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Differential Biological Role of CD3 Chains Revealed by Human Immunodeficiencies[1]

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The biological role in vivo of the homologous CD3γ and δ invariant chains within the human TCR/CD3 complex is a matter of debate, as murine models do not recapitulate human immunodeficiencies. We have characterized, in a Turkish family, two new patients with complete CD3γ deficiency and SCID symptoms and compared them with three CD3γ-deficient individuals belonging to two families from Turkey and Spain. All tested patients shared similar immunological features such as a partial TCR/CD3 expression defect, mild $\alpha\beta$ and $\gamma\delta$ T lymphocytopenia, poor in vitro proliferative responses to Ags and mitogens at diagnosis, and very low TCR rearrangement excision circles and CD45RA$^+$ $\alpha\beta$ T cells. However, intrafamilial and interfamilial clinical variability was observed in patients carrying the same *CD3G* mutations. Two reached the second or third decade in healthy conditions, whereas the other three showed lethal SCID features with enteropathy early in life. In contrast, all reported human complete CD3δ (or CD3ε) deficiencies are in infants with life-threatening SCID and very severe $\alpha\beta$ and $\gamma\delta$ T lymphocytopenia. Thus, the peripheral T lymphocyte pool was comparatively well preserved in human CD3γ deficiencies despite poor thymus output or clinical outcome. We propose a CD3$\delta \gg$ CD3γ hierarchy for the relative impact of their absence on the signaling for T cell production in humans. ***The Journal of Immunology,*** **2007, 178: 2556–2564.**

Mature $\alpha\beta$ T cells detect peptides on MHC molecules by way of a variable cell surface heterodimer termed the $\alpha\beta$ TCR (1). Before reaching the membrane, variable $\alpha\beta$ TCR heterodimers associate in a preferential sequence with three invariant dimers collectively called CD3 (CD3$\delta\varepsilon$, CD3$\gamma\varepsilon$, and $\zeta\zeta$) (2). The stoichiometry of the full $\alpha\beta$ TCR/CD3 complex is most likely $\alpha\beta/\delta\varepsilon\gamma\varepsilon\zeta\zeta$ (3). Once on the cell surface, CD3 proteins translate ligand recognition by $\alpha\beta$ TCR chains into intracellular signals that drive T cell maturation or apoptosis in the thymus, and T cell activation, proliferation, and effector function or anergy/apoptosis in the periphery (4). During early T cell development, some CD3 chains may act alone or assist immature TCR ensembles, such as those containing pre-TCR chains. CD3 chains lack intrinsic enzymatic activity for signal transduction. Rather, they relay on phosphorylation-dependent recruitment and the activation of a number of cytosolic and transmembrane protein tyrosine kinases and adaptors such as Zap-70, Fyn, Lck, TRIM, LAT, SLP76, and SIT (5). The intracellular substrates of each CD3 chain may be different, together with their respective signaling pathways (6).

The complete lack of any of the chains of the invariant dimers causes in humans a group of rare T lymphocyte immunodeficiencies that, in the case of δ and ε, partially resemble their murine models, i.e., severe selective $\alpha\beta$ T lymphocytopenia and absent $\alpha\beta$ TCR/CD3 surface expression, associated with SCID features and early lethality before 3 years of age (for a review, see Ref. 7). In contrast, CD3γ deficiency was reported to allow in humans (8), but not in mice (6, 9), the selection of substantial numbers of polyclonal peripheral T cells that express relatively high levels of functional $\alpha\beta$ TCR/CD3 complexes (10, 11), albeit with an abnormal stoichiometry ($\alpha\beta/\delta\varepsilon\delta\varepsilon\zeta\zeta$) and an impaired association to $\zeta\zeta$ dimers (11, 12). Two healthy, unrelated individuals lacking CD3γ have been reported to reach their second and third decade, respectively, but the defect was lethal in a sibling with SCID symptoms (13). It is not clear why complete human CD3γ deficiencies are clinically milder and phenotypically leakier for T lymphocyte numbers than in the murine model or complete human CD3δ or ε deficiencies, and further cases can help to settle the matter.

In this study we have characterized the clinical, immunological, and genetic features of two new patients with complete CD3γ deficiency, lethal SCID symptoms, and partial T lymphocytopenia and compare them with three previously reported cases and with other CD3 deficiencies. The results shed light on the relative role of each CD3 chain in TCR/CD3 expression and function in humans.

Materials and Methods

Case reports

A 7-mo-old boy (subject VI:1 of family 1; Fig. 1*A*), born in May 2004 to distantly consanguineous parents, suffered chronic diarrhea and pulmonary infections, recurrent otitis media, oral moniliasis, severe diaper dermatitis,

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Table I. *Primers used for PCR amplification of human* CD3G *exons*

Exon and Primer	Sequence
Exon 1	
Forward	5′-CCCAGCCCCAACAGTGATG-3′
Reverse	5′-AGAGGTAGGCTGAAGGTTAGGGATA-3′
Exon 2	
Forward	5′-CCCAGGATACCAGGACAAAGATG-3′
Reverse	5′-ATCCCCGTCATTTCATTCATTCTA-3′
Exon 3	
Forward	5′-TGGTATGCAGAAGCAGGGAGAA-3′
Reverse	5′-TAAAAAGCTCACCAGAACAGCAAAT-3′
Exon 4	
Forward	5′-CCCTCCACAGCGGCATTATT-3′
Reverse	5′-GCACACTAGCAAGAAGCACCAACT-3′
Exon 5	
Forward	5′-GGATTAACTTTGCTTGTTTTTGTCC-3′
Reverse	5′-GTCGAGAGTTTCTTCGTGTGCC-3′
Exon 6	
Forward	5′-CTCCTTGTCCTCTTTCCATCCTC-3′
Reverse	5′-GAAGGGAGCTAGACTTAGAAGCAAT-3′
Exon 7	
Forward	5′-AGGAAGACAGGGTTGAAAGCATTAT-3′
Reverse	5′-TCACTGAATATTTGAGCCATGTGAA-3′

and perianal fistula. The immunological workup with BD Biosciences reagents showed 2180 lymphocytes/μl with partial T lymphocytopenia: 43% $CD19^+$, 18% $CD16^+CD56^+CD3^-$, 21% $CD4^+CD8^-$, 18% $CD8^+CD4^-$, 14% $CD25^+$, low CD3 (27% with SK7) and TCR$\alpha\beta$ (2% with WT31), but not TCR$\gamma\delta$ (7.6% with 11F2, 3.2% also $CD8^+$, 5.6% also $CD3^+$). CD45 and HLA class I expression were both normal as well as IgG, IgM, and IgA (827, 41, and 84 mg/dl, respectively) and IgG1–4 subclasses (606, 69, 34, and <1.4, age-matched ranges 152–951, 18–216, 18–129, and 0–130, respectively). The proliferative responses to PHA and Con A were low compared with the control (6020 vs 43949 and 6504 vs 35760 cpm, respectively). Tested autoantibodies were negative (anti-nuclear Ab, smooth muscle, parietal, thyroglobulin, transglutaminase, and enterocyte). The boy underwent a bone marrow transplant at the age of 13 mo and a retransplant 5 mo later, but he died due to bilateral pneumonia at the age of 20 mo.

