Review Article

Human congenital T-cell receptor disorders

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ABSTRACT

Immunodeficiencies of most T-cell receptor (TCR) components (TCRID) have been reported in almost 40 patients worldwide who have also, at times, shown signs of autoimmunity. We updated their clinical, immunological, and molecular features with an emphasis on practical diagnosis, as the range of the disorder grows in complexity with new partial defects. Cellular and animal models are also reviewed and in some cases reveal their limitations for predicting TCRID immunopathology.

Introduction

T-cell receptors (TCR)

Mature T lymphocytes detect antigens with a variable surface TCR heterodimer, which is either αβ or γδ (Figure 1).

During early T-cell development, the invariant pre-Tα chain is used instead of TCRα. In humans, the variable TCR heterodimers form a complex with 2 invariant heterodimers termed CD3γε and CD3δε and a single invariant homodimer termed ζ or CD247 (Call et al. 2002). These 3 invariant dimers participate in assembly and surface expression of the whole TCR–CD3–CD247 complex, which we will refer to here as TCR. When a TCR interacts with its specific antigen through the variable heterodimers, intracellular signals are transduced to drive T-cell maturation or apoptosis in the thymus and T-cell activation, proliferation, and effector function or anergy–apoptosis in the periphery (Brownlie and Zamoyska 2013). The cytoplasmic tails of CD3ε and CD247 then expose their ITAM sequences (Love and Hayes 2010), which are phosphorylated by Lck, thus allowing the recruitment and Lck-mediated activation of ZAP-70. Activated ZAP-70 phosphorylates LAT, which recruits numerous signaling molecules to form a multiprotein complex termed the LAT signalosome. This signalosome, in turn, propagates signals to 3 major signaling pathways, namely MAPK, PI3K/AKT, and PLCγ-1, leading to the activation of transcription factors that are critical for gene expression and essential for T-cell growth and differentiation. Signals initiated from the TCR also result in actin reorganization and the activation of integrins by inside-out signaling (Kuhns and Davis 2012). Most αβTCR-bearing T cells recognize processed peptides associated with major histocompatibility complex (MHC) molecules, whereas γδTCR-bearing T cells can respond to a wide variety of antigens irrespective of their molecular nature including peptide and nonpeptide antigens, MHC-related proteins, phosphoantigens, and soluble proteins (Chien et al. 2014).

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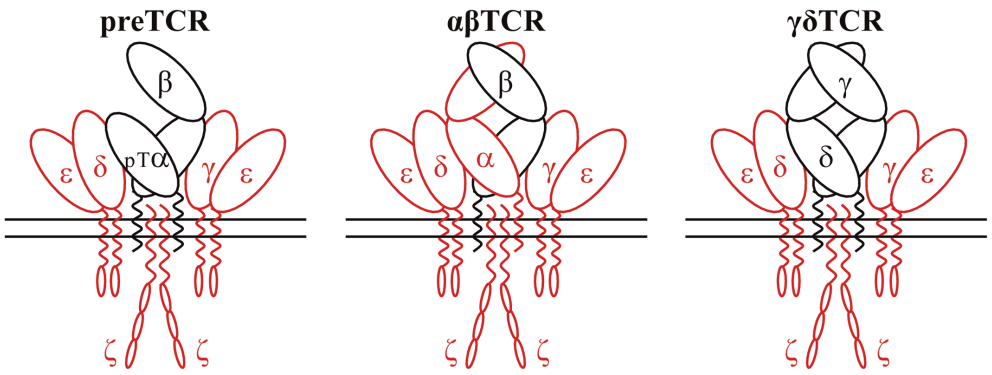

Figure 1: TCR isotypes. Red chains show mutations reported to cause immunodeficiency and (or) autoimmunity (Table 1).

T-cell receptor immunodeficiencies (TCRID)

TCRIDs are low-prevalence autosomal recessive diseases characterized by impaired surface TCR expression, frequently associated with peripheral blood T lymphocytopenia, severe combined ID (SCID) and (or) autoimmune symptoms, but not associated with B or natural killer (NK) lymphocytopenia. The first TCRID was a human familial CD3 expression deficiency in a child with SCID and in his healthy sibling (Regueiro et al. 1986), later shown to be due to a complete CD3γ deficiency (Arnaiz-Villena et al. 1992). A second TCRID was soon reported (Thoenes et al. 1992) and shown to be due to partial CD3ε deficiency (Soudais et al. 1993). Several CD3, CD247, and TCR deficiencies have since been described (Figure 1, Table 1), which can be classified as complete or partial according to the absence or presence of residual levels of the affected protein. Although rare and sometimes based on single cases, TCRID offer rich information about the underpinnings of human TCR structure and function, which in turn impact our understanding of T-cell development and function. Therefore we, as have others (Casanova et al. 2014), believe they merit a detailed study.

Clinical and pathological manifestations

TCRID

Most patients show SCID or CID features such as recurrent respiratory infections, otitis, candidiasis, diarrhea, failure to thrive, and sometimes autoimmune phenomena in the first year of life. Thymus size can be small (CD3γ, δ, or ε deficiency (Arnaiz-Villena et al. 1991; de Saint Basile et al. 2004; Takada et al. 2005)) or normal (CD3γ or CD247 deficiency, unpublished). Chronic pyogenic infections, dysmorphic features, or bone abnormalities were not reported. Stem cell transplantation is indispensable when SCID is present, otherwise most patients die early in life as a consequence of viral infection (cytomegalovirus mainly, but also adenovirus). Autoimmune phenomena have been described in several patients, mainly when T cells are present. Most CD3γ-deficient patients showed autoimmune hemolytic anemia (AHA) or vitiligo (Arnaiz-Villena et al. 1992) and also autoimmune thyroiditis, thrombocytopenia, or autoimmune hepatitis (Gokturk et al. 2014). Two

Table 1: **TCR immunodeficiencies.**

	References		Number of cases	
Protein	Complete	Partial	Families	Patients
CD3γ	Arnaiz-Villena et al. 1992; Sanal et al. 1996; Allende et al. 2000; Recio et al. 2007; Ozgur et al. 2008; Tokgoz et al. 2013; Gokturk et al. 2014	—	5	10
CD3δ	Dadi et al. 2003; de Saint Basile et al. 2004; Takada et al. 2005; Marcus et al. 2011	Gil et al. 2011; de la Calle-Martín, unpublished data	10	19
CD3ε	de Saint Basile et al. 2004; Fuehrer et al. 2014	Soudais et al. 1993	3	5
ζ or CD247	Rieux-Laucat et al. 2006; Roberts et al. 2007; Marin, unpublished data		3	3
TCRα	Morgan et al. 2011		2	2
Total			23	39

TCRα-deficient patients presented with vitiligo, AHA, or autoantibodies (Morgan et al. 2011). These results indicate that both positive selection and negative selection, which are TCR-dependent events, can be impaired in TCRID.

