

Restriction in T-Cell Receptor Repertoire in a Patient Affected by Trichothiodystrophy and CD4⁺ Lymphopenia

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Molecular analysis of T-cell receptor (TCR) repertoire, by measuring the CDR3 heterogeneity length of beta-variable regions (spectratyping), is useful for acquiring novel information on the status of immune system in primary immunodeficiency. Here, we evaluate TCR repertoire in a child with trichothiodystrophy (TTD) and combined immunodeficiency (CID). Spectratyping revealed marked alterations of TCR repertoire distribution: 21 and 10 out of 27 TCR V β (TCRBV) families and subfamilies were skewed in CD8⁺ and CD4⁺ subsets, respectively. These findings revealed, for the first time in a TTD patient with CID, a marked reduction in the TCR repertoire complexity, which may reflect alterations in the mechanisms regulating the generation and homeostasis of T cells.

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INTRODUCTION

Trichothiodystrophy (TTD) is an autosomal recessive disorder owing to alterations in nucleotide excision repair (NER) and basal transcription. Most of TTD patients carry different mutations in the *xp-d* gene. This gene encodes for a subunit of transcription factor II H (TFIIH), a complex involved in NER – a DNA repair mechanism – and basal transcription [1]. Immunological alterations have been reported in patients affected by xeroderma pigmentosum (XP) with genetic defects in the *xp-d* gene but not in TTD patients with mutations in the same gene [2, 3]. Moreover, fatal infections suggesting an immunodeficiency have been reported elsewhere in some TTD patients [4]. Recently, we described a case of TTD showing neurological and immune system abnormalities such as CD4⁺ lymphopenia and defects in dendritic cell maturation [5]. Here, we investigated the T-cell receptor (TCR) repertoire distribution in peripheral blood lymphocytes derived from this patient. For this purpose, we used the CDR3 heterogeneity length analysis, also known as *spectratyping* [6–8].

PATIENT AND METHODS

Case report. We report of a male infant with a TTD admitted at 3 years and 6 months of age to our hospital for severe chronic diarrhoea, failure to thrive and severe interstitial pneumonia, who was identified as affected by a combined immunodeficiency (CID).

Immunological investigations showed normal total lymphocyte numbers ranging from 1920 to 3350/mm³ with a normal number of T cells (up to 2140/mm³). We found a marked reduction of CD4⁺ cell number (25%; 535/mm³) and a slight hypogammaglobulinaemia with a reduction of immunoglobulin G2 (IgG2) level: IgG, 3.68 g/l; IgM, 2.13 g/l; IgA, 0.865 g/l; IgG1, 3.16 g/l; IgG2, 0.10 g/l; IgG3, 0.42 g/l. The memory and naïve percentage were normal in both CD4⁺ and CD8⁺ subsets (CD45RA 69%, CD45Ro 19%, CD4CD45RA 14%, CD8CD45RA 28%, CD4CD45Ro 9%, CD8CD45 Ro 7%) [5]. Patient's lymphocytes showed a reduced mitogenic response to PHA, OKT3, recall antigens and allogenic peripheral blood mononuclear cell (PBMC). The activation markers CD69, CD40L, CD25 and HLA-DR were normally expressed on T lymphocytes upon stimulation with phorbol 12-myristate 12-acetate (PMA)+ionomycin. Over the following 2 years, the patient received monthly Ig infusion and trimethoprim-sulfamethoxazole

prophylaxis. He died at the age of 7 for an untreatable interstitial pneumonia.

TCR repertoire analysis. T cells were obtained from patient's peripheral blood by Ficoll density gradient (Amersham, Pharmacia,

Uppsala, Sweden) and fractionated into CD4⁺ and CD8⁺ subsets using anti-CD4 or anti-CD8 monoclonal antibody-coupled magnetic beads (Dynal AS, Oslo, Norway). The purity of T-cell populations was tested by flow cytometry and ranged between 90 and 95%.

