immunodeficiency • Omenn syndrome • T lymphocytes • HTCRBV • repertoire

Introduction

Omenn's syndrome, also known as combined immunodeficiency with hypereosinophilia, is a primary recessive inherited immunodeficiency with specific clinical and immunological features. In addition to the severe recurrent infections occurring in severe combined immunodeficiency (SCID),[1] patients with Omenn's syndrome rapidly develop diffuse erythroderma and protracted diarrhea, together with marked hypereosinophilia (1). The number of circulating T cells is high but they are poorly responsive to antigens and allogenic cells (2–4), and their cytokine secretion pattern is markedly altered (4, 5). Contrasting with the severe depletion observed in lymphoid

tissues, large numbers of T cells also infiltrate the skin and intestine (6). B cells are absent or scarce in both peripheral blood and tissues, resulting in profound hypogammaglobulinemia (except for IgE) (2).

We and others have found a restricted rearrangement pattern of T cell receptor (TCR) genes in patients with Omenn's syndrome (2, 3), pointing to limited T cell diversity. The presence of other clones in the T cell population could not be ruled out, owing to the limited sensitivity of Southern blot analysis. We now report the results of molecular analysis of the TCRB chain in three patients with Omenn's syndrome, with an anchored-PCR (A-PCR) amplification method. In addition, TCRBV chain diversity in tissue-infiltrating T cells was also determined in one patient.

Methods

Patients and controls

Case reports on the three patients including peripheral blood lymphocyte phenotypes have been published elsewhere (2, 7). The absence of maternal engrafted T cells was confirmed in every case by either minisatellite analysis (2) or HLA typing (7). Briefly all patients had > 80% circulating T cells, comprising 30–57% of activated Dr+ cells. Among CD3+ cells, the percentages of CD4+ and CD8+ were as follows: patient 1, 29 and 54%; patient 2, 55 and 36%; and patient 3, 85 and 5%. Peripheral blood lymphocytes from two healthy volunteers were used as controls for the A-PCR study. Skin biopsy samples from psoriatic patients served as controls for the immunohistochemical study. Samples were obtained from patients before bone marrow transplantation conditioning.

Antibodies

The following mAbs were used: anti-CD3: Leu4 (IgG2a) (Becton Dickinson, Mountain View, CA), anti-CD4: Leu3a (IgG1) (Becton Dickinson), anti-CD8: Leu2a (IgG1) (Becton Dickinson), anti-CD25 (IgG1) (Becton Dickinson), anti–HLA-DR (IgG2a) (Becton Dickinson), anti-TCRBV3 (IgG1), anti-TCRBV5a = anti-TCRBV5.2+5.3 (IgG1), anti-TCRBV5b = anti-TCRBV5.3 (IgG1) (all from T Cell Sciences, Cambridge, MA), anti-TCRBV2 (IgM), anti-TCRBV5.1 (IgM), anti-TCRBV6.1 (IgM), anti-TCRBV8 (IgG2a), anti-TCRBV9 (IgM), anti-TCRBV12 (IgG2a), anti-TCRBV13.1 (IgG2b), anti-TCRBV13.6 (IgG1), anti-TCRBV14 (IgG1), anti-TCRBV16 (IgG1), anti-TCRBV17 (IgG1), anti-TCRBV18 (IgG2b), anti-TCRBV20 (Ig), and anti-TCRBV21 (IgG2b) (all from Immunotech, Marseille, France).

Immunofluorescence study

Lymphocyte phenotyping. Direct immunofluorescence staining was performed using FITC- or PE-conjugated antibodies. An FITC-conjugated goat anti–mouse Ig, GAMIG (Nordic Immunology, Tilburg, The Netherlands), was used for indirect immunofluorescence.

TCRBV repertoire. Peripheral blood lymphocytes were incubated successively with an anti-TCRBV antibody, an FITC-conjugated GAMIG (Nordic), and a PE-conjugated anti-CD4 or anti-CD8 antibody. After each incubation, the cells were washed twice for 10 min at 4°C with 1× PBS. Background staining due to nonspecific fixation of the GAMIG was deducted. Samples were analyzed in a FAC-Scan® flow cytometer (Becton Dickinson).

1. *Abbreviations used in this paper:* A-PCR, anchored PCR; SCID, severe combined immunodeficiency; TCR, T cell receptor.

Immunohistochemistry

Biopsy specimens of lymph node, spleen, skin, and intestine were embedded in Tissue Tek II O.C.T compound (Miles Laboratories, Inc., Elkhart, IN), snap-frozen in liquid nitrogen, and stored at −70°C until use. For simple TCRBV immunostaining, consecutive 5-mm cryostat sections were incubated successively with a primary antibody, a biotin-conjugated rat anti–mouse Ig (Jackson Laboratories, West Grove, Pennsylvania, PA), and finally with alkaline phosphatase–conjugated streptavidin (DAKO Corp., Carpinteria, CA). After each incubation, the slides were washed twice for 5 min at room temperature with Tris-HCl, pH 7.6. Alkaline phosphatase activity was detected with Naphthol-ASMX and Fast-blue (both from Sigma Chemical Co., St. Louis, MO). The results were expressed as − for the lack of positives or + for the presence of positive cells. In the skin, the number of cells bearing a given TCRBV was estimated by counting the cells in five contiguous high-power fields with a 20× objective.

