

γδ T lymphocytes in the diagnosis of human T cell receptor immunodeficiencies

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INTRODUCTION

Human T cell receptor (TCR) immunodeficiencies (TCRID) are rare autosomal recessive disorders caused by mutations affecting TCR, CD3, or CD247 chains, which share developmental, functional, and TCR expression defects (1). Their rapid diagnosis is fundamental for patient survival and early hematopoietic stem cell transplantation. Here, we propose that studying γδ T cells, which are often neglected, can be helpful for a timely diagnosis. We thus offer a diagnostic flowchart and some lab tricks based on published cases.

γδ T CELL AND TCR PHYSIOPATHOLOGY

γδ T lymphocytes are a minor subset (1–10%) of human peripheral blood T cells. Most (>70%) are CD4−CD8− [double negative (DN)], some (30%) are CD8+CD4− and very few (<1%) are CD4+CD8− [CD8+ or CD4+ single positive (SP), respectively]. Most γδ T cells in adults express Vδ2/Vγ9 TCR variable regions (65–90%), the rest being mostly Vδ1+, some Vδ3+ or Vδ5+, all with different Vγ chains (2). As peripheral blood γδ T cells are scarce, their over-representation is more conspicuous than their under-representation, which is very rarely reported and normally associated to a single subset, such as Vδ2+ in granulomatosis (3) or aging (4). Indeed, no selective γδ T cell immunodeficiency (ID) has been reported to date, although absence of γδ T cells has been described together with other lymphocyte derangements in rare primary ID (5). The clinical significance of increased γδ T cells, defined as >10% of peripheral blood T lymphocytes (6), requires clarification in several diseases including infection, autoimmunity, cancer, and primary ID.

The human γδ TCR (Figure 1A inset) is an octameric protein complex composed of three heterodimers (TCRγ/TCRδ, CD3γ/CD3ζ, and CD3δ/CD3ε) and one single CD247 homodimer (also termed ξ/ξ). The complex can be abbreviated as γδTCR/γδζεξ. The TCRγ/TCRδ heterodimer contains variable regions, which allow for antigen recognition, while the other three dimers are invariant and are required for surface TCR expression and for intracellular propagation of the recognition signal (7). Therefore, defects in any chain would expectedly impact γδTCR expression and γδ T cell selection and function.

γδ T CELLS IN TCRID

αβ T cells have been extensively studied in TCRID. In contrast, γδ T cells have been frequently ignored, in part due to their scarcity but also to the lack of markers other than the TCR to identify them when TCR expression is reduced, as is the case in TCRID. Although their functions are still debated, we believe that their accurate study (relative numbers, Figure 1A, TCR expression, Figure 1B, and main subsets) can help to diagnose TCRID, as reviewed below and summarized in a practical diagnostic flowchart in Figure 1C.

αβ T-cell deficient patients showed combined ID and autoimmune features due to a selective block in αβ T cell development, as TCRα is part of the TCRα/TCRβ (αβTCR, Figure 1A inset) antigen-binding heterodimer (9). In contrast, the γδ TCR was unaffected, as demonstrated by normal surface expression (Figure 1B), which allowed for normal absolute but increased relative numbers of γδ T cells (Figure 1A). This is unique among TCRID and thus a useful feature in the diagnostic flowchart (Figure 1C). Such γδ T cells were proposed to be in part protective from infections in the two reported patients. Indeed, γδ T cells are involved in immune responses against a variety of pathogens including virus, bacteria, and parasites, whereas still other act as antigen-presenting cells (10) or B cell helper cells (11). Their beneficial effects in vivo have found recent unexpected recognition in haploidentical allogeneic hematopoietic cell transplantation after depletion of αβ T and B cells (12),
which showed that γδ T cells did not cause graft vs. host disease and may have helped with host immune maintenance and recovery. The fact is that, compared to other complete TCRID, symptoms in both TCRα deficient patients appeared rather late (6 and 15 months of age) and transplantation took place very late (6–7 years of age).