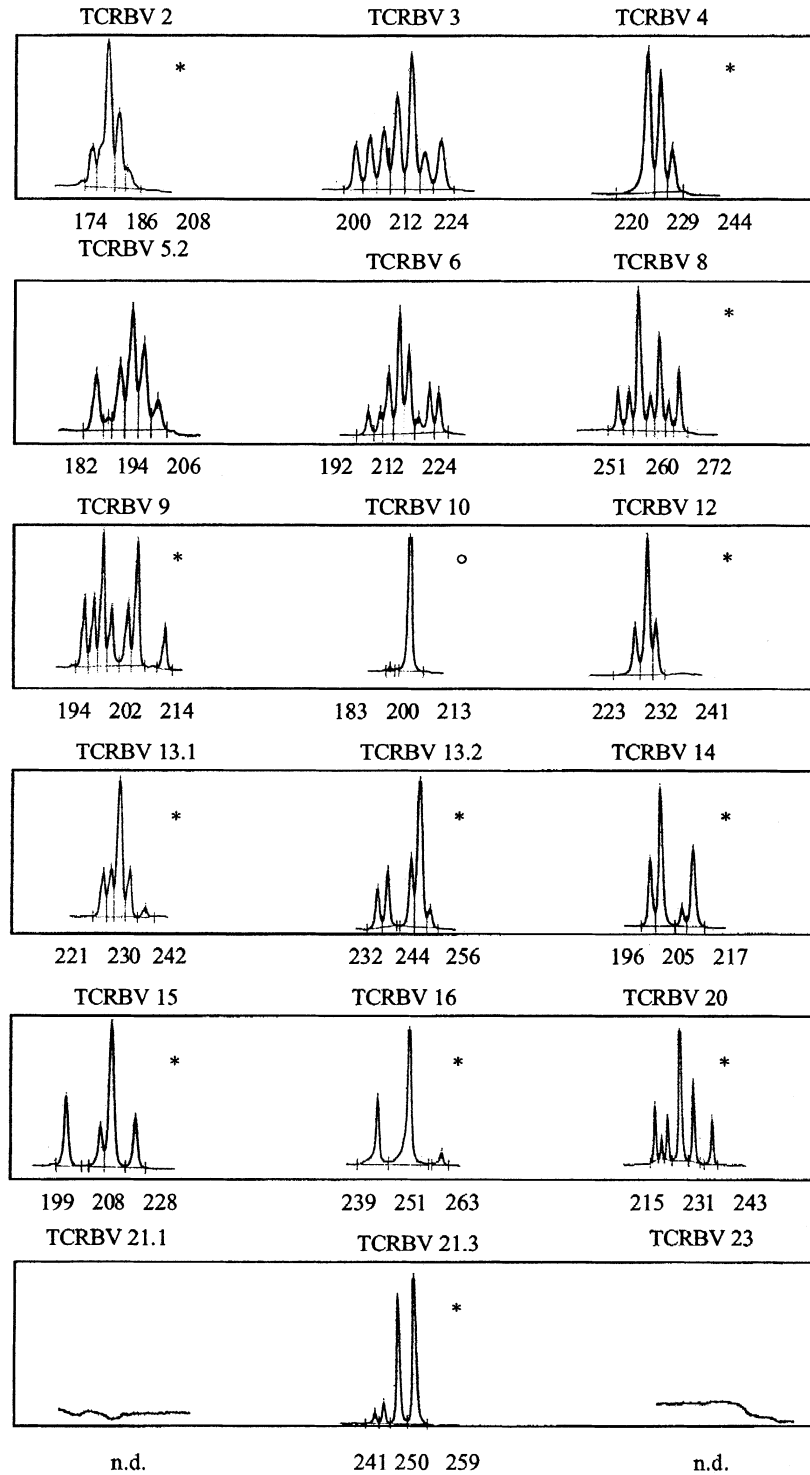


Fig. 1. T-cell receptor (TCR) CD3 length distribution of some TCR V β (TCRBV) families in CD8⁺ subset. Altered (skewed/perturbed) patterns are indicated by '*' and monoclonal by 'o'. On the x-axis bp units are shown.

RNA was extracted by Trizol™ (Gibco-BRL, Bethesda, MD, USA) according to the manufacturer's instructions. Reverse transcription and polymerase chain reaction (PCR) amplification were performed with primers for the 27 TCRBV families and subfamilies combined with one primer for the beta constant region (BC) [6]. Primer sequences for TCRBV from 21.1 to 24 were the following: BV21.1 CTGGTTCAATTTTCAGGATGAGAGT, BV21.2 GATTCGATATGAGAATGAGGAAGC, BV21.3 TCTGATT-CAGTTTCA GAATAACGG, BV22 AAAGAGGGAAACAGC-CACTCTG, BV23 CGCTGTGTCCCCATCTCTAATC, BV24 CAGTGACCCTGAGTTGTTCTCA [9].