Analysis of the TCR repertoire

PBMC were isolated on Ficoll/Hypaque gradients. PBMC were further purified into single positive CD4 and CD8 subsets (patients 1 and 2) by two-color fluorescence-activated cell sorting (FACStarplus®; Becton Dickinson) using FITC-Leu3a and PE Leu2a (Becton Dickinson), respectively. Total RNA from PBMC, CD4$^+$, and CD8$^+$ cells was extracted as described elsewhere (8). 1–5 μg of total RNA was used for cDNA first-strand synthesis with 200 U of MMLV reverse transcriptase (Superscript; GIBCO BRL, Gaithersburg, MD) and Oligo-dT (Pharmacia Biotech, Piscataway, NJ) according to the manufacturers' recommendations. After 1.5 h of elongation at 37°C, cDNA was precipitated in isopropanol at 4°C for 15 min, washed twice in 70% ethanol, and tailed with dGTP for 1 h at 37°C using terminal deoxynucleotidyl transferase (Boehringer Mannheim, Indianapolis, IN) according to the manufacturer's recommendations. The tailed cDNA was extracted with equal volumes of phenol and chloroform, precipitated in ethanol, and resuspended in 40 μl of water. 10 μl of the dG-tailed cDNA was amplified by means of A-PCR using TCRBC antisense primer and a "universal" sense primer containing MluI and SalI restriction sites, respectively. PCR amplifications were performed as described elsewhere (9). After digestion with SalI and MluI restriction enzymes, products corresponding to the mature VDJC transcripts were size-selected (720±70 bp) on a 2% low-melting point agarose gel (Nusieve FMT) and ligated into the SalI-MluI sites of an M13 mp18-derived vector (M13 BM20; Boehringer Mannheim). The ligation products were introduced into XL1 Blue bacteria (Stratagene, La Jolla, CA) by electroporation to create the TCRB minilibraries. Minilibrary screenings were done with a panel of TCRBV- and TCRBJ-specific oligoprobes as described previously. The quantitative and qualitative aspects of this technique were previously demonstrated (9–11). TCRBV and TCRBJ frequencies were analyzed on > 600 TCRBC-positive clones in the same experimental conditions for all probes. The following panel of oligonucleotides (5′–3′) was used for TCRBJ and clone-specific analyses: TCRBJ1.1: AGGCACCAGACTCACAGTTG; TCRBJ1.2: GGGGACCAGGTT-AACCGTTG; TCRBJ1.3: GGGAAGTTGGCTCACTGTTG; TCR-BJ1.4: TGGAACCCAGCTCTCTGTCT; TCRBJ1.5: TGGGACTC-GACTCTCCATCC; TCRBJ1.6: TGGGACCAGGCTCACTGTGA; TCRBJ2.1: AGGGACACGGCTCACCGTGC; TCRBJ2.2: AGGCT-CTAGGCTGACCGTAC; TCRBJ2.3: AGGCACCCGGCTGACAG-TGC; TCRBJ2.4: CGGGACCCGGCTCTCAGTGC; TCRBJ2.5: AGGCACGCGGCTCCTGGTGC; TCRBJ2.6: CGGCAGCAGGCT-GACCGTGC; TCRBJ2.7: CGGGCACCAGGCTCACGGTC; DIV-B13: GCCACCCGAACACTGAA; DIVB14a: GTTACTGCCCCT-GAACAC and DIVB14b: GTTTGGGGTTCGATGAGC in patient 1; DIVB2: TTCCCTAGCGGGAAGACAGA and DIVB14c: TTT-AGCCCCTTTGGAAGAAG in patient 3. Sequence analysis was performed using the T7 polymerase kit (Sequenase) with either vector-specific or TCRBV-specific primers. When CDR3 sequences

were identical, clone independence was assessed by G tail length or by the cDNA start point.

For patient 2, TCRBV- and TCRAV-specific amplifications were performed using panels of primers and conditions described by Genevée et al. (12). Actin-specific primers (actin sense: ATTTGCG-GTGGACGATGGAGGGGC; reverse: GGCATCGTCACCAAC-TGGGACGAC) were used as internal controls. Amplified products were run on a 1.2% agarose gel and detected by Southern blot analysis using either a TCRBC- or a TCRAC-specific oligoprobes (HTC-RBC: CGGGCTGCTCCTTGAGGGGCTGCG; HTCRAC: GTTG-CTCCAGGCCGCGGCACTGTT). Membranes were successively hybridized with an actin-specific oligoprobe (ATTTGCGGTGGAC-GATGGAGGGGC).

Definition of CDR3 region

For TCR junction comparisons we used the CDR3 definition of Moss and Bell (13). The CDR3 boundaries are determined as the fourth residue after Cys 90 and the last position preceding the consensus TCRBJ-contained Phe.

Results

Restricted TCRB and TCRA repertoire in Omenn's syndrome. In controls, all 24 TCRBV families were expressed normally in CD4$^+$ and CD8$^+$ T cells. Most frequencies were between 1 and 10% (range 0.5–18%) (Fig. 1 *A*). These results were in keeping with previous studies on normal human TCRBV gene usage (9, 10, 14).

