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Human γδ T cells: From a neglected lymphocyte population to cellular immunotherapy: A personal reflection of 30 years of γδ T cell research☆

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1. Prologue

The structural basis for antigen recognition by T lymphocytes has been a mystery for many years. We have come a long way from the initial studies by Binz and Wigzell showing the shared idiotypic determinants on B and T cells [1] to the identification of the CD3-associated heterodimeric αβ polypeptide constituting the T-cell antigen receptor (TCR) [2,3]. The enigmatic issue of how the plethora of antigen receptors on T lymphocytes is generated appeared to be solved with the discovery of genes encoding the TCR α and β subunits [4–6]. The Kunkel laboratory contributed to the characterization of the human TCR at this early stage by developing monoclonal anti-TCR antibodies (mAb) using peripheral blood cells from leukemic patients as immunogens [7,8]. It was soon recognized, however, that a third rearranging gene is present in murine and human thymocytes [9–11]. It thus came as a surprise that there is indeed a second cell surface-expressed TCR consisting of a heterodimeric γ and δ chain complex [12–14]. The availability of mAb with specificity for expressed Vγ and Vδ genes of the human γδ TCR opened the way to investigate the tissue distribution of γδ T cells and to isolate γδ T cell subpopulations for functional analysis [15,16]. My first encounter with γδ T cells happened when I analyzed T cell clones that I had established from fetal human tissue. We found that a significant proportion of $CD3^+$ T cells in fetal spleen and thymus lacked cell surface CD2 expression [17]. Several of the established $CD2^-CD3^+$ clones did not react with the Cβ-specific mAb BMA031 but were stained by mAb Ti-γA [17], which later was shown to be specific for Vγ9 [16]. The absence of CD2 on some cells is not a selective feature of γδ T cells as we were also able to identify $CD2^-CD3^+$ subsets among peripheral blood αβ T cells [18]. With the discovery of γδ T cells as a distinct T cell population, many groups set out to investigate their antigen specificity and functional activities. There are excellent recent reviews [19,20], and a recently published compendium contains a collection of 27 articles which provide a state-of-the-art overview on recent advances in γδ T cell biology [21]. In the following sections I will concentrate on a few issues where my laboratory has contributed over the years. This is not meant to provide a comprehensive review, and thus I regret omitting the mention of many γδ T cell *aficionados* who have made important contributions to this field.

2. The human γδ TCR repertoire in health and disease

In contrast to the αβ TCR, the available number of expressed Vγ and Vδ genes is small. In humans there are 6 expressed Vγ genes and a few additional pseudogenes, and the number of Vδ genes is similarly low [22]. We generated mAb against Vγ9 (7A5), Vγ2/3/4 (23D12) and Vγ3/5 (56.3) by immunizing mice with thymic or blood-derived γδ T cell lines [23–25]. These antibodies proved useful for characterization of the human TCR Vγ repertoire [26]. Together with an available anti-Vγ8 mAb [27], it was possible to analyze the complete expressed human TCR Vγ repertoire by flow cytometry. Such studies revealed that γδ T cells expressing the Vγ9 element (usually paired with Vδ2) dominate among γδ T cells in the peripheral blood of healthy adults, at least in the Caucasian population [25]. Vγ9 cells usually account for anywhere between 50 to >95% of the peripheral blood γδ T cell population. The TCRγδ repertoire is different in infants where non-Vγ9Vδ2 cells usually predominate. The alteration in the expression of the VγVδ repertoire occurs during childhood and has been attributed to the exposure to environmental stimuli (in particular microbes) that might shape the TCRγδ repertoire in early life [28]. However, this view has been recently challenged by findings demonstrating that phosphoantigen- (see below) reactive Vγ9Vδ2 are already present in the blood of the second-trimester fetus, *i.e.* independent of postnatal microbial exposure [29]. Interestingly, the peripheral blood TCRγδ repertoire is different in healthy adults from West Africa where Vδ1 cells dominate over Vδ2, with no apparent or known influence of environmental factors [30]. As has been elegantly shown in mice [31,32], the TCRγδ repertoire is also compartmentalized in humans. In the small intestine and colon, Vδ1 predominates, and again the TCRγδ repertoire is

Abbreviations: APC, antigen-presenting cell; BTN3A, butyrophilin 3A; HDAC, histone deacetylase; IPP, isopentenyl pyrophosphate; n-BP, nitrogen-containing bisphosphonate.