The temperature profile included the following steps: 95°C 45 s, 55°C 45 s, 72°C 45 s for 32 cycles, followed by a final extension at 72°C for 10 min. PCR products were loaded on a 6% polyacrylamide gel, run for 400 min at 42°C in a DNA automatic fluorescence sequencer (Pharmacia ALF DNA Sequencer), and analysed with a specific software (Pharmacia DNA Fragment Manager 2.0). Briefly, each TCRBV family is resolved by this technique as a series of bands representing the length of the cDNA fragments showing a Gaussian distribution. The size distribution displayed two principal patterns: one consisting of a multipeak pattern and the other consisting of 1–4 dominant peaks. The multipeak pattern (5–8 peaks corresponding to sequences spaced by three nucleotides) was considered normal when the distribution showed a Gaussian-like shape; whenever the polyclonality showed an irregular distribution (defined as peaks' signal deviating more than twice compared with that of a corresponding position in a Gaussian distributed sample), or the signal consisted of one or more solitary peaks (defined as >50% of the total peak area), it was classified as altered [9, 10]. The distribution of the peaks was defined as follows: polyclonal (P), skewed/perturbed (SP), monoclonal (M). Each alteration, in either the distribution or the intensity of single bands, represents a perturbation in the given TCRBV family, which may reflect a reduction of the TCR repertoire complexity or an immune response toward a persisting immunodominant antigen.

RESULTS

Analysis of TCRBV repertoire

The analysis of TCR repertoire was performed at the age of 4 years and 10 months – at that time, the child was free from any chronic and acute infectious diseases.

The analysis of TCR repertoire showed abnormalities in the CD8 + T-cell population in 21 out 24 TCRBV families and subfamilies (Fig. 1). Among them, a monoclonal expansion of the pseudogene TCRBV 10 was also detected. The CD4⁺ population showed skewed distribution in 10 TCRBV out 27 families and subfamilies (Table 1, Fig. 2).

Two male healthy age-related controls and two age-related children (one female and one male) affected by hypogammaglobulinaemia without defects in T-cell compartment, treated by Ig infusion, were also analysed, showing a normal CDR3 length distribution in all the TCRBV families in both CD8⁺ and CD4⁺ subsets.

Table 1. CDR3 length profiles in T-cell subsets*

	CD4 ⁺	CD8 ⁺
TCRBV 1	SP	SP
TCRBV 2	P	SP
TCRBV 3	P	P
TCRBV 4	P	SP
TCRBV 5.1	P	SP
TCRBV 5.2–3	P	P
TCRBV 6.1–3	P	P
TCRBV 7	P	SP
TCRBV 8	P	SP
TCRBV 9	P	SP
TCRBV 10	SP	M
TCRBV 11	SP	SP
TCRBV 12	SP	SP
TCRBV 13.1	P	SP
TCRBV 13.2	P	SP
TCRBV 14	P	SP
TCRBV 15	P	SP
TCRBV 16	SP	SP
TCRBV 17	n.d.	SP
TCRBV 18	P	SP
TCRBV 20	SP	SP
TCRBV 21.1	SP	n.d.
TCRBV 21.2	SP	n.d.
TCRBV 21.3	P	SP
TCRBV 22	SP	SP
TCRBV 23	SP	n.d.
TCRBV 24	P	SP

*Polyclonal, P; skewed/perturbed, SP; monoclonal, M; non-detectable, n.d.

DISCUSSION

We report of a marked restriction in TCR repertoire distribution in a patient affected by TTD and CD4 lymphopenia. The skewed TCR CDR3 profiles, observed in this patient, may reflect either antigen-driven clonal expansions or defects in mechanisms regulating T-cell rearrangement and maturation [11, 12]. It is noteworthy that these alterations were not related to infections, as reported to happen during human immunodeficiency virus (HIV) infection or Epstein–Barr (EBV) infection [13, 14].

Neither severe nor frequent alterations of TCR repertoire have ever been reported in healthy children [10], even in patients with humoral immunodeficiency exposed to recurrent infections [15], as confirmed by the analysis performed on our controls. The skewed TCR repertoire distribution observed therefore may reflect alterations in the generation and survival of T-lymphocyte subsets, as reported in other primitive T-cell immunodeficiencies [11, 12, 15]. The marked CD4⁺ lymphopenia and the profound defect in the proliferative capability of