In striking contrast, the pattern of TCRBV family expression was drastically restricted in both CD4$^+$ and CD8$^+$ T cells from patients 1 and 2, as well as in unsorted T cells from patient 3. Fewer than 10 families were represented in each case (Fig. 1 *B*). Moreover, major imbalances were observed in the frequency of these TCRBV families. Some accounted for up to 60–70% of the CD4$^+$ (TCRBV13 in patient 1 and TCRBV6 in patient 2) or CD8$^+$ subset (TCRBV14 in patient 1). Another intriguing feature observed was the recurrent expression of TCRBV families 6, 14, and 21 in all three patients. Several TCRBV families contain numerous gene members. Therefore, the restricted TCRBV pattern could have resulted from a selection process towards particular families, V gene usage being diverse. Sequencing the clones of each family showed that this restriction occurred at the level of TCRBV genes themselves. For example, the TCRBV6 family, which consists of nine functional gene segments, was overrepresented in patients 1 and 2, but the TCRBV6S1 and 6S4 genes alone accounted for all of the TCRBV6$^+$ clones (see sequence analyses below). TCRBV-specific amplifications further illustrated the restricted pattern of TCRBV usage in patient 2. Indeed, this method detected 9 TCRBV families (TCRBV 1, 3–6, 9, 12, 14, and 15) in patient 2 (Fig. 2). TCRBV15 products were detected by bispecific PCR but not by A-PCR. This could reflect either the presence of few cells bearing these TCRBV specificities or weak cross-amplifications related to the extreme conditions (see Methods and reference 12). This last explanation is probably true for TCRBV 8, 11, and 17, which gave very weak signals but remained undetectable both by A-PCR and immunofluorescence techniques. On the other hand, TCRBV21 was detected by A-PCR only, probably reflecting a poor TCRBV 21 primer amplification as observed in controls (Fig. 2 *A*). In our conditions, the TCRBV-specific amplification is rather qualitative than quantitative. The most striking evidence for this aspect is
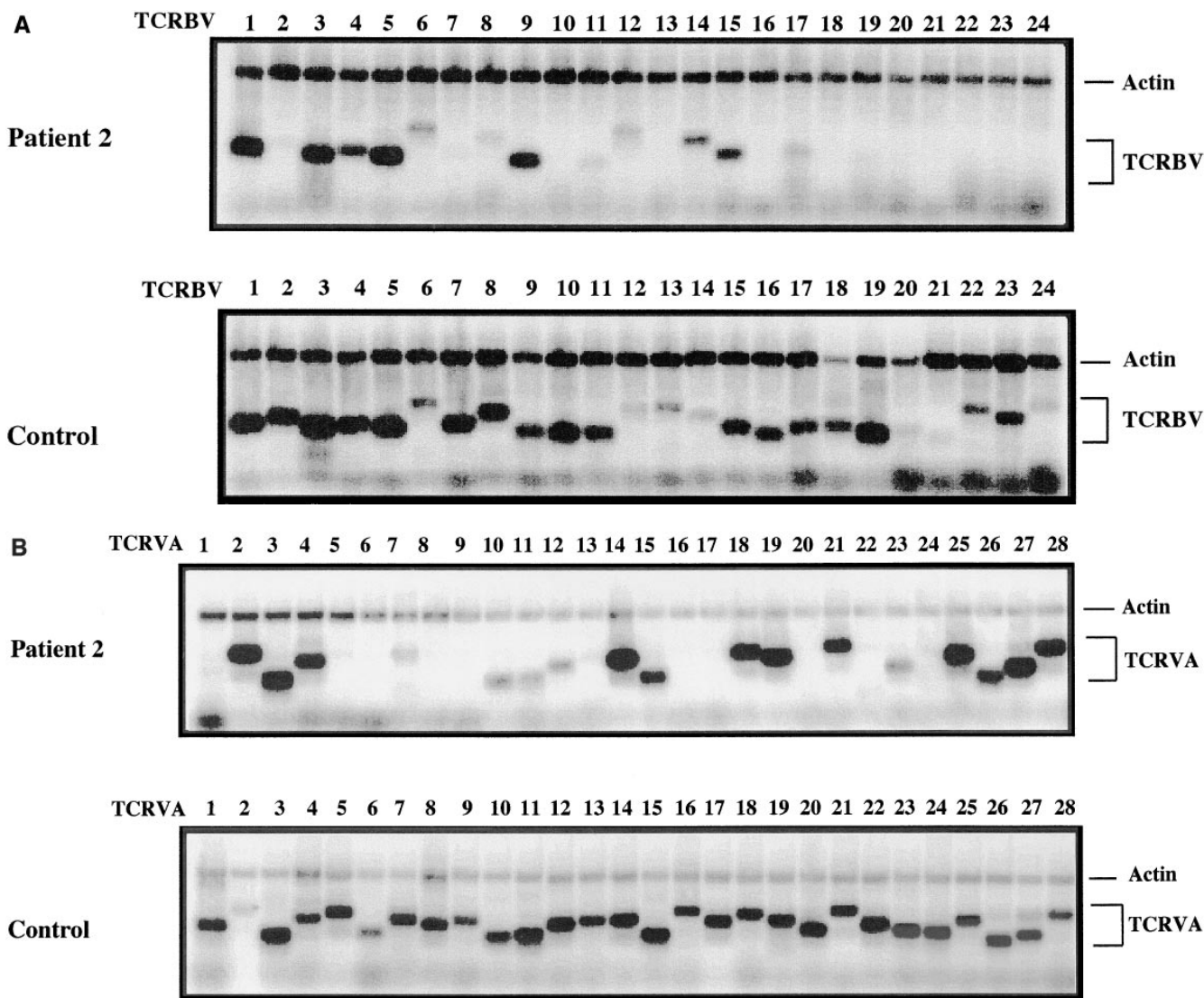
A

B

C
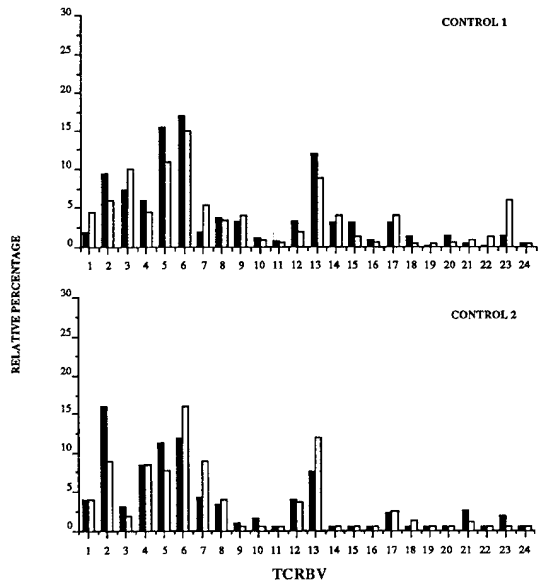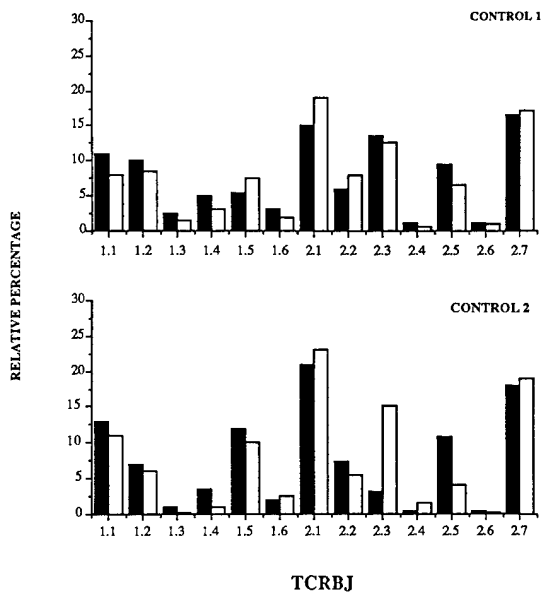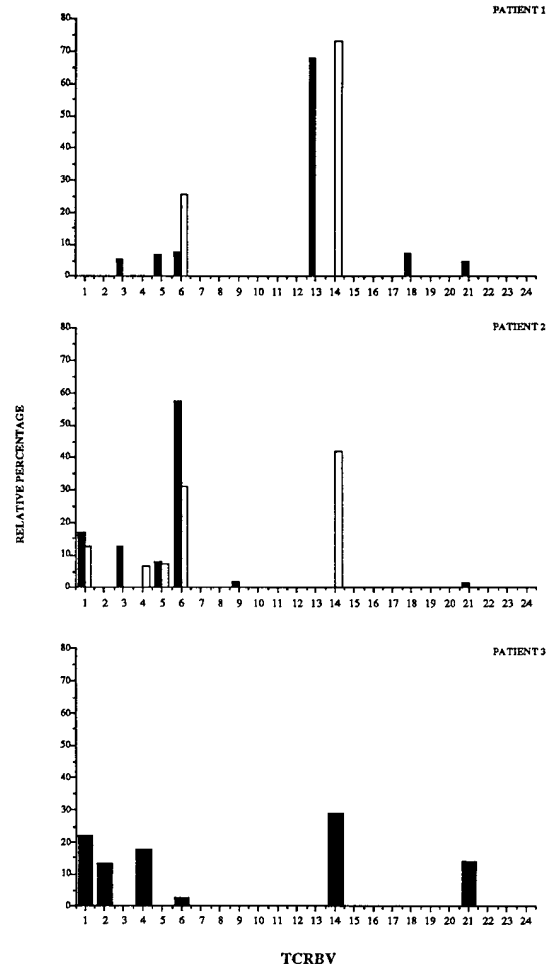
D
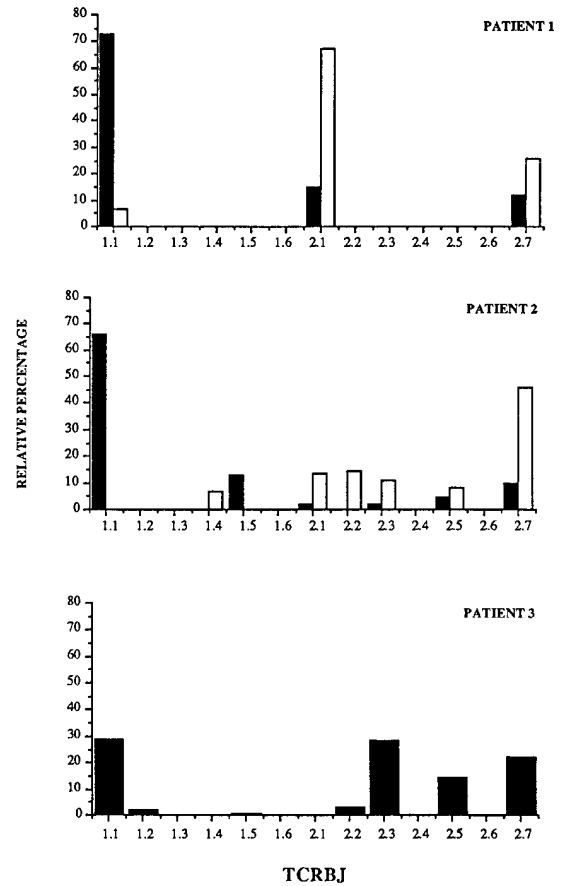
Figure 2. TCRBV (*A*) and TCRAV (*B*) amplifications detected by Southern blot analysis on PBMC from patient 2 (*top*) or control (*bottom*).