His older brother (VI:3) showed very similar symptoms (diarrhea since birth, oral moniliasis, and perianal fistula since 3 mo of age) and immunological findings at 5–6 mo: normal IgG, IgM, and IgA (2010, 229, and 223 mg/dl, respectively); 2473 lymphocytes/μl with partial T lymphocytopenia (26% $CD19^+$, 29% $CD16^+CD56^+CD3^-$, 18% $CD4^+CD8^-$, 11% $CD8^+CD4^-$, 2% $CD4^+CD25^+$, low CD3 (37% with SK7) and TCR$\alpha\beta$ (1% with WT31), but not TCR$\gamma\delta$ (6.7% with 11F2). CD18 expression and neutrophil chemotaxis were normal. Stool cultures were negative. As the diarrhea did not improve, he was referred to Ankara for bone marrow transplantation but died due to sepsis and diarrhea at 9 mo of age.

Molecular genetics

Genomic DNA was extracted following standard methods from peripheral blood samples of patients and unaffected relatives from families 1 and 2 (Fig. 1). Primers were designed to PCR-amplify all exons and the flanking intronic sequences of *CD3G* (GenBank accession no. NM_000073), *CD3D* (GenBank accession no. NM_000732), and *CD3E* (GenBank accession no. NM_000733) genes (see Table I for *CD3G*). PCR products were purified with the QIAquick PCR purification kit (Qiagen) followed by direct sequencing using the BigDye Terminator V3.1 cycle sequencing ready reaction kit in the ABI PRISM 3100 automatic sequencer (Applied Biosystems).

Members of family 1 were screened for the *CD3G* mutation c.205A→T by digesting exon 3 amplimers with the *Tru*9I restriction enzyme and running the digestion products in 3% agarose gels.

Subjects from both families were genotyped for microsatellite markers spanning the genetic interval that contains the *CD3G*, *CD3D*, and CD3*E* genes on chromosome 11q23 (D11S898, D11S4111, D11S1356, MICD3E, GDB:179879, D11S1364, D11S925, D11S4089, and D11S1336). The novel MICD3E marker is intragenic to *CD3E* and was developed by searching for tandem repeats of CA dinucleotide in the sequence contig (GenBank accession no. NT_033899; *Homo sapiens* genome view, build 35.1) and by designing flanking primers (forward: 5′-ATAGCCCCAAACTT TGCTCAC-3′; reverse: 5′-CATTAACTCTTTGTTACCCCAACTC-3′). The order of markers was established by integrating genetic and physical maps (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov) (Table II).

Table II. *Haplotype associated with the c.205A→T pathogenic mutation in* CD3G[a]

Marker	Family 1	Family 2	Genotype for CEPH Individual 134702	Heterozygosity (%)
D11S898	144	150	146/152	79
D11S4111	210	206	208/210	84
D11S1356	195	195	201/205	85
MICD3E	152	152	152/152	ND
GDB:179879	121	121	125/125	ND
D11S1364	138	142	142/142	71
D11S925	173	173	173/173	84
D11S4089	205	207	199/207	74
D11S1336	242	248	236/244	67

[a] Markers comprising the shared core haplotype are depicted in boldface. Relative order and physical distances are as follows: D11S4111-(2 Mb)-**D11S1356**-(270 kb)-**MICD3E**-(25 kb)-**GDB:179879**-(10 kb)-***CD3G:*205A→T**-(274 kb)-D11S1364. The marker heterozygosity was obtained from published sources (52). To allow other laboratories to compare their data with those reported in this work, we provide allele sizes for individual 134702, available from Center d'Etude du Polymorphisme Humain (52).

Abs and flow cytometry

The expression of different surface markers was studied by flow cytometry using standard procedures (10). The following mAb were used: anti-CD3$\varepsilon\gamma/\varepsilon\delta$ (UCHT-1) from Immunotech and anti-CD3 (Leu4), anti-CD3 (SK7), anti-CD4 (Leu2a), anti-CD8 (Leu3a), anti-CD45RA (Leu-18), anti-CD45RO (UCHL-1), anti-TCR$\alpha\beta$ (WT31), anti-TCR$\gamma\delta$ (11F2), and anti-CD8 (SK1) from BD Biosciences. An anti-CD3$\varepsilon\gamma/\varepsilon\delta$ (F101.01) hybridoma supernatant was a gift from Dr. B. Rubin (Centre Hospitalier Universitaire de Purpan, Toulouse, France). The mAb were FITC- or PE-conjugated or purified, and in the latter case a PE-conjugated goat anti-mouse IgG (H+L) from Caltag Laboratories was used as a secondary Ab. Background fluorescence was defined in all cases with an isotype-matched irrelevant mAb from Caltag Laboratories. Briefly, for single- and two-color immunofluorescence, 5×10^5 cells were incubated for 30 min at 4°C with the appropriate mAb in PBS buffer containing 1% FCS. After two washes with PBS, cells were analyzed in an Epics Elite Analyzer cytofluorometer (Coulter Electronics). For comparative stainings we used the mean fluorescence intensity, defined as the average fluorescence value of the corresponding mAb referred to the logarithmic scale of fluorescence intensity along the *x*-axis of the histograms.

TCR Vβ repertoire usage (~70% coverage) was determined within $CD3^+$ lymphocytes with the IOTest Beta Mark kit (Beckman Coulter) following the manufacturer's instructions.