☆ Dedicated to the memory of Henry Kunkel's 100th birthday.

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shaped during fetal to adult development [33,34]. Although human skin does not harbor the dense network of specialized dendritic epidermal T cells (DETC) that represent γδ T cells with a clonotypic TCR in the mouse [35], human skin does have γδ T cells with a restricted TCR repertoire which is different from peripheral blood [36]. Taken together, the TCRγδ repertoire varies in different anatomical localizations (blood, intestine, skin) but is oligoclonal in each case, pointing to recognition of different yet limited sets of ligands by the γδTCR [37].

Alterations in the expressed TCRγδ repertoire suggesting a role for γδ T cells in pathophysiology have been described in a number of diseases including inflammatory bowel disease and rheumatoid arthritis [27,38]. We studied the TCR repertoire and function of γδ T cells in HIV-1 infected individuals. We and others observed a selective reduction of Vγ9Vδ2 T cells while the frequency of Vδ1 γδ T cells was preserved and in many cases even increased [39–41]. Alterations in the γδ T cell compartment were not limited to a shift in the Vδ2/Vδ1 ratio. Rather, we also found reciprocal changes in Th1 *versus* Th2 functions of γδ T cells in HIV-1 infected donors [42]. Vγ9Vδ2 T cells respond to non-peptidic mycobacterial antigens [43]. γδ T cells have a low capacity to produce IL-2 and depend on IL-2 production by CD4 T cells (or exogenous supply of IL-2) for proliferative expansion. Our functional studies demonstrated that the deficient responsiveness of Vγ9Vδ2 T cells from HIV-1 infected individuals to mycobacterial antigens was due to reduced CD4 helper activity and not to an intrinsic defect of the remaining γδ T cells [44]. Given that Vγ9Vδ2 T cells produce CC chemokines including CCL5 (RANTES) with known capacity to inhibit cellular HIV entrance *via* CCR5 [45], a sufficient quantity of functional Vγ9Vδ2 T cells appears to be an important part of the anti-HIV immune response. In fact, it has been shown that antiretroviral therapy that results in prolonged virus suppression is associated with a reconstitution of the Vγ9Vδ2 T cell population; whereas, severe defects in the Vγ9Vδ2 T cell pool remain in patients with chronic HIV viremia [46]. In addition to the direct anti-HIV effect of the Vγ9Vδ2 T cells, the expanded Vδ1 T cells also contribute to immune homeostasis in the context of HIV-1 infection by producing interferon-γ and IL-17 in response to opportunistic microbes such as *Candida albicans* [47]. A depletion of the Vγ9Vδ2 T cell subset is also seen in a few other diseases. Interestingly, we observed such a selective depletion in patients with granulomatosis with polyangiitis (GPA, previously termed Wegener's granulomatosis), an autoimmune disease characterized by anti-neutrophil cytoplasmatic autoantibodies directed against proteinase-3 and associated with multiple alterations in the T cell compartment [48]. Even though currently not proven, a possible contributing factor to the progressive depletion of Vγ9Vδ2 T cells might be chronic overexposure to microbial phosphoantigens [48].