given by the TCRBV9 subfamily detection. This subfamily is detected below 10% using both A-PCR and immunofluorescence (5 and 7%, respectively) as opposed to the major signal obtained using the TCRBV-specific amplification. Altogether these methods allowed us to detect 12 out of 24 TCRBV families in patient 2. Hence, nondetection reflects an absence of cells bearing the corresponding TCRBV family. As an example, TCRBV2 is consistently expressed in ∼ 10% of normal peripheral lymphocytes (9, 10, 14, 15), and this was confirmed in our controls. The absence of TCRBV2⁺ T cells in patient 2 was confirmed both by TCRBV2-specific PCR (Fig. 2) and by anti-TCRBV2–specific immunofluorescence (Table I).

HLA-DR staining of CD3⁺ cells indicated that 30–57% of patients' T cells were activated, compared with only 5% of control T cells. Therefore, it was important to assess separately the TCRBV repertoire in these HLA-DR⁺ and HLA-DR⁻

subsets. Comparative analysis of six different TCRBV families (TCRBV1-6) used in sorted CD4⁺, CD4⁺DR⁺, and CD4⁺DR⁻ cells from patient 2 yielded no significant difference using A-PCR. Therefore, these activated HLA-DR⁺ T cells did not seem to bias the overall TCRBV repertoire, despite their elevated number and potentially higher mRNA content (16).

The TCRBV repertoire in patient 2 was also determined by immunofluorescence analysis using a panel of 18 anti-TCRBV monoclonal antibodies. The results for most TCRBV families closely correlated with those obtained by the A-PCR–based method (Fig. 1 *B* and Table I). TCRBV12, which was not detected by A-PCR but represented only 3% of CD4⁺ T cells in the immunofluorescence study, was the only exception.

Then, it was of interest to assess the TCRAV repertoire. Since few anti-TCRAV antibodies are available and given the

Figure 1. Relative TCRBV (*A* and *B*) and TCRBJ (*C* and *D*) transcript levels in peripheral CD4⁺ (*black bars*) and CD8⁺ (*white bars*) T cells from (*A* and *C*) representative control and (*B* and *D*) three patients with Omenn's syndrome.

	Patient 2		Controls*
TCRBV	CD4^{+}	CD8^{+}	CD3^{+}
2	0	0	7.5–10.9%
3	4	0	0–8%
5.1	0	0	4.2–7.2%
5.2+5.3	5	5	1–5%
5.3	0	0	1–5%
6.1	42	17	2.5–4.4%
6.7	0	0	1–5%
8	0	0	2.6–5.1%
9	7	0	1.6–3.2%
12	3	0	1.1–1.9%
13.1	0	0	1.8–3.3%
13.6	0	0	0.9–1.9%
14	5	47	2.2–5.6%
16	0	0	0.4–1.1%
17	0	0	3.3–7%
18	0	0	0.7–1.4%
20	0	0	0–2.4%
21	0	0	2.2–3.6%
Total	66	69	34–82%

*According to the manufacturer.

fact that up to one-third of peripheral T cells can express two productive alpha chains (17), we could not use a quantitative approach. Therefore, we limited our study to a qualitative analysis of patient 2. Results of a typical experiment are depicted in Fig. 2 *B*. All TCRAV subfamilies tested can be amplified in controls, but only a restricted pattern of TCRAV was detected in patient 2. Comparing results obtained with the same approach showed that 17 out of 28 TCRAV subfamilies (2–4, 7, 10–12, 14, 15, 18, 19, 21, 23, 25–28) and 12 out of 24 TCRBV subfamilies (1, 3–6, 8, 9, 11, 12, 14, 15, 17) were detected to different extents (as compared with controls). This is in keeping with 30% of T cells expressing two productive alpha chains.