TCR rearrangement excision circles (TRECs)[4]

Thymic function was evaluated by peripheral blood $CD45RA^+$ T cell numbers or TCR Vβ usage (see above) or by quantifying TRECs, the highly specific markers for T cells recently produced by the thymus. The δ deletion (signal joint) TRECs formed by δRec-ψJα rearrangement were amplified and quantified in genomic DNA from PBMC by real-time quantitative PCR using a LightCycler system (Roche) as previously described (14). Briefly, fluorescently labeled oligonucleotides were used as reporter probes for standard curve generation and quantification with the manufacturer's reagent kits, protocols, and software. Serial dilutions of a plasmid clone containing a 375-bp fragment of the δ TREC sequence (supplied by D. Douek, National Institute of Allergy and Infectious Diseases, Bethesda, MD) (15) were used to generate standard curves for quantification, and different amounts of a template were used to ensure a linear response. TREC abundance was normalized to cell number by a parallel amplification for a single copy/chromosome gene (β-globin), a plasmid of which was also used to generate standard curves. Data are expressed as TRECs per 10^5 cells using mean values from duplicate or triplicate assays for both TRECs and β-globin, which never varied >10%. Because there are two globin copies per cell, the TRECs content was calculated as [(mean TRECs quantity/mean globin quantity) (2×10^5)]. The detection limit was two copies. Standard stocks were characterized for total cell content and PCR

[4] Abbreviation used in this paper: TREC, TCR rearrangement excision circle.

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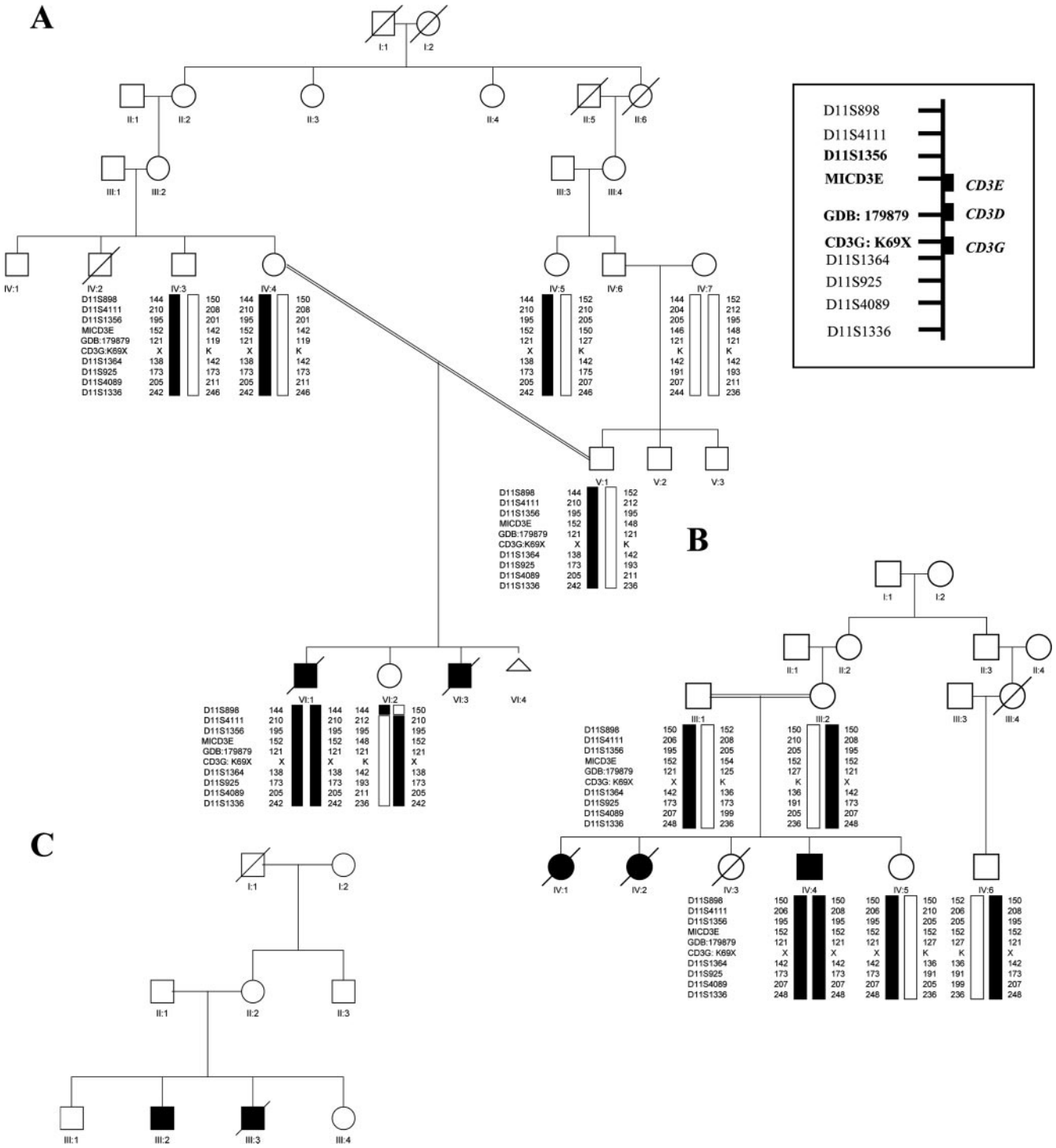

FIGURE 1. Pedigrees and haplotype analysis of families 1 (*A*) and 2 (*B*) based on the indicated polymorphic markers spanning the CD3*GDE* region on chromosome 11q23 (*inset*, shared core haplotype in boldface). Affected status and disease haplotype are depicted in black. The shared mutation is denoted as CD3G:K69X. Homozygous haplotype segregation was observed in patient VI:1 from family 1 and patient IV:4 from family 2. *C*, Pedigree of a Spanish family (family 3) characterized previously (17).

amplicon quantities by repeated measures over time and were saved as a quality assurance measure of all reagents for run-to-run variability.