The CD3γ deficiency exception

In contrast with all other reported TCRID due to complete protein deficiencies (Table 1), most CD3γ deficient individuals reported to date (7 out of 10) are presently alive and well without transplantation (Table 2), including some in their thirties. Late diagnosis, mild T lymphopenia, low TCR expression, and autoimmune features are common (see "TCRID" section). These limited immunological consequences are at odds with what is observed when the very homologous CD3δ chain is absent: early diagnosis, severe T lymphopenia, no TCR expression, and urgent transplantation. This clearly shows that CD3γ is not critical for survival in most cases, as compared with other TCR proteins, and particularly with CD3δ. The most likely molecular explanation is that CD3δ can replace CD3γ in the TCR, as has been reported (Zapata et al. 2004), whereas the reverse is not possible. As we review in the "Animal models" section, this is not the case in CD3γ Knock-Out (KO) mice. However, 3 out of 10 CD3γ-deficient patients, even though they had received transplants, did not survive to adulthood, thus suggesting that therapeutic decisions must be taken on a case by case basis.

Diagnosis

Differential diagnosis

A male or female infant showing T lymphopenia could be a result of TCRID, but also of mutations in IL7Rα, FOXN1, Coronin-1A, Zap70, TAP, MHC class II, transcription factors, PNP, ADA, or 22q11.2 (DiGeorge syndrome) (Al-Herz et al. 2014); however, only TCRID show TCR expression defects when T cells are present. Thus, a careful flow cytometry study is essential for the early differential diagnosis of TCRID, which is critical for patient survival, and should include both αβ and γδ T-cell subsets.

Sample quality

Samples are often received from abroad and travel long distances at changing temperatures before they reach the diagnostic lab. In such cases it is important to include age-matched controls that suffer similar shortcomings, so that comparisons can be meaningful.

T-cell subset definition and lymphopenia

TCRID may have no T cells and are thus properly called $T^{-}B^{+}NK^{+}$. But often some T cells are present ($T^{\pm}B^{+}NK^{+}$), although their identification using CD3 as an extracellular marker can be difficult owing to their reduced TCR expression. Indeed, some authors have reported $T^{-}B^{+}NK^{+}$ phenotypes despite the presence of abundant $CD4^{+}$ and $CD8^{+}$ lymphocytes expressing very low levels of surface CD3 (Roberts et al. 2007). As intracellular CD3 is not normally used for screening TCRID, we used extracellular CD4 and CD8 to identify T cells in TCRID patients. We have shown that essentially all $CD4^{+}$ and $CD8^{bright}$ cells within the lymphocyte gate are αβ T cells and, conversely, most double negative cells (DN, $CD4^{-}CD8^{-}$) in the same gate are γδ T cells (Muñoz-Ruiz et al. 2013). $CD8^{dull}$ lymphocytes are mostly NK cells. For comparative purposes, we have considered $CD3^{+}$ lymphocytes in Table 2 because they are widely reported, although it is likely an underestimation of the true number of T cells. Chimerism should be excluded using standard fingerprinting procedures, to avoid for instance counting the mother's T cells, as reported recently in a new case of CD3ε deficiency (Fuehrer et al. 2014).

Using these criteria, most patients with complete defects show selective peripheral blood T lymphopenia, either $T^{-}B^{+}NK^{+}$ or $T^{\pm}B^{+}NK^{+}$ depending on the affected TCR chain, which is a first clue to the molecular basis of the pathology. $T^{-}B^{+}NK^{+}$ has been associated with complete CD3ε or CD3δ defects, with less than 2% peripheral blood T cells. $T^{\pm}B^{+}NK^{+}$ has been reported in complete CD3γ, TCRα, or CD247 defects. All partial TCRID show a $T^{\pm}B^{+}NK^{+}$ or even a $T^{+}B^{+}NK^{+}$ immunophenotype.

TCRID are best diagnosed if both αβ and γδ T cells are studied, as shown for complete TCRα deficiency (Morgan et al. 2011) and for partial CD3δ deficiency (Gil et al. 2011), both of which shared a $T\alpha\beta^{-}\gamma\delta^{+}B^{+}NK^{+}$ immunophenotype despite their disparate molecular basis. Figure 2 (top) summarizes the effects of TCRID mutations in T-cell development.

Surface TCR expression in patients' lymphocytes

We have listed the monoclonal antibodies used in our lab for surface staining of peripheral blood lymphocytes from possible TCRID patients (Table 3, extracellular).

Table 2: **TCRID clinical and immunological data.**

Deficiency	CD3δ																		
Ethnic background	Mennonites (Canada, USA, Germany)										France			Japan			Ecuador		
Family	1					2	3	4			5		6	7			8	9	10
Patient/sex	1F	2M	3M	4F	5M	6F	7M	8F	9M	10F	11F	12F	13M	14F	15M	16M	17M	18M	19F
Consanguineous	Yes										Yes			No			No		No
Mutation	c.202C>T										c.279C>A			c.IVS2-2A>G			c.IVS2+5G>A		
Predicted effect (leaky)[†]	Truncation										Truncation			Skip3			*Skip2*		
Diagnosis at (m)	0	2	2	9	0	0	0	10	13	0	3	0	2	3	0	0	14	4	10
% CD3$^+$ cells[‡]	0.3	0.1	0.6	2	0	0	1	0	27	1	<1		0	1.7	0.1	0	14	30	2
HSCT[§] at (m)	MB4m	No	No	MB16m	MB4m	IDB1m	HP6m	HP12m	HB14m	HB2m	No	H	H	HP4m	MIC1m	MC1m	IDP23m	HP8m	No
Cause of death[‖]	AW	ADV	CMV	AW	AW	AW	GvHD	HHV6	CMV	AW	CMV	Asp	EBV	CMV	AW	AW	AW	CMV	A