To further characterize TCRB chain structure in Omenn's syndrome, the TCRBC libraries were screened with a panel of TCRBJ-specific probes. As expected from the results of previous studies (11, 14, 18), all TCRBJ segments were expressed in CD4^{+} and CD8^{+} T cells from controls, with most frequencies comprised between 1 and 10% (range 0.5–20%). Expression of TCRBJ2S1 and TCRBJ2S7 predominated (Fig. 1 *C*). TCRBJ segment usage was drastically restricted and biased in CD4^{+} and CD8^{+} T cells from patients 1 and 2 and in unsorted PBMC from patient 3 (Fig. 1 *D*). Superimposition of hybridization patterns obtained consecutively on the same TCRBV libraries with TCRBV- and TCRBJ-specific probes indicated that most of the TCRBV segments were preferentially associated with one TCRBJ segment. For example, the 171 TCRBV13^{+} clones in the CD4^{+} T cell library of patient 1 all hybridized with the TCRBJ1S1 probe, thus suggesting monoclonality within this TCRBV family. Other predominant, although not exclusive, associations were suggestive of oligoclonal TCRBV families, such as TCRBV14-TCRBJ2S7 (71/115 TCRBV14^{+} clones

were TCRBJ2S7^{+}) in CD8^{+} T cells of patient 2. Such a predominant TCRBV-TCRBJ association was never found in controls with the same screening approach.

Taken together, the pattern of the TCRBV and TCRBJ repertoire suggested a highly restricted diversity of the TCRB chain and, consequently, of the T cell population in Omenn's syndrome, and prompted us to examine the sequence of these TCRBC-positive clones in more detail.

Sequence analysis of TCRBVJ junctions in Omenn's syndrome. To confirm the oligoclonal nature of the T cell population in Omenn's syndrome, the nucleotide sequences of most TCRBV[+] clones were determined in the three patients (Table II). When redundant CDR3 structures were found, the 5′ ends of the corresponding clones (containing the G tail and the cDNA start) were sequenced. Differences in G tail length or a different cDNA start site always confirmed that these clones arose from independent PCR products (data not shown). The control TCRBC libraries generated by A-PCR were also free of redundant clones, as reported previously (11). All the sequenced clones resulted from in-frame rearrangements, and were thus likely to correspond to productive TCRB transcripts. The structure of the junctions appeared to be normal, with N nucleotides between V and D or D and J regions in most cases.

The most striking finding concerned patient 3, in whom sequence analysis demonstrated a single junctional sequence for each of the five TCRBV expressed in T cells. This argued that the T cell population in this patient consisted of only five different T cell clones. In the case of TCRBV1 and TCRBV14, monoclonality was further demonstrated with the CDR3-specific probes DIVB1 and DIVB14, which hybridized, respectively, 67 out of 67 TCRBV1^{+} and 55 out of 55 TCRBV14^{+} clones (Table II). In patient 1, the TCRBV13 family accounted for 68% of the CD4^{+} T cell population. Sequence analysis of 11 independent TCRBV13^{+} clones indicated a single VDJ structure. The monoclonality was again confirmed by hybridization with the DIVB13 CDR3-specific probe, which detected 160 out of 160 TCRBV13^{+} clones. The same was true for TCRBV14, which accounted for as many as 75% of CD8^{+} T cells in the same patient. Sequence analysis of five independent clones identified only two different junctions, and the DIVB14a and DIVB14b probes specific for each CDR3 hybridized 160 out of 176 and 16 out of 176 TCRB14^{+} clones, respectively. This confirmed the absence of other junctional sequences in these clones. According to the other sequences (except for the TCRBV6 family in CD4^{+} T cells, which comprised two clones) the other five TCRBV families detected in patient 1 were most likely monoclonal (Table II). In patient 2, sequence analysis showed that each of the seven TCRBV families detected by hybridization reflected the presence of one (TCRBV3 and TCRBV9 in CD4^{+} and TCRBV1 in CD8^{+}), two (TCRBV1, TCRBV5, and TCRBV21 in CD4^{+}), or at most three (TCRBV6 in CD4^{+} and TCRBV6 and TCRBV14 in CD8^{+}) different clones (Table II). Comparisons of CDR3 sequences revealed three types of homologies. As a first type we found homologies between clones of the same patient. They used the same TCRBV segment and exhibited an identical CDR3 length. Those are represented by sequences 17 and 18 (TCRBV6S1; 12 amino acids) and sequences 20 and 21 (TCRBV21; 10 amino acids) in the CD4^{+} subpopulation of patient 2. A second group of homologies is made of sequences found in different patients and exhibiting the previous charac-

Table II. VDJ Junction Sequences in Omenn's Syndrome

Patient 1 CD4+

Seq#	TCRBV	CDR3	TCRBJ	1	2
		C A S ... S S Q G Q V Y E Q Y F			
1	3S1	TGTGCCAGC AGTTCCCAgggacaggTCTACGAGCAGTAC TTC	2S7	4/4	
		C A T ... G T D I S Y E Q Y F			
2	5S1	TGCGCCA CA GGAACCgacaTCTCCTACGAGCAGTAC TTC	2S7	5/5	
		C A S S L T R D G Y N E Q F			
3	6S1	TGTGCCAGC AGCTTAACgagggACGGTTACAATGAGCAG TTC	2S1	2/6	
		C A S S D G A H N E Q F			
4	6S1	TGTGCCAGC AGCGACggggcCCACAATGAGCAG TTC	2S1	4/6	
		C A S S H P N T E A F			
5	13	TGTGCCAGC AGCCACCCGAACACTGAAGCT TTT	1S1	11/11	160/160
		C A S S P T S G G P Y Y N N E Q F			
6	18S1	TGTGCCAGC TCACCCACGTCTGgggggcCTTATTATAACAATGAGCAG TTC	2S1	3/3	
		C A S S L G G A E A F			
7	21S1	TGTGCCAGC AGCTTAGggggcGCTGAAGCT TTT	1S1	3/3	

Patient 1 CD8+

Seq#	TCRBV	CDR3	TCRBJ	1	2
8		C A S S L S Y E Q Y F			
	6S1	TGTGCCAGC AGCTTGTCCTACGAGCAGTAC TTC	2S7	5/5	
		C A S R L L P L N T E A F			
9	14S1	TGTGCCAGC AGGTTACTGCCCCTGAACACTGAAGCT TTT	1S1	2/10	16/176
		C A S S L G F D E Q F			
10	14S1	TGTGCCAGC AGTTTggggTTCGATGAGCAG TTC	2S1	8/10	160/176