Results

Mutation screening and haplotype analysis

Selective partial T lymphocytopenia ($T^{+/-}B^{+}NK^{+}$ phenotype) in patients VI:1 and VI:3 from family 1 (Fig. 1*A*), together with low surface CD3 expression, was suggestive of a hereditary recessive form of CD3 deficiency. Therefore, we sequenced all exons and intron/exon boundaries of *CD3G*, *CD3D*, and *CD3E* genes in patient VI:1. No mutations were detected in *CD3D* and *CD3E*, but the analysis revealed a homozygous A to T transversion at nucleotide 205 in exon 3 (c.205A→T; Fig. 2, *A* and *B*) of *CD3G*. This mutation results in the substitution of the lysine codon at position 69 by a premature stop codon (p.K69X), which is predicted to truncate the CD3γ protein shortly after the leader peptide and thus

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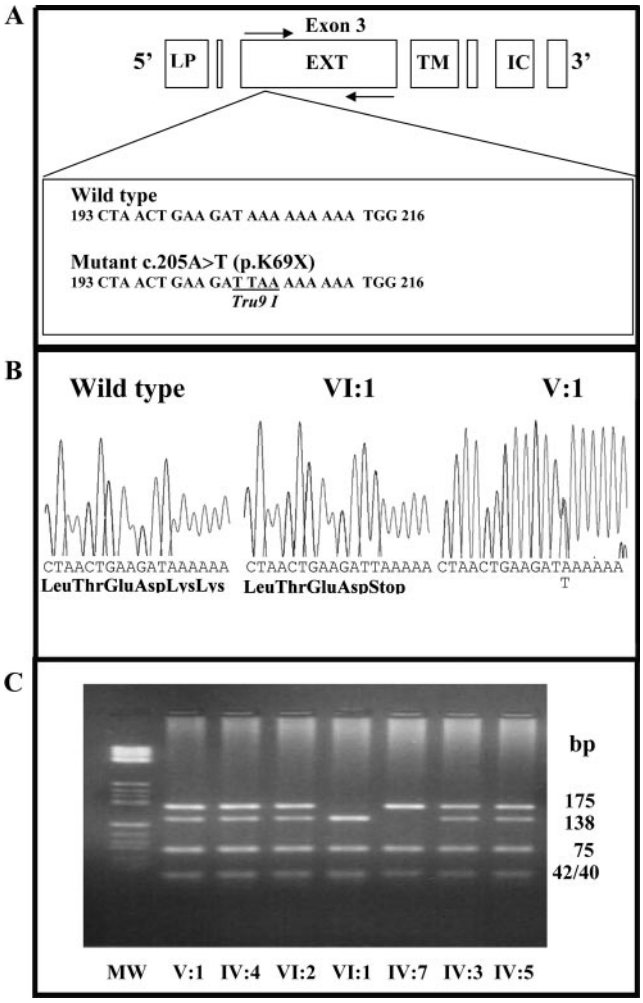

FIGURE 2. Mutation analysis of family 1. *A*, Wild-type and mutant sequences (with the *Tru9 I* restriction site underlined) are localized within *CD3G* exon 3. The arrows in exon 3 identify PCR primers. LP, EXT, TM, and IC indicate the leader peptide, extracellular, transmembrane, and intracellular domains of the protein, respectively. *B*, Electropherograms depicting the *CD3G* exon 3 sequence fragment that contains the mutation obtained from an unaffected subject (wild-type allele), a patient (VI:1, homozygous for the c.205 A→T mutation), and an heterozygous carrier (V:1). *C*, *Tru9 I* digestion of exon 3 PCR products (365 bp) from several family members. A wild-type amplimer (such as in IV:7) yielded five fragments of 175, 75, 42, 40, and 33 bp (only the first four are visible in this 3% agarose gel). When the c.205A→T mutation is present, the 175-bp fragment is cleaved in two parts of 138 and 37 bp (not visible). Several family members were heterozygous, whereas only the patient was homozygous for the c.205A→T mutation. MW, molecular mass marker.

cause the pathology. The mutation creates an additional restriction site for the enzyme *Tru*9I at exon 3. We took advantage of this finding to develop a screening test specific for the mutation (*Materials and Methods*, and Fig. 2). This test allowed us to follow the segregation of the mutation in the family and showed that it was, as expected, in a heterozygous state in both parents (obligate carriers) and, additionally, in three healthy relatives tested, including the patient's sister (Fig. 2*C*).

The mutation had been reported in another family (no. 2; Fig. 1*B*) described previously (16). Both families 1 and 2 came from the city of Diyarbakir in Southeast Turkey. Genealogic data, as far as available, revealed no relationship between them, but the geographic and ethnic origin of both families suggested the possibility of a common founder. This was investigated by haplotype analysis of microsatellite markers spanning the *CD3* region (Fig. 1). These results showed that both families shared a core haplotype associated with the c.205A→T mutation composed of alleles 195, 152, and 121 of markers D11S1356, MICD3E and GDB:179879, respectively, where the last two are in close proximity to the mutation (Table II and Fig. 1). These data suggest the possibility of a common founder allele bearing the c.205A→T mutation causing the CD3γ deficiency in the Turkish population.

Clinical and immunological spectrum of human CD3γ deficiencies

Patient VI:1 (and probably VI:3) from family 1 and patient IV:4 from family 2 therefore carried identical mutations in *CD3G*. However, their clinical features were disparate: patients VI:1 and VI:3 had clinical signs of SCID starting at 7 or 3 mo of age, respectively, whereas patient IV:4 is presently healthy and well into his teens (Table III). In a previous work, we reported a similar situation in two *CD3G* compound heterozygous [c.1A→G] plus [c.IVS2-1G→C] brothers belonging to a nonconsanguineous Spanish family (17) (Table III and Fig. 1*C*). Indeed, one of the siblings died early in life with SCID features (III:3), whereas his older brother (III:2) has reached his third decade in good health. The comparative immunophenotype of the five individuals (Figs. 3 and 4*A*) did not reflect substantial differences in TCR/CD3 expression levels between them, although the shared selective partial αβ (and γδ) T lymphocytopenia was more intense early in life (an average 3- to 9-fold in VI:1, VI:3, and III:3 vs 2- to 3-fold in IV:4 and III:2; Fig. 4*B*). There was no obvious correlation between in vitro functional assays and SCID vs non-SCID condition, because proliferation to several TCR-dependent stimuli was depressed in all patients at diagnosis (Table III). We also measured peripheral blood TRECs, $CD45RA^+$ T cells, and TCR Vβ usage as a means to estimate thymic output and function (18, 19) (Fig. 5). Unfortunately, early transplantation (patient VI:1) or decease (IV:3, III:3) precluded a more complete analysis of these parameters in SCID patients. Nevertheless, the results indicated that all tested patients had very few peripheral blood thymus emigrants ($CD45RA^+TREC^+$) as compared with age-matched controls, although the difference for TRECs was more meaningful in younger patients because TRECs are strongly reduced in normal adults (Fig. 5, *A* and *B*). In contrast, the memory ($CD45RO^+$) T cell pool and TCR Vβ usage were essentially normal by RT-PCR and/or flow cytometry (Ref. 11; Fig. 5, *A* and *C*). The size and survival of the naive T cell pool is more dependent on thymus output and TCR/MHC interactions, respectively, than the size and survival of the memory T cell pool (20). Thus, the results indicated that the lack of CD3γ impairs thymus production but not the peripheral expansion or accumulation of mature polyclonal T cells, which correlates with the presence of a small thymus in two tested individuals (13). Therefore, the lack of CD3γ can give rise in man to relatively similar immunological features with substantial peripheral T cells despite poor thymus output but with a wide spectrum of clinical symptoms.