Deficiency	CD3γ										CD3ε					ζ/CD247			TCRα	
Ethnic background	Turkey			Spain		Turkey					France				Germany	Caribbean	Hawaii	Turkey	Pakistan	
Family	1		2	3		4		5			1	2			3	1	2	3	1	2
Patient/sex	1M	2M	3M	4M	5M	6F	7F	8F	9M	10F	1M	2F	3M	4F	5F	1M	2F	3F	1F	2M
Consanguineous	Yes			No		No		Yes			No	Yes			No	NR[‖]	No	Yes	Yes	
Mutation	c.205A>T			c.1A>G and c.IVS2-1G>C		c.IVS2-1G>C		c.IVS2-1G>C			c.IVS7+2T>C and c.230G>A	c.128_129del			c.IVS2+1G>C	c.207C>T	c.411insC	c.2T>C	c.*1G>A	
Predicted effect (*leaky*)[†]	Truncation			Trunc, Het		Truncation		Truncation			*Skip7, Tr, Het*	Truncation			Skip 2	Trunc	Insertion	Trunc	Skip3	
Diagnosis at (m)	3	7	48	12	48	12	240	84	132	14	48	5	1	0	2	4	10	2	15	6
% CD3$^+$ cells[‡††]	37	27	30	10	18	40	45	33	32	45	63	NR[¶]	NR[¶]	<1	100[‡‡]	21	64	60	21	50
HSCT[§] at (m)	No	ID	No	No	No	No	No	No	No	No	No	No	No	H	H7m	H30m	H>12m	H18m	IDB6y	IDB7y
Cause of death[‖]	Sepsis	Pneum	AW	Pneum	AW	AW	AW	AW	AW	AW	AW	Pneum	CMV	ADV	AW	AW	AW	A	AW	AW

[†]Leaky refers to wild type protein levels. (Skip7, exon 7 skipping; Tr/Trunc, protein truncation; Het, compound heterozygote.)
[‡]At diagnosis. Normal values 60%–85%, includes dull and bright CD3$^+$ cells.
[§]First hematopoietic stem cell transplantation from related (matched, ID; haploidentical, H) or unrelated (matched, M; mismatched, MI) donor bone marrow (B), peripheral blood (P), or cord blood (C).
[‖]AW, alive and well; A,alive; ADV, CMV, EBV, and HHVviral infection; Pneum,pneumonia; Asp aspergillus; GvHD graft vs host disease
[¶]NR,not reported.
[††]For TCRα defects mostly γδTCR$^+$ T cells, few αβTCR^{low} T cells.
[‡‡]T cells from maternal origin.

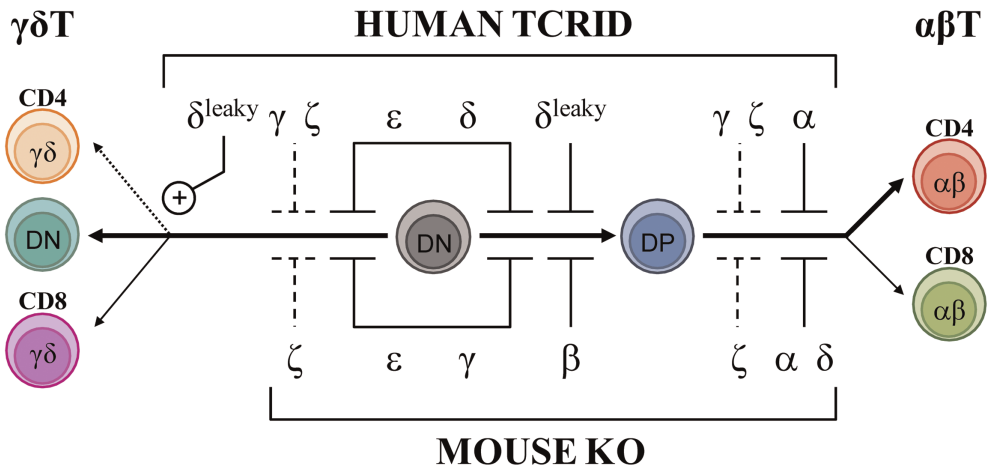

Figure 2: Impact of mutations causing complete TCRID on human αβ (right) and γδ (left) T-cell development (top), as compared with knock-out (KO) mice (bottom). Isolated Greek letters except α and β stand for invariant CD3 or CD247 chains, all other are variable TCR chains. (DN, double negative ($CD4^-CD8^-$); DP, double positive ($CD4^+CD8^+$)).

We start with just UCHT-1 (anti-CD3ε), 11F2 (anti-γδTCR), and any anti-CD4 and anti-CD8α antibodies, and in a different staining IP26 (anti-αβTCR) and 11F2 (anti-γδTCR). Within a subset (for instance, $CD4^+$ lymphocytes), TCR Mean Fluorescence Intensity is evaluated with any of those CD3- or TCR-specific antibodies in comparison with that of an age-matched healthy control (Figure 3, top center). The expression of CD4 itself can serve as an internal control (Figure 3, top left). We normally use a range of CD3-specific monoclonal antibodies, such as those listed in Table 3 (extracellular), to confirm any TCR expression defect.

Table 3: **Monoclonal antibodies used for TCRID diagnosis.**

Specifity	Name	Species	Source
Extracellular			
αβTCR	IP26	Mouse	Biolegend
αβTCR	BMA031	Mouse	Miltenyi Biotec
αβTCR	WT31	Mouse	BD Biosciences
γδTCR	11F2	Mouse	BD Biosciences
γδTCR	Inmu510	Mouse	Beckman Coulter
CD3ε	Leu-4	Mouse	Beckton Dickinson
CD3ε	S4.1	Mouse	Invitrogen
CD3ε	UCHT-1	Mouse	Beckman Coulter
Intracellular			
CD3ε	UCHT-1	Mouse	Beckman Coulter
CD3δ	EP4426	Rabbit	Abcam
CD3γ	EPR4517	Rabbit	Abcam
ζ/CD247	6B10.2	Mouse	Biolegend
TCRβ	Jovi-1	Mouse	Non commercial
TCRβ	βF1	Mouse	Non commercial

Family members are not appropriate controls, because in some cases they also show (partial) TCR expression defects that may underestimate the patient's defect (see Figure 3 and Muñoz-Ruiz et al. 2013, top center and "Surface TCR expression in carriers' lymphocytes"). If no TCR expression defect is observed, TCRID can be excluded. If a TCR expression defect is ascertained, both αβ and γδ T cells should be analyzed. If αβ T cells (defined as $CD4^+$ and (or) $CD8^{bright}$) show impaired TCR expression and $γδTCR^+$ show normal surface TCR expression, TCRα deficiency is likely. If αβ and γδ T cells share a surface TCR expression defect, a CD3 or CD247 defect may be suspected and further studies are required to identify the culprit protein (see "Protein identification in patients' lymphocytes"), as indicated in the diagnostic flow chart (Figure 3, bottom). The following TCR expression hierarchy may be useful for the diagnosis of TCRID due to the lack of the indicated chains: CD3ε=CD3δ=TCRα≥CD247>CD3γ (our experience). Also, studying NK cells can be informative, as CD247 deficiency affects the expression of NK surface receptors such as CD16 or NKp30 (Reyburn, unpublished data). It is important to note that in contrast to αβ T cells, the range of surface TCR levels in γδ T cells in different individuals is quite heterogeneous (Garcillán et al. 2014), a fact that must be taken into account when analyzing the overlays of γδ T cells.