Patient 2 CD4+

Seq#	TCRBV	CDR3	TCRBJ	1	
		C A S S P R T G Q W P Q H F			
11	1	TGTGCCAGC AGCCCGAggacaggCCAATGGCCCCAGCAT TTT	1S5	3/5	
		C A S S V E E T Q Y F			
12	1	TGTGCCAGC AGCGtagAAGAGACCCAGTAC TTC	2S5	2/5	
		C A S S L L G G A K A F			
13	3	TGTGCCAGC AGCTTATTGgggggcGCGAAAGCTTTC TTT	1S1	3/3	
		C A Q G F G E R N Q P Q H F			
14	5	TGTGCC CAG GGCTTCGgggagAGGAATCAGCCCCAGCAT TTT	1S5	2/4	
		C A Q G L S Y E Q Y F			
15	5	TGTGCC CAA GGATTGTCCTACGAGCAGTAC TTC	2S7	2/4	
		C A S S T S Y E Q Y F			
16	6S6	TGTGCCAGC AGCACCTCCTACGAGCAGTAC TTC	2S7	4/4	
		C A S S L R P G Q G N T E A F			
17	6S1	TGTGCCAGC AGCTTAAGACCgggacagggGAACACTGAAGCTTTC TTT	1S1	5/8	
		C A S S L V L P V G R T E A F			
18	6S1	TGTGCCAGC AGCTTAGTACTCCCAGTggggaggACTGAAGCTTTC TTT	1S1	3/8	
		C A S S Y S N Q P Q H F			
19	9	TGTGCCAGC AGCTATAGCAATCAGCCCCAGCAT TTT	1S5	5/5	
		C A S S S R R M N T E A F			
20	21S1	TGTGCCAGC AGCTCGCGAcggATGAACACTGAAGCTTTC TTT	1S1	1/3	
		C A S S L D L G T D T Q Y F			
21	21S1	TGTGCCAGC AGCTTAGATTTGGGCACAGATACGCAGTAT TTT	2S3	2/3	

(*Continued*)

teristics (same TCRBV segment, identical CDR3 length). Two subgroups were found in clones using TCRBV14. Sequences 10 (CD8+ T cells from patient 1) and 27 (CD8+ T cells from patient 2) with a CDR3 length of 7 amino acids, and sequences 9 (CD8+ T cells from patient 1), 28 (CD8+ T cells from patient 2), and 32 (PBL from patient 3) with a CDR3 made of 9 amino acids. These two types of homologies could be representative

of antigen-driven selections. A third type of CDR3 homology was observed for sequences 8, 15, 16, and 26 (bottom of Table II). Although these clones belong to different subpopulations and expressed different TCRBV, they all used the TCRBJ2S7 segment and exhibited a 7–amino acid long CDR3. More strikingly, an hydroxylic residue (serine or threonine) was always found at position 3 in these CDR3s. Two additional sequences

Table II (Continued)

			CDR3				

Patient 2 CD8+

Seq#	TCRBV				TCRBJ		
		C A S	S V P S V E Q Y	F			
22	1	TGTGCCAGC	AGCGTCCCAAgcgTCGAGCAGTAC	TTC	2S7	3/3	
		C A S	S L Y Q E T Q Y	F			
23	6S1	TGTGCCAGC	AGCCTGTACCAAGAGACCCAGTAC	TTC	2S5	2/10	
		C A S	S Y T G E L F	F			
24	6S1	TGTGCCAGC	AGCTACACCGGGGAGCTGTTT	TTT	2S2	6/10	
		C A S	R P P Y T D T Q Y	F			
25	6S1	TGTGCCAGC	AGACCTCCTTACACAGATACGCAGTAT	TTT	2S3	2/10	
		C A S	S T T Y E Q Y	F			
26	14S1	TGTGCCAGC	AGTACGACCTACGAGCAGTAC	TTC	2S7	5/11	
		C A S	S F S G G R F	F			
27	14S1	TGTGCCAGC	AGTTTTTcaggggGGCGGTTC	TTC	2S1	3/11	
		C A S	S V P T R E T Q Y	F			
28	14S1	TGTGCCAGC	AGCGTACCTACtagGGAGACCCAGTAC	TTC	2S5	3/11	

Patient 3 PBL

Seq#	TCRBV				TCRBJ	1	2
		C A S	S V G G F N E Q Y	F			
29	1	TGTGCCAGC	AGCTTGGgcgggTTCAACGAGCAGTAC	TTC	2S7	4/4	67/67
		C S A	R F P S G K T D T Q Y	F			
30	2	TGCAGTGC	TAGGTTCCCtagcgggaAGACAGATACGCAGTAT	TTT	2S3	5/5	
		C S S	G T L A G G T D T Q Y	F			
31	4	TGCAGCTCC	GGGACCctagcgggGGGCACAGATACGCAGTAT	TTT	2S3	T/T	
		C A S	S L A P L E E A F	F			
32	14S1	TGTGCCAGC	AGTTTAGCCCCTTTggaAGAAGCTTTC	TTT	1S1	2/2	55/55
		C A S	S L G L A G E E T Q Y	F			
33	21S1	TGTGCCAGC	AGCTTAggactagcgggagAAGAGACCCAGTAC	TTC	2S5	3/3	

CDR3 homologies

Seq#	TCRBV	CDR3 positions		TCRBJ
		1 2 3 4 5 6 7		
8	6S1	S L [S] Y E Q Y		2S7
15	5	G L S Y E Q Y		2S7
16	6S6	S T S Y E Q Y		2S7
26	14S1	S T T Y E Q Y		2S7
24	6S1	S Y T G E L F		2S2
27	14S1	S F S G G R F		2S1

1, X/Y, X times found in Y sequences of a given TCRBV. 2, x/y, x times found in y TCRBV clones after hybridization with junction-specific primer.

(24 and 27) showed the same (7 amino acids and a serine or a threonine at position 3) although using another TCRBJ segment. These homologies between clones belonging either to the CD4 or the CD8 subpopulation would rather suggest a superantigen-driven selection or a nonconventional antigen stimulation. It was shown recently that *Mycoplasma* or *Urtica doica* superantigens could select the CDR3 and TCRBJ segment, respectively (19, 20).