Discussion

Founder effect for the c.205A→T CD3G *mutation*

We have genetically characterized a new familial case of CD3γ deficiency in Turkey. The fact that the *CD3G* mutation (c.205A→T) is associated with the same CD3-region haplotype in two affected families strongly supports a common founder allele causing CD3γ deficiency in the Turkish population. Therefore the

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Table III. *Summary of clinical and immunological data of human CD3γ deficiencies at diagnosis*

	Turkey			Spain	
	Family 1	Family 1	Family 2	Family 3	Family 3
Consanguinity	+	+	+	−	−
Individual[a]/sex	▼ VI:3/male	▲ VI:1/male	Δ IV:4/male	■ III:3/male	□ III:2/male
Deceased siblings	+	+	+	−	+
CD3G mutation(s)	Not tested	c.205A→T	c.205A→T	[c.1A→G] + [c.IVS2-1G→C]	[c.1A→G] + [c.IVS2-1G→C]
Predicted mutation(s)		p.K69X	p.K69X	[p.M1V] + [p.N28V/H29X]	[p.M1V] + [p.N28V/H29X]
Clinical status/age (†, at death)	Severe/†9 mo	Severe/†20 mo[b]	Mild/15 years	Severe/†32mo	Mild/24 years
Cause of death (†)	Sepsis	Pneumonia	Living	Pneumonia	Living
Age at diagnosis	3 mo	7 mo	4 years	1 year	4 years
Enteropathy[c]	+ (IBD)	+ (IBD)	−	+ (AE)	−
Autoimmunity[d]	Not tested	−	+	+	+
Lymphocyte no.[e]	Low	Low	Normal	Normal	Normal/low
T cells	Low	Low	Normal/low	Normal/low	Normal/low
B cells	Normal	Normal	Normal	Normal	Normal
NK cells	Normal	Normal	Normal	Normal	Normal
CD3 expression[e]	Low	Low	Low	Low	Low
PBMC responses to:					
Ags[f]	ND	ND	Low	Low	Low[g]
Anti-CD3	ND	ND	Low	Low	Low[g]
Lectins[f]	ND	Low	Low	Low	Low
IL-2	ND	ND	Normal	Normal	Normal
PMA + Ion[f]	ND	ND	Normal	ND	Normal

[a] Filled symbols identify SCID patients as in Figs. 3–5.
[b] Bone marrow transplantations at 13 and 18 mo of age.
[c] IBD, Inflammatory bowel disease; AE, autoimmune enteropathy.
[d] Vitiligo and low titer thyroglobulin and thyroid peroxidase autoantibodies after 17 years of age (III:2), gut epithelial cell, smooth muscle, and mitochondrial autoantibodies, and autoimmune hemolytic anemia (III:3), psoriasis, and low titer microsomal and thyroglobulin autoantibodies at diagnosis (IV:4).
[e] Figs. 3 and 4.
[f] Ags, tetanus toxoid or alloantigens; lectins, PHA or Con A; Ion, ionomycin.
[g] In vitro evaluation of PBMC response to bacterial (tuberculin, tetanus toxoid, and diphtheria) and viral (CMV, HSV, rubella, varicella, mumps, measles, influenza A and B) Ags or anti-CD3 was normal at 21–24 years of age.

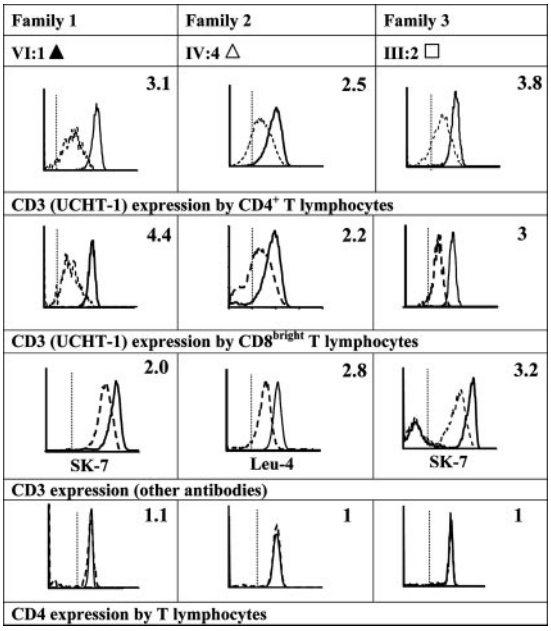

FIGURE 3. Comparative CD3 and CD4 expression between human CD3γ-deficient (dashed line) and control (solid line) T lymphocytes. Similar results were obtained with other CD3 (F101.01), TCRγδ (11F2), and control (CD45) Abs (data not shown). The vertical dotted line in each panel indicates the upper limit of background fluorescence using isotype-matched irrelevant mAb. The numbers in each histogram indicate the mean fluorescence intensity ratio between control and CD3γ-deficient cells, which are useful for comparative purposes between samples analyzed at different times and in different flow cytometers.

initial molecular diagnosis of Turkish patients with an immunophenotype suggestive of CD3γ deficiency regardless of the accompanying spectrum of clinical symptoms should consider screening for the c.205A→T mutation. The specific screening test developed here may be useful for the genetic diagnosis of this mutation in other laboratories.

Clinical spectrum of CD3γ deficiencies

Despite their relatively similar immunological features, there is clear clinical heterogeneity among human CD3γ deficiencies, ranging from healthy ($n = 2$, subjects IV:4 of family 2 and III:2 in the Spanish family) to life-threatening SCID cases ($n = 2$, Spanish subject III:3 and subject VI:1 of family 1; or $n = 3$, if we consider patient VI:3 of family 1). In contrast, human complete CD3δ or CD3ε deficiencies ($n = 8 + 2$, respectively) were all SCID infants with essentially no T cells (100- to 4600-fold reduction; Fig. 4*B*) (21).

Thus, two sets of CD3γ-deficient individuals sharing the same truncating mutations, similar immunological characteristics, and comparable medical care belonged to both clinical extremes. We believe their disparate clinical features reflect the involvement of either environmental or genetic factors (modifying genes) (22, 23). In the case of family 1 (and presumably also family 2, because it shared the same disease core haplotype), *CD3D* and *CD3E* were excluded as modifying genes, as they were shown to lack additional pathogenic mutations or nonsynonymous single nucleotide polymorphisms.