Lastly, it is advisable to grow T cells using IL-2 plus allogeneic feeder cells or immortalize them with *Herpesvirus saimiri* (HVS) or Human T-cell leukemia virus type 1 (HTLV-1), (see "Functional studies" and "Cellular models"), so that the detected TCR expression defects can be ascertained and further analyzed.

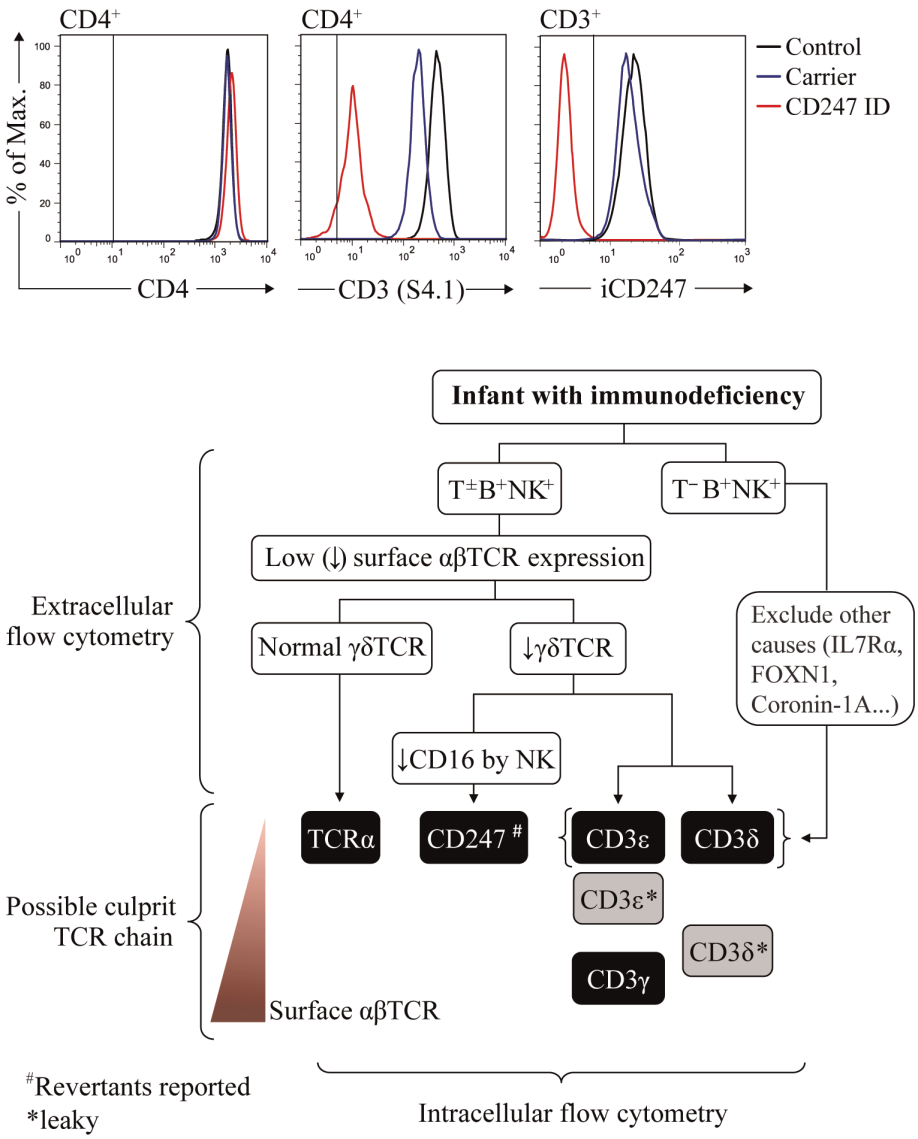

Figure 3: TCRID diagnosis by flow cytometry. Top: example of histogram overlays of a control, a carrier and a CD247 deficiency patient (black, blue, and red line, respectively) using extracellular (left and center) and intracellular (right) staining with the indicated antibodies and gates. Vertical lines are the isotype control fluorescence upper limit. Bottom: diagnostic flow chart using cytometric data. Keys represent expected defects, as αβTCR expression values were not available in these TCRID.

Surface TCR expression in carriers' lymphocytes

The detection of TCRID mutation carriers using flow cytometry is difficult because surface TCR expression is often within the normal range. Again, overlays of extracellular CD3ε staining can be used to compare several family members, as shown in Figure 3 (top center), for CD247 (Marin and Garcillán, unpublished data). Using similar strategies, we have shown that CD3γ$^{+/-}$, CD3δ$^{+/-}$ (and even CD3δ$^{+/leaky}$) carriers can be identified, although the difference is not so clear and can be confounded by normal surface TCR variation (Muñoz-Ruiz et al. 2013). Of course, a genetic analysis is required for confirmation.