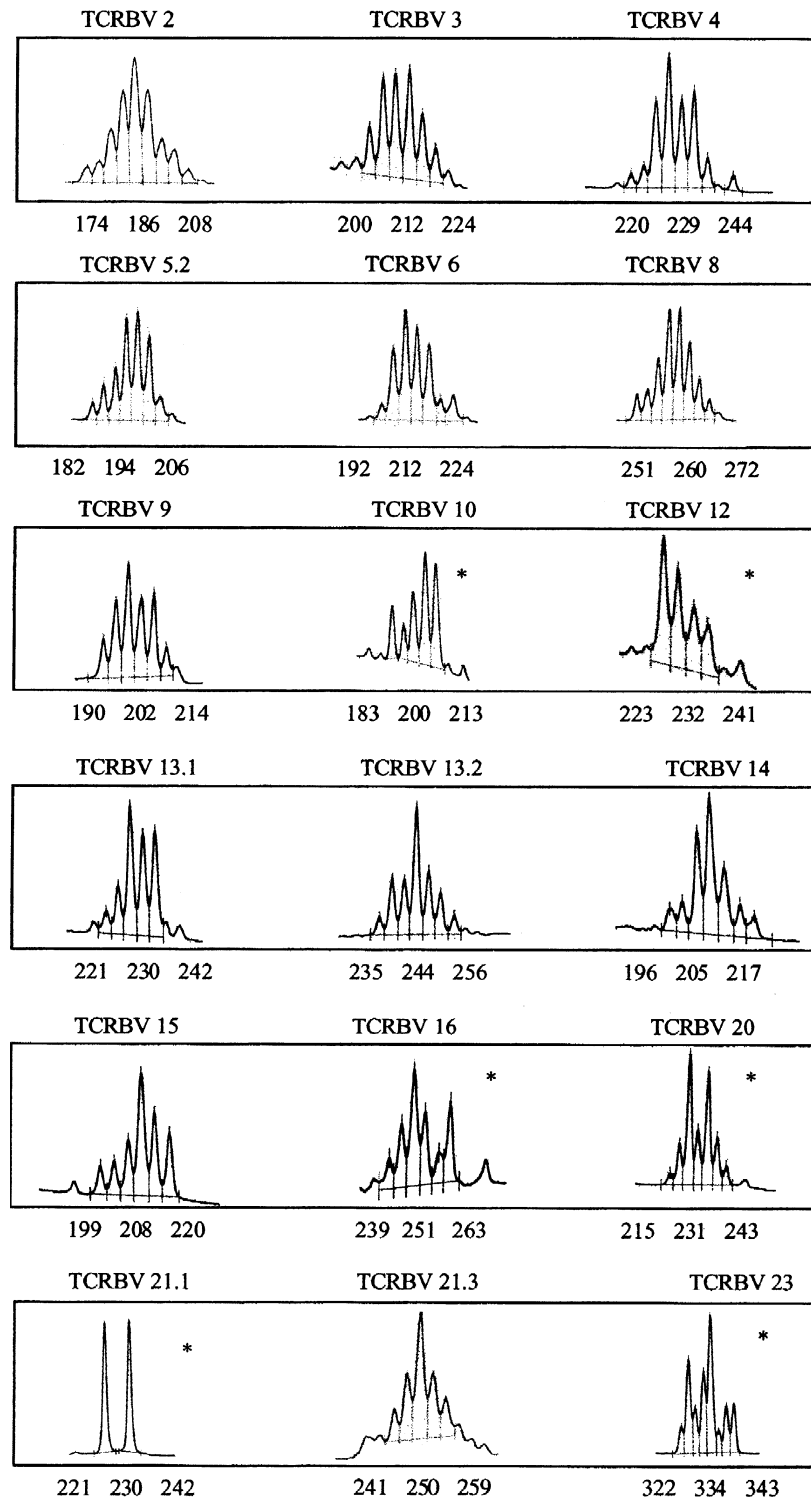


Fig. 2. T-cell receptor (TCR) CD3 length distribution of some TCR Vβ (TCRBV) families in CD4⁺ subset. Altered (skewed/perturbed) patterns are indicated by '*' and monoclonal by 'o'. On the x-axis bp units are shown.

T lymphocytes, strongly support the hypothesis that a defect in mechanisms regulating the generation as well as the survival of this lymphocyte subset may account for the reduction

of TCR complexity. Conversely, despite the CD8⁺ number was normal in TTD patient, we observed more severe alterations in CD8 TCR repertoire compared with the CD4⁺ subset.

Indeed, alterations of TCR repertoire have been reported to persist longer and to be more severe in CD8⁺ than in the CD4⁺ subset [11, 12]. This could be due to the different homeostasis of CD4⁺ and CD8⁺ cells. The marked alterations in TCR complexity may reflect the accumulation of few mature CD8⁺ T cells responding to homeostatic compensatory mechanisms triggered by CD4⁺ loss [16]. A progressive decrease of TCR repertoire complexity, more evident in CD8⁺ subset than CD4⁺, has been observed with age, as reported in adults [17]. This gradual loss could be evident earlier in TTD–CID patient, revealing that the size and the complexity of TCR repertoire may be differentially regulated in immunodeficiency conditions.