Although the functional data are unfortunately incomplete, both SCID and non-SCID patients showed poor PBMC proliferative responses to Ags and mitogens at diagnosis and persistent low TRECs. These results argue against a correlation of poor proliferative responses with the SCID condition. However, we cannot rule out that differences in thymus function (as observed between VI:1

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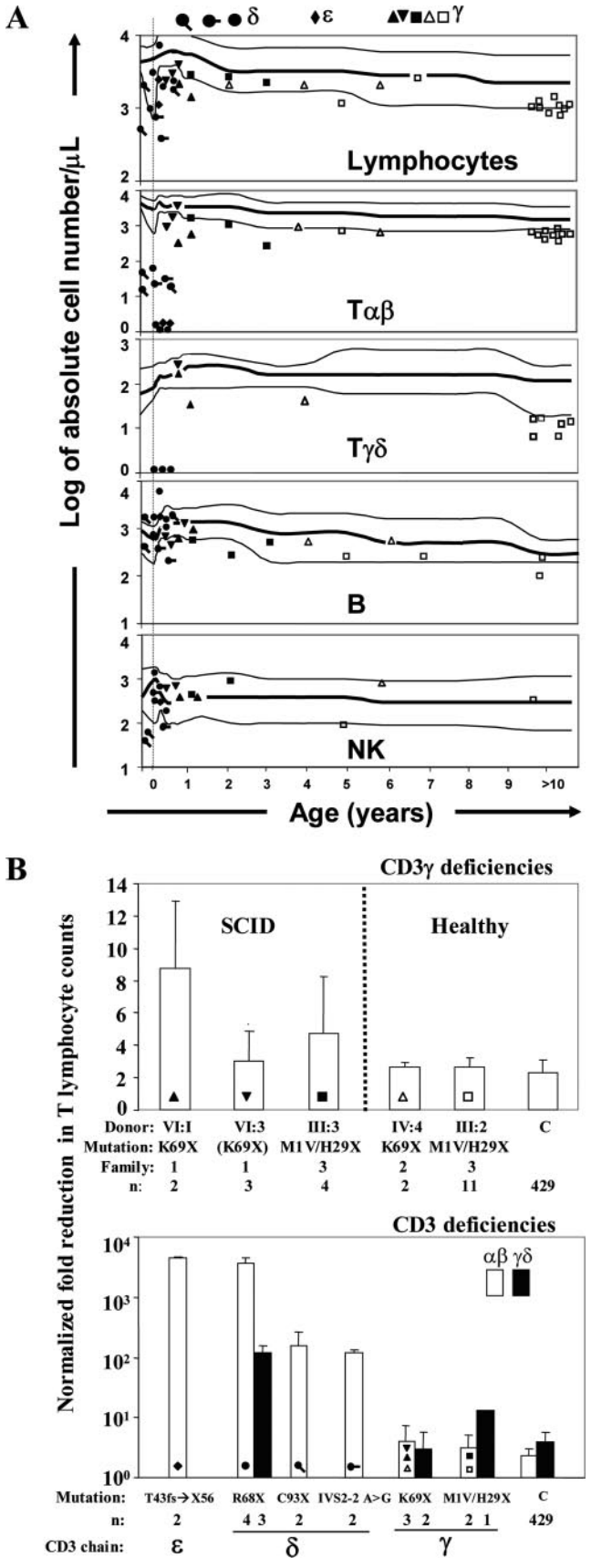

FIGURE 4. Comparative absolute lymphocyte counts in human complete CD3 deficiencies. Black symbols identify SCID patients. *A*, Individual total, T, B, and NK lymphocyte counts are plotted as a function of age in comparison with the normal distribution (P5, median, P95) (38, 39). Due to the CD3 expression defect, $\alpha\beta$ T lymphocytes were defined as $CD4^+$ plus $CD8^+$ or $CD8^{bright}$, thus excluding most $\gamma\delta$ T cells (<8% in all cases). T $\gamma\delta$, B, and NK lymphocytes were defined as $11F2^+$, $CD20^+$ or surface Ig, and $CD56^+$ or $CD16^+CD56^+$, respectively. Thus, $\gamma\delta$ T cells may be underestimated due to the TCR/CD3 expression defect. Data from five, eight, and two different individuals or fetuses from three, three, and one unrelated families for CD3γ (▲▼△ ■□), CD3δ (●, ● with diagonal bar, and ● with horizontal bar) and CD3ε (♦) deficiencies, respectively (8, 40–46). The specific mutations are listed in *B*. *B*, Normalized fold reductions in absolute T lymphocyte counts (cell/μl) were calculated from the above data as a ratio relative to the normal median in age-matched controls for each data point for the indicated individuals (*top*, CD3γ deficiencies, where *n* is the number of experiments) or disorders (*bottom*, CD3 deficiencies, where *n* is the number of different patients sharing the indicated mutations). The control range (labeled C) is P5/median. Subject VI:3 is presumed to carry the p.K69X mutation, hence the brackets. The average normalized reductions in $\alpha\beta$ T lymphocyte counts ranged 2- to 9-fold for CD3γ deficiencies but 100–4600 for CD3δ or CD3ε deficiencies. Reductions in $\gamma\delta$ T lymphocyte counts are also shown for comparison, although they are less reliable as explained above.

and IV:4; Figs. 4*B* and 5, *B* and *C*) may have caused the disparate clinical outcomes. Collectively, it seems that thymus output is poor in all patients in terms of naive ($CD45RA^+TREC^+$) T cells and, thus, functional T cells are scarce early in life. With time, however, as observed in surviving patients the functional polyclonal memory T cells ($CD45R0^+$) accumulate and expand to close to normal numbers. This may explain the normalization of in vitro proliferation assays in non-SCID patients (Table III, footnote *g*).

Interestingly, a boy with partial CD3ε deficiency was also healthy and is now in his late teens despite a comparatively more severe TCR/CD3 expression defect (~10-fold compared with 3-fold in CD3γ deficiency). These results support the idea that partial but not complete CD3ε deficiency has low clinical penetrance (24). Last, the fact that the three patients with SCID features shared severe enteropathy suggests that the CD3γ-chain, and thus T cell function, is particularly important for survival when the intestinal mucosal barrier is breached.