At the clonal level, T cells including γδ T cells occasionally express unconventional TCR configurations. Transrearrangements between distinct TCR genes may result in surface expression of hybrid TCR chains, and rare T cells can escape allelic exclusion and express two separate TCR molecules. We identified a significant proportion (1/10.000) of peripheral blood T cells which expressed a productive Vγ-JβCβ rearrangement giving rise to αβ T cells that were stained with the Vγ-specific mAb 23D12 [49]. The hybrid VγCβ chains comprising Vγ3 or Vγ4 genes were paired to classical VαCα chains and could be identified by simultaneous staining with Vγ2/3/4-specific mAb 23D12 and Cβ-specific mAb BMA031 [49]. In further studies we found that productive Vγ-JβCβ transrearrangements can also involve the Vγ9 element. In-frame transcripts of Vγ9-JβCβ transrearrangements were detected in healthy individuals and in patients with ataxia telangiectasia, where increased chromosomal instability is a characteristic feature [50,51]. In these instances, cell surface-expressed transrearranged TCR are functional and thus contribute to enlargement of the TCR repertoire [49,50]. We also observed that some γδ T cells can actually express two distinct TCR on the cell surface. We thus identified a patient with a lymphocytosis where the clonally expanded γδ T cells expressed two distinct in-frame Vγ4Vδ1 and Vγ5Vδ1 TCR rearrangements and corresponding cell surface receptors [52]. Moreover, we characterized a patient where a hepatosplenic γδ T cell lymphoma developed two years after complete remission of acute myelogenous leukemia (AML). In this case, the malignant T cell clone expressed Vγ3Vδ1 and Vγ9Vδ1 TCR [53]. However, the occurence of dual TCR is not limited to (malignant) γδ T cells but is also found among αβ T cells. Rare examples of dual TCR-expressing T cells have also been described in inflammatory bowel diseases and in HIV infection. While it is not clear why non-transformed dual TCR expressing cells have escaped allelic exclusion during intrathymic development, these occurrences can theoretically (depending on the antigen specificity of the two TCRs) give rise to pathological conditions such as autoimmunity [54].

3. Functional plasticity of Vγ9 T cells

Conventional T cells expressing the αβ TCR and CD4 or CD8 molecules as coreceptors recognize myriads of peptides in the context of appropriate MHC class II or class I molecules. Apart from these conventional T cells which (together with B cells) constitute the adaptive "specific" immune system, various groups of unconventional lymphoid cells provide additional layers of cell mediated immunity. Such cells may (*e.g.*, NKT cells, MAIT cells, γδ T cells) or may not (*e.g.*, NK cells, ILC) express TCR molecules [55–60]. These unconventional lymphoid cells rapidly respond to stress- or infection-induced signals or cytokines and play a major role in local immune surveillance. In some cases, redundancy between activation of distinct subsets might ensure an appropriate overall response. A short overview is presented in Table 1. In the following, I concentrate on the Vγ9Vδ2 subset of human γδ T cells.

Activation of γδ T cells is triggered by TCR/CD3 stimulation but also by alternative signaling pathways. αβ T cells can be stimulated in a CD3-independent pathway *via* the cell surface CD2 molecule by a combination of two anti-CD2 mAb directed against distinct epitopes [61]. We found that γδ T cells have a reduced CD2 pathway activation threshold, because single anti-CD2 mAb were sufficient to activate γδ but not αβ T cells [62]. On the other hand, the kinetics and intensity of signaling *via* the CD3/TCR complex seem to be stimulus-dependent and sustained compared to αβ T cells [63,64]. Furthermore, the γδ TCR also differs from the αβ TCR in its capacity to initiate a conformational change in CD3 subunits upon antigen recognition. This conformational change is absolutely required for activation of αβ T cells but is dispensable for induction of cytokine production and proliferation by γδ T cells [65]. The functional plasticity of conventional αβ T cells correlates with the differentiation-dependent expression of specific transcription factors dictating the production of pro- *versus* anti-inflammatory cytokines. Appropriate priming conditions can also induce a comparable spectrum of cytokines in γδ T cells. While peripheral blood Vγ9 T cells activated with microbial phosphoantigens are prone to produce TNFα and IFN-γ [66], they can be easily driven toward IL-4 secreting Th2 phenotype [67]. In

Table 1
Human unconventional lymphoid cells.

Lymphoid cell	Expressed TCR	Activating ligands/signals	Reference
Invariant NKT cell	Vα24-Jα18	Glycolipids + CD1d	[56,57]
MAIT cells[a]	Vα7.2-Jα33	Riboflavin derivatives	[56,57]
Non-Vδ2 γδ T cells	Non-Vδ2, various Vγ	CD1d, lipids + CD1d	[55]
		Endothelial protein C receptor	[93]
Vγ9Vδ2 T cells	Vγ9Vδ2	Pyrophosphates	[19,20]
NK cells	No TCR	MICA/B, ULBPs	[60]
		HLA class I	
ILC1,2,3[b]	No TCR	Microbial signals, IL-23, IL-25, IL-33, TSLP[c]	[58,59]

[a] MAIT, mucosa-associated T cell.
[b] ILC, innate lymphoid cell.
[c] TSLP, thymic stromal lymphopoietin.