TCRBV repertoire of tissue-infiltrating lymphocytes. To determine whether the highly restricted number of T cell clones observed in peripheral blood of patients with Omenn's syndrome was representative of the whole T cell population, we performed an immunohistochemical study with 18 anti-TCRBV mAbs on lymph node, spleen, skin, and intestine samples available from patient 2 (Table III). The results were compared with the peripheral blood lymphocyte TCRBV repertoire. Six different TCRBV families (TCRBV 3, 5.2, 6.1, 9, 12, and 14) were detected in tissue-infiltrating lymphocyte. Only a few cells were stained by the anti-TCRBV14 antibody, but the TCRBV14S1J1S1 amplification products using genomic DNA from spleen and skin (data not shown) indicated that TCRBV14 T cells were actually present in tissues. The other 12 anti-TCRBV mAbs which reproducibly stained positive controls did not reveal the presence of additional TCRBV families in tissue-infiltrating lymphocytes as compared with PBMC from this patient.

In the intestine, the T cell infiltrate, mostly located in the lamina propria with a few scattered intraepithelial cells, was too sparse to allow comparison of TCRBV families. Histology of lymph nodes and spleen showed the atrophy of follicles. The T cell infiltrate was dense in the skin, with a majority of dermal T cells but also a significant fraction of T cells in the epidermis

Table III. Immunohistochemical Study of TCRBV Repertoire in Tissue-infiltrating Lymphocytes from Patient 2

TCRBV	Lymph node	Spleen	Skin (dermis/epidermis)	Intestine (lamina propria)
2	−	−	−	−
3	+	+	+/−	+
5.1	−	−	−	−
5.2+5.3	+	+	+/+	+
5.3	−	−	−	−
6.1	+	+	+/+	+
6.7	−	−	−	−
8	−	−	−	−
9	+	−	−	−
12	+	+	+/−	+
13.1	−	−	−	−
13.6	−	−	−	−
14	+	+	+	+
16	−	−	−	−
17	−	−	−	−
18	−	−	−	−
20	−	−	−	−
21	−	−	−	−

(Fig. 3 *A*). Immunophenotyping showed that the dermal infiltrate comprised both CD4$^+$ and CD8$^+$ T cells, whereas epidermal T cells were almost exclusively CD8$^+$ (not shown). TCRBV3, 5S2, 6S1, and 12 were detected in the dermal infiltrate (Fig. 3, *B–E*). Lack of TCRBV3 and TCRBV12 in epidermal T cells was coherent, as these TCRBV segments were expressed only by CD4$^+$ T cells in peripheral blood (Table I). TCRBV5S2 and 6S1 were detected in the dermis and the epidermis (Fig. 3, *C* and *D*). Rare TCRBV 14 cells were detected in the epidermis (not shown).

Counting of TCRBV-positive cells in the skin showed approximately the same number (10–20/HPF) of TCRBV3-, 5S2-, and 6S1-positive cells. TCRBV12 (5–10/HPF) and TCRBV14 (1–5/HPF) positive cells were less numerous. It was in contrast to peripheral blood where TCRBV6S1- and TCRBV14S1-positive cells were much more numerous than cells of any other TCRBV family. This could suggest either a preferential homing of TCRBV3- and TCRBV5S2-positive cells in the skin or a homing of TCRBV6S1 and TCRBV14S1 in other tissues.

Discussion

The T cell population is profoundly abnormal in Omenn's syndrome. Thymus is virtually devoid of T cells. Lymphoid organs are atrophic. Activated T cells are numerous in blood and infiltrate mostly the skin and intestine to a lesser extent (2). These T cells are poorly responsive to antigens and allogeneic cells in vitro (2–4) and exhibit an abnormally biased Th2 cytokine pattern (4, 5). Their CD4 or CD8 phenotype is heterogeneous and mostly unpredictable. Some patients exhibit either a hyper-CD4$^+$ (as patient 3 herein) or CD8$^+$ (as patient 1 herein) lymphocytosis or, sometimes, present a main CD4$^-$CD8$^-$ T cell population (3, 4).

Discrete rearrangement patterns detected by Southern blot analysis of T cell DNA from four patients suggested that the

TCRBV repertoire might be restricted in Omenn's syndrome (2). Given the limited sensitivity of this technique, together with the impossibility of ascribing TCRBV segments, we used an A-PCR–based amplification method to analyze the entire TCRBV repertoire and the molecular structure of the TCRB chain in three patients with Omenn's syndrome. Also, we had the opportunity to analyze qualitatively the TCRVA usage in one case. An immunofluorescence study was also performed in this case. The close correlation between the results obtained by A-PCR and FACS® analyses ruled out PCR-related artifacts.

The drastic restriction of TCRBV usage found in the three patients confirmed our previous findings (2). We analyzed the TCRBV repertoire of CD4$^+$ and CD8$^+$ T cells separately, in patients 1 and 2, and found that the two subsets were equally affected by this restriction. Therefore, any conclusion can be driven related to CD4 or CD8 status. The TCRBV repertoire of HLA-DR$^+$ and HLA-DR$^-$ CD4$^+$ T cells from patient 2 was similar, ruling out the possibility that activated cells biased the global repertoire because of their potentially higher mRNA content (16). These skewed profiles of TCRBV usage, together with the expression of common TCRBV families 6, 14, and 21 in the three patients, are reminiscent of superantigen-mediated stimulation (15, 21). Interestingly an evaluation of TCRBV repertoire by RT-PCR in a child with Omenn's syndrome previously showed a dramatic expansion of TCRBV14$^+$ cells (4). However, lack of TCRAV and TCRBJ repertoire analysis and sequencing did not allow for the ascription of this TCRBV profile to a superantigen or an antigen-mediated stimulation. In the present study the comparable restriction in TCRBJ usage, and the small number of CDR3 sequences within each TCRBV family, pointed to expansion of a limited number of clones possibly stimulated by a few recurrent antigenic determinants, as the CDR3 junction is generally associated with antigen recognition (22–24). This was supported by the identical CDR3 length among clones from patient 2 (TCRBV21 and TCRBV6S1) and among clones found in the three patients (TCRBV14 with a CDR3 made of 9 amino acids). However, the most striking finding concerned the CDR3 homology observed within a group of six clones. Although different TCRBV segments are used in these clones, they share similarities in length and amino acid composition with a conserved hydroxylic residue at position 3. Since these clones belonged to the CD4 or the CD8 subpopulation, this similarity could reflect an unusual mechanism of selection. Superantigens were shown, in some instances, to influence both the CDR3 structure (19) and the TCRBJ segment usage (20). Therefore, finding a conserved position within CDR3s using in most cases the same TCRBJ segments could reflect a superantigen selection. On the other hand it could also reflect an expansion of T cells in response to a nonconventional antigen and/or in the context of a defective negative selection. Finally, the finding of a restricted TCRAV repertoire in patient 2 is in keeping with a proliferation of few T cell clones. This scarcity of TCR diversity, despite large numbers of T cells, probably explains the defective in vitro T cell responses to conventional antigens in Omenn's syndrome (2).