A hierarchy for structural and functional defects in human CD3 deficiencies

The new family confirms that, in sharp contrast to the lack of CD3δ or CD3ε, the complete lack of CD3γ allowed in all cases for the production of substantial numbers of peripheral polyclonal $\alpha\beta$ (and $\gamma\delta$) T cells (Fig. 4 and Ref. 11) expressing relatively high levels of $\alpha\beta$ (and $\gamma\delta$) TCR/CD3 (Fig. 3 and our unpublished results). Thus, the TCR (and the pre-TCR) can be quite functional in humans without CD3γ, but not without CD3δ or CD3ε. This could be due to structural reasons precluding TCR or pre-TCR assembly in the absence of CD3δ or CD3ε as described originally for all $\alpha\beta$ TCR/CD3 chains (25). However, it was later shown in human non-T cells (HeLa) that the expression of incomplete human $\alpha\beta$ TCR/CD3 complexes is possible when either CD3δ or CD3γ (but not ε or any other chain) is absent (26). This suggested a $\varepsilon \gg \delta = \gamma$ hierarchy for impact of the missing chain on structure. Such a hierarchy could be expected given the fact that ε is shared by both CD3γε and δε dimers in the normal complex (Fig. 6*A*). Thus, the structural constraint argument holds for $CD3\varepsilon^{-/-}$ but not for $CD3\delta^{-/-}$ humans. In a lymphoid context (B cell microsomes), δε dimers were shown to associate to TCRα or TCRβ, whereas γε dimers could only bind to TCRβ (27), supporting a $\delta > \gamma$ structural hierarchy. Also, although both $\gamma^{-/-}$ and $\delta^{-/-}$ TCR/CD3 are structurally viable, only the former can signal for T cell selection (and function) in humans despite the high homology of the two chains (66%) (28). A boy with partial CD3ε deficiency showed

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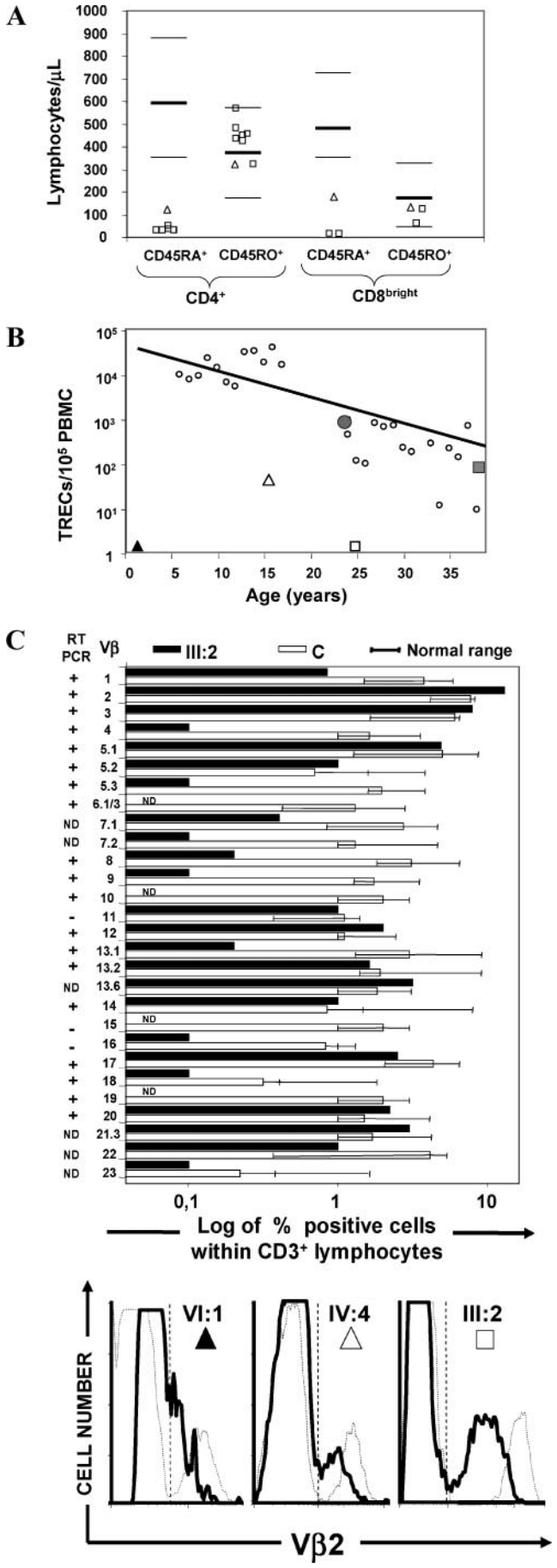

FIGURE 5. Peripheral blood TRECs, CD45RA$^+$ T lymphocytes, and TCR Vβ usage as a measure of thymus function in human complete CD3γ deficiencies. *A*, T cell subsets were defined as indicated. Lines indicate normal ranges (P5, median, P95; Ref. 38). Symbols identify patients as in Fig. 4. *B*, The δ deletion (signal joint) TRECs were determined as indicated in *Materials and Methods*. Circles are healthy donors (the gray circle was run with patient samples) and the gray square is the mother of IV: 4 ($\gamma^{+/-}$ control). For further reference, the thick line represents normal TRECs levels as a function of age (47). *C*, TCR Vβ mAb binding within CD3$^+$ lymphocytes. *Top*, Data from patient III:2 (black bars) at 24 years of age are shown in comparison with a normal age-matched control (C; Empty bars) and with the normal range (P10–P90) (48). TCR Vβ usage by RT-PCR at 18 years of age is shown for comparison (reported previously; Ref. 11). Note that Vβ usage may be underestimated due to the TCR/CD3 expression defect. Discordant PCR/mAb binding results may reflect normal age-dependent fluctuations (Vβ11) or impaired recognition of the mutant TCR/CD3 complex by the mAb involved (Vβ4) as reported for framework (BMA031; Ref. 11) or Vβ-specific (Vβ18; Ref. 49) mAb recognition of mutant TCR/CD3 complexes (without CD3γ or with TCRδ replacing TCRα, respectively). *Bottom*, Reactivity patterns for TCR Vβ2 mAb binding in the indicated patients (thick histograms) at 1, 16, and 24 years of age, respectively, as compared with a control (thin dotted histograms). The profiles are shown as logarithm of relative fluorescence vs cell number. The vertical dotted line in each panel indicates the upper limit of background fluorescence using isotype-matched irrelevant mAbs.

substantial T cell numbers and function despite a severe TCR/CD3 expression defect (10-fold) (24), supporting the idea that CD3ε is structurally critical but less so for signaling when CD3γ and CD3δ are present, because even minute amounts of TCR sufficed for T cell selection/expansion in that case. Therefore we believe that there must be some signaling difference between $\gamma^{-/-}$ and $\delta^{-/-}$ TCR/CD3 mutant complexes, as proposed in Fig. 6*A* ($\varepsilon \gg \delta \geq \gamma$ hierarchy for impact on structure, but $\delta \gg \gamma$ for impact on signaling). In fact, mammalian CD3δ resembles the chicken CD3$\gamma\delta$ precursor more closely than CD3γ (29), suggesting that the latter became specialized later in evolution. Indeed, specialized signaling functions for each CD3 chain have been reported previously (30). The proposed hypothesis could be tested by retroviral transduction of 2A peptide-linked human TCR/CD3 constructs lacking either γ or δ and functional analyses of their signaling potential (31).