Protein identification in patients' lymphocytes

In the past, immunoprecipitation (Perez-Aciego et al. 1991) or Western blot techniques (Gil et al. 2011) had to be used for the identification of protein defects. But these approaches require large amounts of T cells, which are difficult to obtain when samples are scarce (as is

Table 4: **Mutations found in TCRID.**

Mutation	LOCUS	Site	Sequence variation	Expression	References
I. Missense					
p.M1T†	*CD247*	exon 1	c.2T>C	CD247 WT detected in revertant T cells (lower TCR/CD3 expression levels, only for compensatory mutant)	(Marin, unpublished)
p.M1V	*CD3G*	exon 1	c.1A>G	ND	(Arnaiz-Villena et al. 1992)
II. Nonsense					
p.W59X	*CD3E*	exon 6	c.230G>A	NR	(Soudais et al. 1993)
p.Q70X†	*CD247*	exon 3	c.207T>C	CD247 WT detected in revertant T cells (normal TCR/CD3 expression levels)	(Rieux-Laucat et al. 2006)
p.K69X	*CD3G*	exon 3	c.205A>T	ND	(Recio et al. 2007)
p.R68X	*CD3D*	exon 2	c.202C>T	Truncated CD3δ protein was not detected	(de Saint Basile et al. 2004), (Dadi 2003)
p.C93X	*CD3D*	exon 2	c.279C>A	NR	(de Saint Basile et al. 2004)
III. Splice-site					
Deletes exon 2	*CD3E*	intron 2	c.IVS2+1G>C	Mutant CD3ε protein was not detected (Kozak consensus sequence at position 82 in exon 4 could not be used as alternative stop codon)	(Fuehrer et al. 2014)
Deletes exon 7	*CD3E*	intron 7	c.IVS7+2T>C	Mutant CD3ε protein was not detected	(Soudais et al. 1993)
Cryptic splice site	*CD3G*	intron 2	c.IVS2-1G>C	ND	(Arnaiz-Villena et al. 1992, Tokgoz et al. 2013, Gokturk et al. 2014)
Deletes exon 3	*CD3D*	intron 2	c. IVS2-2A>G	Truncated CD3δ protein was not detected	(Takada et al. 2005)
Deletes exon 2	*CD3D*	intron 2	c.IVS2+5G>A	Truncated CD3δ protein was detected in transfected HeLa cells but not in PBMC from patients	(Gil et al. 2011)
Deletes exon 3/ p.T107fsX56	*TCRA*	exon 3	c.*1G>A	Reduced levels of TCRα and TCRβ expression/ no colocalization	(Morgan et al. 2011)
IV. Deletions/insertions					
p. T43fsX56	*CD3E*	exon 6	c.128_129del	NR	(de Saint Basile et al. 2004)
p. D138fsX272	*CD247*	exon 7	c.411insC	CD247 protein was not detected	(Roberts 2007)

†Reversions reported.
ND, not done; NR, not reported

often the case with infant patients), and even more difficult in cases with severe T-cell lymphopenia. Generation of T-cell lines can be a solution, but they take precious time, thereby delaying diagnosis. CD3 chain-specific antibodies for intracellular staining have become available lately, and in our hands simplified the diagnosis of TCRID, which can now be done rapidly. Table 3 (intracellular) lists such antibodies and Figure 3 (top right) shows a recent example of their use. Once the culprit protein is defined, genetic validation is indispensable to establish the molecular basis of the protein defect.

In summary, excellent reagents are now available to diagnose TCRID defects in a record time, even when very few T cells are present.

Mutation analysis

A mutation database has been established that contains most described mutations in the genes encoding for TCR chains (Piirilä et al. 2006). Mutations causing TCRID include missense, nonsense, and splice-site mutations as well as less common genomic deletions and insertions (Table 4). Figure 4 illustrates their

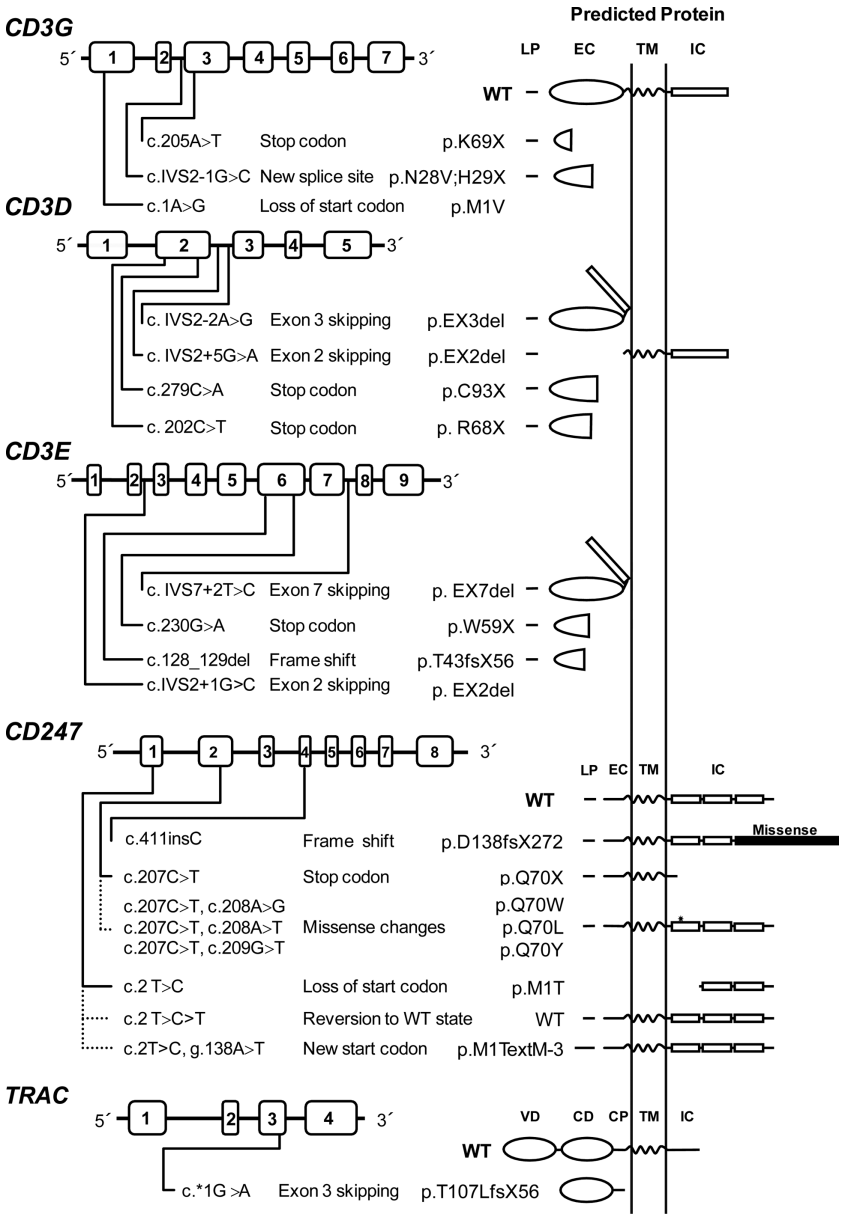

Figure 4: Graphical representation of mutations causing TCRID and their predicted protein products, including reversions (dotted lines). (LP, leader peptide; EC, extracellular; TM, transmembrane; IC, intracellular; VD, variable domain; CD, constant domain; CP, connecting peptide.)

presumed protein products that, when truncated, are often unstable.