The presence of abundant activated T cells in skin and gut in Omenn's syndrome suggests that these tissues may express target antigen(s). Therefore, it was of interest to determine the TCRBV repertoire of the infiltrating T cells. The TCRBV families detected in the tissues of patient 2 were also very re-
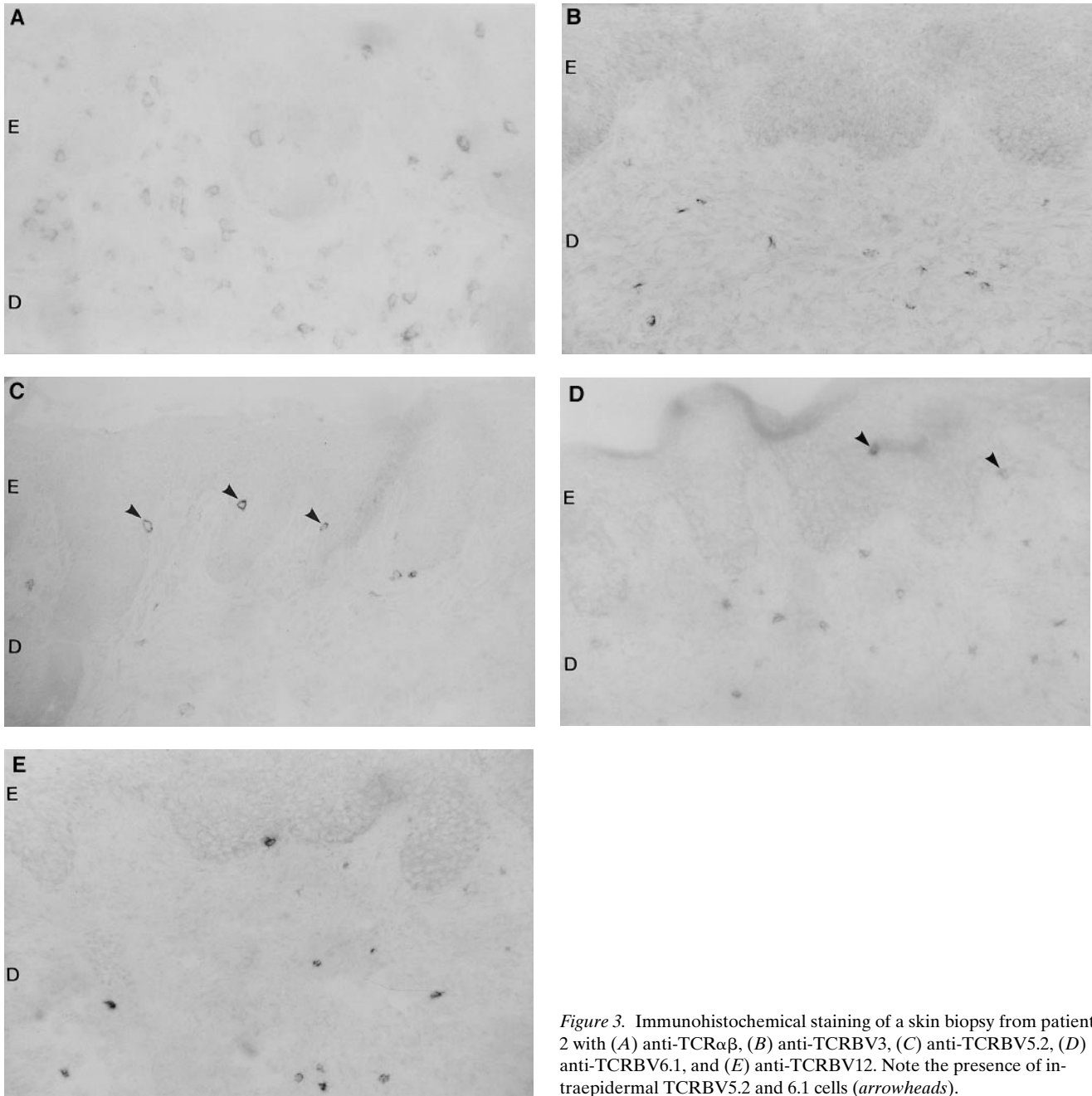
Figure 3. Immunohistochemical staining of a skin biopsy from patient 2 with (*A*) anti-TCRαβ, (*B*) anti-TCRBV3, (*C*) anti-TCRBV5.2, (*D*) anti-TCRBV6.1, and (*E*) anti-TCRBV12. Note the presence of intraepidermal TCRBV5.2 and 6.1 cells (*arrowheads*).

stricted and similar to those in this patient's peripheral blood. The overall T cell population consisted of no more than 11 different CD4^{+} and 7 different CD8^{+} clones in this particular patient. The relative overexpression of TCRBV3 and TCRBV5.2 in skin relative to peripheral blood indicates that accumulation of these TCRBV specificities may not simply result from nonspecific influx of circulating T cells but rather reflect antigen-driven homing. Moreover, the selective location of different TCRBV clones in the dermis or the epidermis is also in accordance with a nonrandom tissue migration of these cells. Selective expansion of T cells in target organs has been observed in various autoimmune diseases (25–27). Part of the TCRBV profile in Omenn's syndrome might thus be attributed to an autoimmune reaction towards undetermined antigen(s). How-

ever, this would not explain the absence of an otherwise normal and diverse T cell population as seen in autoimmune diseases (28, 29) and after superantigen stimulation (15). A more likely explanation is that these clones arise in the context of a major defect in T cell development as in SCID condition. Omenn's syndrome would then represent either a "mild" form or a partial reversion of such SCID conditions. Omenn's syndrome may thus correspond to the phenotype induced by an incomplete blockade of T cell differentiation, followed by the expansion of a small number of autoreactive T cell clones that are poorly responsive in vitro. Additionally the stochastic coreceptor phenotype observed in patients suggest a T cell development in absence of either central or negative selection. Obtention of cellular clones from patients will certainly be

helpful to get insights into functions of these peculiar lymphocyte populations.

Acknowledgments

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