Mice are not humans

Gene targeting of CD3 components in mice has shown that the ablation of any CD3 protein essentially blocked T cell development, although at different intrathymic checkpoints and to a different extent (30). Indeed, all CD3 proteins except CD3δ are required for T cell selection at the pre-TCR (TCR-β) checkpoint (double-negative/double-positive transition) with an $\varepsilon > \gamma > \zeta \gg \delta$ rank (Fig. 6*B*). Thus, contrary to what we propose in humans, mice hierarchy for impact on signaling would be $\gamma \gg \delta$. However, as observed in humans in vitro, the expression of incomplete murine $\alpha\beta$ TCR/CD3 complexes in non-T cells (3T3) is possible to substantial levels when either CD3δ or γ (but not ε or any other chain) is absent (Ref. 31 and D. Vignali, unpublished observations), supporting the $\varepsilon \gg \delta \geq \gamma$ hierarchy for structural constraints. Similar results were obtained in vivo (6, 9, 32). Interestingly, CD3δ is also dispensable for mature $\gamma\delta$ (but not $\alpha\beta$) T cell selection and for $\gamma\delta$TCR surface expression in mice, supporting substantial signaling through certain $\delta^{-/-}$ TCR/CD3 isotypes (33). But the proposed $\gamma\delta/\gamma\varepsilon\gamma\varepsilon\zeta\zeta$ stoichiometry for the murine $\gamma\delta$ TCR/CD3 clearly does not hold in humans. $\varepsilon^{-/-}$ humans resemble the mice model, but $\gamma^{-/-}$ and $\delta^{-/-}$ human do not (34). For instance,

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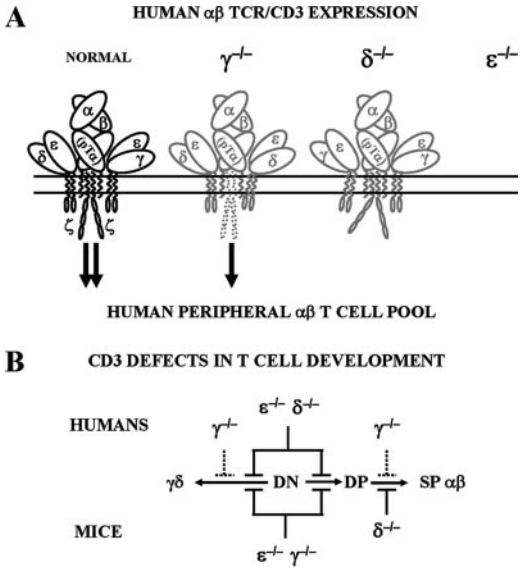

FIGURE 6. The normal human $\alpha\beta$ TCR/CD3 (or pre-TCR) complex and proposed hierarchy for structural ($\varepsilon \gg \delta \geq \gamma$) and functional ($\delta \gg \gamma$) defects in mutant TCR complexes. *A*, Surface expression is based on published data using transfected HeLa cells (26) or human complete $\gamma^{-/-}$ or partial CD3ε -deficient T lymphocytes (12, 17, 24). The normal structure follows published models (50, 51). Faint receptors indicate low expression as detected by flow cytometry. The $\zeta\zeta$ dimers were shown to associate poorly to $\gamma^{-/-}$ TCR/CD3 complexes in human T cells (11, 27). Promiscuous binding of human $\delta\varepsilon$ but not $\gamma\varepsilon$ dimers to TCRα or TCRβ is based on in vitro translation data (27). Similar structural but not functional constraints apply to mice. The peripheral $\alpha\beta$ T cell pool is indicated by arrows that represent absolute numbers of peripheral blood mature T lymphocytes (Fig. 4; complete $\delta^{-/-}$ and $\varepsilon^{-/-}$ humans have virtually no T cells). *B*, Comparative partial (- - -|) or complete (—|) impact of CD3 defects in human and murine T cell development, illustrating that $\delta \gg \gamma$ in humans, whereas $\gamma \gg \delta$ in mice for signaling defects at the double-negative (DN)/double-positive (DP) transition ($\gamma^{-/-}$, $\delta^{-/-}$, and $\varepsilon^{-/-}$ mice have very few or no single-positive (SP) $\alpha\beta$ T cells).

humans but not mice lacking CD3δ have no double-positive thymocytes and no $\gamma\delta$ T cells, and mice but not humans lacking CD3γ have very few peripheral $\alpha\beta$ or $\gamma\delta$ T cells (Figs. 4 and 6*B*). Also, in a murine $\gamma^{-/-}\delta^{-/-}$ double knockout model (35), human but not mouse CD3δ restored signaling at the TCR-β checkpoint (36). Similarly, a human $\delta\varepsilon$ heterodimer restored pre-TCR functions in $\gamma^{-/-}$ $\delta^{-/-}$, $\gamma^{-/-}$, and (partially) $\varepsilon^{-/-}$ mice (37). Thus, human CD3δ carries critical signaling cues that can mimic murine CD3γ. Taken together, these results suggest a different role for CD3γ and CD3δ in humans and mice in pre-TCR and TCR function during $\alpha\beta$ T cell development. Nevertheless, lymphocyte selection and expansion mechanisms may differ between species, because other immunodeficiencies show also dramatic differences as compared with mice models. For instance, Zap70- or CIITA-deficient humans show normal or substantial numbers of peripheral $CD4^+$ T cells, respectively, and γc- or Jak3-deficient humans have normal numbers of B cells, whereas their murine counterparts do not. Finally, CD3 gene inactivation in mice, even when kept in pathogen-free facilities, may cause pathological manifestations (enteropathy in CD3ζ- or CD3δ-deficient mice) that resemble those observed in some humans (CD3γ or δ deficiency).

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Disclosures

The authors have no financial conflict of interest.

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