Most mutations drastically affect the expression of the protein (complete defects), whereas in some leaky splice-site mutations, small amounts of normal transcripts were produced, allowing for the expression of some normal protein and thus causing a partial phenotype. This is the case for CD3δ and CD3ε TCRID.

Potentially truncated proteins have also been reported and were likely unstable, as they were undetectable in T cells. Indeed, the in-frame deletion of exon 7 predicted a mutant CD3ε protein that was not found even with very sensitive techniques (Thoenes et al. 1992). Also, a predicted mutant CD3δ protein selectively lacking the extracellular domain encoded by exon 2 was undetectable in peripheral blood mononuclear cells (PBMC) from the patient or carriers, although it could be shown in transfected non-T cells (Gil et al. 2011). This may be mutation specific, as shown recently in a new splicing mutation in *CD3E* intron 2, which was predicted to delete exon 2 and thus the start codon. As a consequence, no protein was detected by Western blot

either in carrier PBMC or in transfected non-T cells (Fuehrer et al. 2014). In TCRα deficiency, a c.*1G>A mutation resulted in an aberrant transcript joining exon 2 to the normally untranslated exon 4. In the predicted translation product, the 35 C-terminal amino acids would be replaced by 56 amino acids encoded by exon 4. This would result in partial loss of the connecting peptide domain and abolition of the transmembrane and cytoplasmic domains of the TCRα chain. In PBMC, no TCRα or TCRβ proteins were detected by Western blotting with specific antibodies, but minute amounts of both chains were detectable by immunofluorescence microscopy using a different set of antibodies. The authors concluded that the residual predicted truncated TCRα failed to bind normally to TCRβ, because in the latter experiment the TCR chains did not colocalize. This was later confirmed in transfected HeLa cells (Morgan et al. 2011).

Nonsense mutations are clearly deleterious and frequently associate with severe TCRID immunophenotypes, except for complete CD3γ and partial CD3ε deficiencies. In CD3δ deficiency, a microarray analysis in thymocytes revealed more than 2-fold lower specific transcripts, but no CD3δ protein was detected by Western blot analysis of the same lysates or by thymus immunohistochemistry (Dadi et al. 2003).

Somatic mosaicism caused by reversions can ease otherwise severe clinical phenotypes. Reversions have been reported in several disorders, including dermatological, metabolic, and immunological disorders such as ADA deficiency, X-linked SCID, and Wiskott–Aldrich syndrome (Hirschhorn 2003; Wada and Candotti 2008).

Reversion of some T cells to carrier TCR expression levels has been shown to be the consequence of *CD247* germ-line mutations when studied (Figure 4). However, TCR signaling after stimulation with phytohemagglutinin (PHA) or anti-CD3 of revertant T cells can be impaired (Rieux-Laucat et al. 2006) or restored to carrier levels (Marin, unpublished data) for reasons yet to be defined. Interestingly, no reversions have been reported in other TCRID, suggesting that *CD247* is more prone to somatic mutations than the *CD3* or *TRAC* genes.

CD3GDE haplotype analysis using polymorphic markers can be useful (*i*) to rule out CD3 mutations, if segregation does not fit the pedigree; (*ii*) to establish the existence of founder alleles even when families are unaware of such connections, as we have reported for *CD3G* in Turkey (Recio et al. 2007) and for *CD3D* in Ecuador (Gil et al. 2011); and (*iii*) for carrier detection and (or) prenatal diagnosis, because recombination within the *CD3* gene complex is rare.

Screening tests specific for some founder *CD3G* and *CD3D* mutations using TaqI and BsaAI (Table 5) have been developed for several Turkish and Ecuadorian families, respectively, which help to reach quick diagnoses and segregation data for genetic counselling (Recio et al. 2007; Gil et al. 2011). Therefore, in Turkish or Ecuadorian patients with an immunophenotype suggestive of CD3γ or CD3δ deficiency, regardless of the accompanying spectrum of clinical symptoms, screening for such mutations should be considered in their molecular diagnosis.

Functional studies

T-cell functional analyses both in vivo and in vitro are useful, but not critical, for TCRID diagnosis, as they are frequently impaired even when T cells are abundant or in healthy siblings, as reported for CD3γ deficiencies (Recio et al. 2007). More often, lymphopenia precludes functional studies in primary T cells, so it becomes necessary to use immortalized cell lines to explore the functional impact of the mutations in T-cell function. For this purpose, T cells can be immortalized with HVS or HTLV-1 (Pacheco-Castro et al. 1998).

Table 5: **Screening test for carrier detection in CD3γ and CD3δ deficiencies.**

					Restriction fragments (bp)	
Enzyme	Restriction site	Mutation / LOCUS	Position	PCR primers	Homozygous	Heterozygous
Tru9I	TT**A**A	c.205A>T / *CD3G*	exon 3	5′-TGGTATGCAGAAGCAGGGAGAA-3′ 5′-TAAAAAGCTCACCAGAACAGCAAAT-3′	138, 37	175, 138, 37[†]
BsaAI	TAC**G**TG	*c.IVS2+5G>A* / *CD3D*	intron 2	5′-TGAGCTTCCGCAGAACAAAGG-3′ 5′-CACATCCAGAAGCCCTATCCATT-3′	345	345, 295, 50[†]

[†]37 and 50 bp fragments were not visible in agarose gel

Additionally, fresh PBMC can be cultured with allogeneic feeder cells (a γ-irradiated mixture of EBV-transformed lymphoblastoid cells and PBMC from several healthy donors).

TCR-triggered early activation events (such as Ca^{2+} influx and CD69 or CD25 induction) and late activation events (such as proliferation induced by mitogenic, antigenic, or allogeneic stimuli) sometimes show contradictory results. For instance, T cells from CD3δ^{leaky} patients showed strongly reduced induction of CD69 and CD25 but normal proliferation when anti-CD3 or PHA stimulation were used (Gil et al. 2011). In other cases, although anti-CD3 responses were reduced, costimulation with CD28 or IL-2 restored proliferative responses (Le Deist et al. 1991; Rieux-Laucat et al. 2006).