Similar to TCRα deficient patients, patients with partial CD3δ deficiency (CD3δ* in Figure 1) due to a leaky splicing mutation showed strongly reduced αβ T cell numbers and normal absolute but high relative numbers of γδ T cells (Figure 1A), although with low surface TCR expression [113] and Figure 1B]. In contrast to TCRα deficiency, partial CD3δ deficiency showed early severe combined ID (SCID) features and required very early transplantation (before 2 years of age), thus their γδ T cells were not protective, perhaps as a consequence of their impaired TCR expression and function [13]. Unexpectedly, partial CD3δ deficiency caused a stronger impact in γδ (Figure 1B) than in αβ TCR surface expression (25 vs. 55% relative to controls [13]). A detailed study of their γδ T cells showed an enrichment in a subset of otherwise rare CD4+ γδ T cells, which exhibited an activated phenotype and were refractory to further TCR stimulation [14]. This CD4-expressing γδ T cell subset seems to be pathognomonic for partial CD3δ deficiency, since: (i) it has been ascertained in three of three tested patients with this condition and (ii) it was not found in other TCRID [14,15]. Its developmental origin deserves further comment. αβ and γδ T cells differentiate within the thymus from a late DN common progenitor [16]. In humans, development of most γδ T cells seems to mimic that of αβ T cells: from DN progenitors through a CD4+CD8+ double positive (DP) pathway [17, 18], to DN and either CD4+ or CD8+ SP populations. DN and CD8+ SP γδ T cells are minor intrathymic subsets but become the major γδ T cell subsets in the periphery, while CD4+ SP are the main intrathymic subset, followed by DP [19]. Notably, the last two subsets can be found in peripheral blood in pathological conditions, and most bone marrow and peripheral blood γδ T cells from patients with γδ T cell acute lymphoblastic leukemia are either CD4+ SP or DP [20]. Thus, we believe that the 10-fold enrichment of CD4+ SP γδ T cells observed

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**FIGURE 1 | γδ T cells in TCRID** (A) Proportion of γδ T cells within the T cell compartment. The percentage of γδ T cells (mean ± SEM) was defined as γδ TCR+ using 11F2, IMMU510, or anti-TCRδ monoclone antibody (mAb) within the T lymphocyte gate (defined as CD3+ and ordered from left to right in decreasing values. The gray band indicates the normal range for infants [1]). Inset: human TCR isotypes. NA: not analyzable (no T cells); *: leaky mutations (partial defects); n: number of patients for which data was available. (B) % TCR surface expression (mean ± SEM) in γδ or αβ T cells relative to healthy donors. TCR surface expression was determined by flow cytometry using different anti-CD3 mAb, γδ T cells were identified as in (A) and αβ T cells as γδ TCR CD3+ or CD4+ cells. ND: not determined. (C) Our suggested TCRID diagnostic flowchart using absolute lymphocyte counts for T−B−NK+ or T−B−NK+ phenotype and basic flow cytometry data (top) to point to the most likely culprit TCR chain (bottom). TCR chains are represented by black boxes arranged according to the proportion of γδ T cells from (A) and their surface TCR expression relative to normal controls from (B). The white box indicates normal value. * as in (A). Brackets represent expected defects, as γδ T cells values were not available in these TCRID.
in patients with partial CD3δ deficiency is due to low TCR-dependence for positive selection of CD4+ γδ T cells and disrupted negative selection of CD4+ γδ T cells (14).

CD3γδ deficient patients, most of which showed mild ID (21), had normal numbers of polyclonal peripheral blood γδ T cells (absolute and relative, (22) and Figure 1A) with low surface TCR [around 30% of control levels (22) and Figure 1B], similar to their γδ T cell counterparts (23), likely with an abnormal γδ TCR/δδδδ̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅̅"


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