Mutations in the *xp-d* gene impair TFIIH function involved in DNA repair and basal transcription [1–4]. In TTD, the transcription deficiency may become manifest at the end of cell differentiation process when the transcriptional load is high, as proposed in a TTD mouse model by de Boer *et al.* [18]. As well, in this patient, an efficient level of transcription of newly synthesized molecules involved in T-cell maturation and survival could be affected. Therefore, a deficiency in TFIIH activity may impair the process of TCR gene rearrangement as well as the differentiation and survival of T lymphocytes, and in turn affect the complexity of T-cell repertoire.

In conclusion, the severe restriction observed in TCRBV repertoire might better explain the development of immunodeficiency in this child.

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REFERENCES

- Berneburg M, Lehmann AR. Xeroderma pigmentosum and related disorders: defects in DNA repair and transcription. *Adv Genet* 2001;43:71–102.
- Mariani E, Facchini A, Honorati MC *et al.* Immune defects in families and patients with xeroderma pigmentosum and trichothiodystrophy. *Clin Exp Immunol* 1992;88:376–82.
- Gaspari AA, Fleisher TA, Kraemer KH. Impaired interferon production and natural killer cell activation in patients with the skin cancer-prone disorder, xeroderma pigmentosum. *J Clin Invest* 1993;92:1135–42.
- Botta E, Nardo T, Broughton BC, Marinoni S, Lehmann AR, Stefanini M. Analysis of mutation in the gene in Italian patients with trichothiodystrophy: site of mutation correlates with repair deficiency but gene dosage appears to determine clinical severity. *Am J Hum Genet* 1998;63:1036–48.
- Racioppi L, Cancrini C, Romiti ML *et al.* Defective dendritic cell maturation in a child with nucleotide excision repair deficiency and CD4 lymphopenia. *Clin Exp Immunol* 2001;126:511–8.
- Choi Y, Kotzin B, Herron L, Callan MFC, Marrack P, Kappler J. Interaction of *Staphylococcus aureus* toxin ‘superantigens’ with human T-cells. *Proc Natl Acad Sci USA* 1989;86:8941–5.
- Gorski J, Yassai M, Zhu X *et al.* Circulating T-cell repertoire complexity in normal individuals and bone marrow recipients analysed by CDR3 size spectratyping. *J Immunol* 1994;152:5109–19.
- Pannatier C, Even J, Kourilsky P. T-cell repertoire diversity and clonal expansion in normal and clinical samples. *Immunol Today* 1995;4:176–81.
- Romiti ML, Cancrini C, Castelli-Gattinara G *et al.* Kinetics of the T-cell receptor CD4 and CD8 V β repertoire in HIV-1 vertically infected infants early treated with HAART. *AIDS* 2001;15:2075–84.
- Halapi E, Jeddi-Tehrani M, Blucher A. Diverse T-cell receptor CDR3 length patterns in human CD4⁺ and CD8⁺ T lymphocytes from newborns and adults. *Scand J Immunol* 1999;49:149–54.
- Signorini S, Pirovano S, Fiorentini S *et al.* Restriction of T-cell receptor repertoires in idiopathic CD4⁺ lymphocytopenia. *Br J Haematol* 2000;110:434–7.
- Brooks EG, Fillipovic AH, Padget JW, Mamlock R, Goldblum RM. T-cell receptor analysis in Omenn’s syndrome: evidence for defects in gene rearrangements and assembly. *Blood* 1999;93:2042–50.
- Callan MFC, Steven N, Krausa P *et al.* Large clonal expansion of CD8⁺ T cells in acute infectious mononucleosis. *Nat Med* 1996;2:906–11.
- Pantaleo G, Soudeyans H, Demarest JF *et al.* Evidence for rapid disappearance of initially expanded HIV-specific CD8⁺ T cell clones during primary HIV infection. *Proc Natl Acad Sci USA* 1997;94:9848–53.
- Serrano D, Becker K, Cunningham-Rundles C, Mayer L. Characterization of the T cell receptor repertoire in patients with common variable immunodeficiency: oligoclonal expansion of CD8 (+) T cells. *Clin Immunol* 2000;97:248–58.
- Fry TJ, Mackall CL. Interleukin-7: master regulator of peripheral T-cell homeostasis? *Trends Immunol* 2001;22:564–71.
- Hall MA, Reid JL, Lanchbury JS. The distribution of human TCR junctional region lengths shifts with age in both CD4 and CD8 T cells. *Int Immunol* 1998;10:1407–19.
- de Boer J, de Wit J, van Steeg H *et al.* A mouse model for the basal transcription/DNA repair syndrome trichothiodystrophy. *Mol Cell* 1998;1:981–90.