Thymus function, often measured as output of peripheral blood recent thymic emigrants ($CD4^+CD45RA^+CD31^+$ lymphocytes), is frequently impaired in TCRID. Additionally, TCR diversity is commonly poor, as measured looking at *TCRB* clonality and TCRVβ diversity (Gil et al. 2011).

Despite impaired T-cell responses, TCRID are not always associated with diminished B-cell memory subsets, reduced immunoglobulin (Ig) levels, or abnormal vaccination responses, as reported in TCRα deficiency (Morgan et al. 2011) and in CD3δ^{leaky} deficiency (Gil et al. 2011).

Treatment and prognosis

The only effective treatment is hematopoietic stem cell transplantation (HSCT), and the sooner the better (Marcus et al. 2011). Patients with successful transplants have been shown to be healthy up to 18 years post-transplantation. Otherwise, the prognosis is very poor for those with complete or even some partial defects such as CD3δ^{leaky} (Table 2), with some exceptions (see next paragraph). Matched related, haploidentical mismatched related, matched unrelated, and mismatched unrelated donors have all been used for HSCT, generally after myeloablative conditioning, but matched related donor HSCT is also used without conditioning when SCID is diagnosed. Complete T-cell reconstitution is generally observed (including normalized B cell function), but split-chimerism has been recently reported for CD3ε deficiency, which 15 years later resulted in the need of intravenous Igs owing to of lack of switched memory B cells (Fuehrer et al. 2014). A diminished switched memory B-cell subset is a common finding in other SCID patients (Sarantopoulos et al. 2009). A frequent complication of HSCT is graft versus host disease, which can be treated with immunosuppression as was done recently in a case with CD247 deficiency (Aydogmus, unpublished data). When autoimmunity is present, immunosuppression is also required (steroids and cyclosporin A), as reported recently for Evans syndrome in CD3γ deficiency (Tokgoz et al. 2013).

Some CD3γ- and a partial CD3ε-deficient patient without ID symptoms did not receive HSCT, but rather antibiotics only when symptoms developed (Allende et al. 2000) or prophylactic intravenous Igs with antibiotics (Le Deist et al. 1991), respectively.

Models of TCRID

Animal models

Knock-Out (KO) mouse models (Table 6) have been used to replicate human congenital TCRID, because mouse and human TCR and CD3 chains share significant sequence and functional homology (Dave 2009). Indeed, a block in intrathymic T-cell development and reduced TCR expression was reported in all cases, although sharp differences were found for CD3γ and CD3δ (Figure 2).

CD3δ$^{-/-}$mice and humans shared a severe selective αβ T lymphocytopenia, but this was due to a block at different checkpoints (DP in mice vs DN in humans, Dave 2009). However, although CD3δ-deficient patients also lacked γδ T cells, CD3δ$^{-/-}$mice showed no obvious defect in γδ T-cell development or TCR expression (Dave et al. 1997). The mechanism became clear when stoichiometry studies showed that in humans, but not in mice, the γδTCR incorporates CD3δ (Hayes and Love 2006; Siegers et al. 2007).

Table 6: **TCRID mouse models.**

	Reference	
Protein	Complete	Partial
CD3γ	Haks et al. 1998	
CD3δ	Dave et al. 1997	
CD3ε	Malissen et al. 1995; DeJarnette et al. 1998	Wang et al. 2009
ζ/CD247	Malissen et al. 1993; Liu et al. 1993; Love et al. 1993	
TCRα	Mombaerts et al. 1992	
TCRβ	Mombaerts et al. 1992	

CD3γ$^{-/-}$mice showed very few peripheral T cells (around 10% of normal values) with low surface TCR and abnormal stoichiometry (αβ/δεδεζζ) owing to a severe block at the DN stage (Haks et al. 1998). By contrast, CD3γ deficiency in humans allows the selection of substantial numbers of peripheral T cells (Recio et al. 2007), which in the case of αβ T lymphocytes expressed relatively high levels of functional αβTCR complexes (Pacheco-Castro et al. 1998), albeit with an abnormal stoichiometry (αβTCR/CD3δεδεζζ) and an impaired association to CD247 (Zapata et al. 2004). Therefore, the lack of CD3γ or CD3δ during αβ T-cell development gives rise to inverted phenotypes in mice versus humans, often precluding a meaningful comparison (Siegers et al. 2007).

CD3δ and CD3γ are the most homologous CD3 proteins. Yet, although patients lacking CD3δ are devoid of mature T cells, CD3γ-deficient individuals do have seemingly functional αβ and γδ T lymphocytes and generally less severe clinical features (Table 2). These differential phenotypes indicate that in humans CD3δ replaces CD3γ in the pre-TCR and TCR for stable expression on and signalling from the cell surface, allowing thymic development of fairly functional T cells. In support of this notion, expression of a human, but not a mouse, CD3δ transgene rescued αβ and γδ T-cell development in mice that were deficient for both CD3δ and CD3γ (Fernández-Malavé et al. 2006; Siegers et al. 2007). Because pre-TCR and γδTCR complexes in mice do not seem to incorporate CD3δ (Malissen et al. 1999), these data further suggest that CD3δ has evolved distinct features in humans that allows it to substitute CD3γ in both species.

CD3ε$^{-/-}$mice showed abrogated T-cell development with a severe block at the DN stage (DeJarnette et al. 1998) (Figure 2), supporting a shared critical role on thymocyte development versus humans (de Saint Basile et al. 2004).

As observed in humans, CD247$^{-/-}$mice (Liu et al. 1993) showed a partial block at the DP stage with weak transition to SP T cells and very low surface TCR in both thymocytes and peripheral T cells, which are nonfunctional (Liu et al. 1993).

T-cell development in TCRα$^{-/-}$mice is blocked at the DP stage, indicating that TCR gene rearrangements are critical for αβ T-cell differentiation, whereas γδ T cells are unscathed (Mombaerts et al. 1992).

Cellular models

The complex structure of the TCR and the severe T-cell lymphopenia normally associated to TCRID (Mazariegos et al. 2013) has hampered the dissection of the precise role of each TCR chain for TCR assembly and T-cell selection and function. KO mice, TCRID patients, mutagenized T cells lacking certain chains, TCR-transfected non-T cells and, more recently, Knocked-Down (KD) T cells tell different yet complementary tales in each species, as we review here for cellular models.

Human T cells

Jurkat mutants

Several variants of the αβ T-cell leukemia line Jurkat, lacking TCRα, TCRβ, or CD3γ have been obtained over the years (Table 7). All lacked surface TCR, thereby supporting the idea that these proteins are required for TCR expression.

In 1992, Geisler described a Jurkat cell line, termed Jurkat Gamma Negative (JGN), deficient for surface TCR expression owing to the lack of CD3γ (Geisler 1992). Using JGN and site-directed mutagenesis, they identified a di-leucine motif in the intracellular (IC) domain of CD3γ implicated in TCR internalization (Dietrich et al. 1996a), two EC interaction sites of CD3γ with CD3ε, and one transmembrane (TM) residue involved in binding to TCRβ. They also demonstrated that N-glycosylation in CD3γ is not required for TCR assembly and expression, and that the IC domain of CD3γ is not required for surface TCR expression (Dietrich et al. 1996b). Experiments using chimeric constructions of CD3γ and CD3δ demonstrated that the IC and TM regions of these highly homologous proteins were interchangeable (Wegener et al. 1995). TCRα- or TCRβ-deficient Jurkat cell lines have been also used to define important assembly residues in TCR chains, TCR assembly hierarchy, endoplasmic reticulum

Table 7: **Jurkat variants.**

Name	Lacks	Reference
JGN	CD3γ	Geisler 1992
R3.20.11a	TCRα and TCRβ	Arnaud et al. 2001
JR3.11	TCRα	Arnaud et al. 2001
JRT-T3.1	TCRα	Weiss and Stobo 1984
18.B3	TCRα	Saito et al. 1987
J76.2	TCRβ	Arnaud et al. 2001
JRT3-T3.5	TCRβ	Weiss and Stobo 1984
JBN	TCRβ	Saito et al. 1987

retention of partial complexes, and degradation of TCR chains (Dietrich et al. 1999; Martin et al. 1999; Arnaud et al. 2001).

Knocked-Down (KD) Jurkat

We have generated Jurkat KD lines for CD3δ or CD3γ that show that both chains, although highly homologous, are essential for surface TCR expression (Garcillán, unpublished data). This observation is in agreement with the profound T-specific lymphopenia found in CD3δ KO mice and CD3δ-deficient patients, although residual surface TCR was found in T cells from both mutants. However, cellular models do not always recapitulate the human TCRID, as CD3γ-deficient patients (but not KO mice) showed abundant T cells and surface TCR expression (Perez-Aciego et al. 1991), whereas JGN lacked surface TCR. We believe that intrathymic selection pressure can explain this discrepancy, which also shows that human and murine TCR chains have acquired different roles upon speciation (see "Animal models").

Immortalized T cells from TCRID patients

Both HVS- and HTLV-1-immortalized T-cell lines have been derived from CD3γ-deficient patients (Pacheco-Castro et al. 1998; Torres et al. 2003), which preserved the TCR expression features of their donors. These cell lines have been instrumental to show that despite decreased surface TCR expression several mature T-cell responses were normally induced via their mutant TCR, including calcium flux, cytotoxicity, upregulation of CD69, proliferation, and synthesis of specific cytokines, such as TNFα and IFNγ. In contrast, TCR-induced IL-2 synthesis, adhesion, tyrosine phosphorylation, and PMA-induced TCR downregulation were impaired (Torres et al. 2002; Torres et al. 2003; Reine et al. 2011).

Cell lines derived from patients with partial CD3δ or complete CD247 deficiency have been also generated using allogeneic feeder cells and IL-2 (Gil et al. 2011; Garcillán et al. 2014; Marin, unpublished data). These cell lines also preserved the features of the primary lymphocytes and have been useful for biochemical and functional analyses.

Mouse T cells

Several murine T-cell lines deficient in one of the TCR chains have been also studied. In 1988, a CD247 deficient cell line (MA5.8) was generated by chemically mutagenesis of the antigen-specific 2B4 T-cell hybridomas (Sussman et al. 1988). MA5.8 cells were able to express small amounts of surface TCR but failed to respond normally to antigens. MA5.8 has been widely used to establish the role of CD247 in TCR assembly (Dietrich et al. 1999; Delgado and Alarcon 2005) and to study CD247 splicing variants present in systemic lupus erythematosus patients (Tsuzaka et al. 2003; Tsuzaka et al. 2005). Later, a CD3δ-deficient T-cell line was obtained by repetitive subcloning at limiting dilution of the 2B4 T-cell hybridoma line. Studies with this cell line showed that, in the absence of CD3δ, surface TCR expression was abolished and the other TCR chains were retained in the endoplasmic reticulum (Bonifacino et al. 1989). Another study corroborated these results analyzing a murine cytotoxic T lymphocyte (CTL) clone that selectively lacked expression of the CD3δ mRNA (Buferne et al. 1992). Lastly, studies in a CTL clone deficient in both CD3δ and CD3γ showed that expression of either CD3δ or CD3γ alone failed to reconstitute surface TCR expression, but reconstitution with cytoplasmically truncated CD3δ and CD3γ led to reexpression of the TCR, demonstrating that both chains are required

Table 8: **Cellular models to study the role of CD3γ and CD3δ in TCR expression.**

Cells	TCR species	Conclusion	Reference
Jurkat (and KD)	Human	CD3γ required for TCR expression	Geisler 1992
Primary CD3γ def.	Human	CD3γ NOT required for TCR expression	Perez-Aciego et al. 1991
CD3γ def. mouse	Mouse	CD3γ required for TCR expression	Haks et al. 1998
HeLa (TCR transfected)	Mouse	CD3γ or CD3δ NOT required for TCR expression in non-T cells	Kappes and Tonegawa 1991
293T (TCR transfected)	Mouse	CD3γ or CD3δ NOT required for TCR expression in non-T cells	Szymczak et al. 2004
2B4 CD3δ def.	Mouse	CD3δ required for TCR expression	Bonifacino et al. 1989
CD3δ def. clone	Mouse	CD3δ required for TCR expression	Buferne et al. 1992
CD3δ def. mouse	Mouse	CD3δ required for αβTCR expression	Dave et al. 1997
B cell microsomes (TCR transfected)	Human	CD3γ & CD3δ required for association to ζζ homodimer	Call et al. 2002

for surface TCR expression, whereas their cytoplasmic domains are dispensable (Luton et al. 1997).

A controversy about the requirement of CD3γ or CD3δ for TCR assembly and surface expression has been active for years. Several studies using primary cells, cell lines or TCR-transfected non-T cells have led to different conclusions, both for and against their critical role (Table 8).

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