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REVIEW

Defining the nature of human γδ T cells: a biographical sketch of the highly empathetic

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The elusive task of defining the character of γδ T cells has been an evolving process for immunologists since stumbling upon their existence during the molecular characterization of the α and β T cell receptor genes of their better understood brethren. Defying the categorical rules used to distinctly characterize lymphocytes as either innate or adaptive in nature, γδ T cells inhabit a hybrid world of their own. At opposing ends of the simplified spectrum of modes of antigen recognition used by lymphocytes, natural killer and αβ T cells are particularly well equipped to respond to the 'missing self' and the 'dangerous non-self', respectively. However, between these two reductive extremes, we are chronically faced with the challenge of making peace with the 'safe non-self' and dealing with the inevitable 'distressed self', and it is within this more complex realm γδ T cells excel thanks to their highly empathetic nature. This review gives an overview of the latest insights revealing the unfolding story of human γδ T cells, providing a biographical sketch of these unique lymphocytes in an attempt to capture the essence of their fundamental nature and events that influence their life trajectory. What hangs in their balance is their nuanced ability to differentiate the friends from the foe and the pathological from the benign to help us adapt swiftly and efficiently to life's many stresses.
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PROLOGUE

Since the time of their accidental discovery during the molecular characterization of the α and β T cell receptor (TCR) genes belonging to their relatively unambiguous brethren,[1] 'enigmatic' is still one of the most commonly used adjectives to describe γδ T cells. This can be attributed to the fact that these unconventional lymphocytes have thus far been remarkably successful in thwarting most attempts at definition. Much of what we know about T cell biology and function comes from what has been discerned through studies done on αβ T cells; however, one thing that is certain is that the majority of the rules governing the lives of αβ T cells are surprisingly irrelevant when it comes to understanding the elusive character of γδ T cells. Table 1 highlights some of the major differences between these two T cell subsets in humans.

Recognition by γδ T cells is not typically in the context of classical major histocompatibility complex (MHC) class I or II molecules and neither is antigen processing required.[2,3] These observations are corroborated by the fact that the majority of γδ T cells are CD8 and CD4 negative. Crystal structure analysis of the γδ TCR determined that the length and conformation of γδ TCR resemble immunoglobulins (Ig) more than the αβ TCR, which was taken to suggest that antigen recognition by γδ T cells may be more similar to the binding of antibody to antigen rather than the MHC/peptide complex recognized by αβ T cells.[4] The very basis of foreign antigen recognition by lymphocytes of the adaptive immune system is a contentious point for these unique T cells. Although γδ T cells appear to have antigen recall memory by their demonstrated rapid expansion and antimicrobial response upon reinfection with a particular pathogen, much like lymphocytes of adaptive immunity,[5–7] clonally expanded γδ T cells with very restricted receptor repertoires are able to respond readily to a wide range of both infectious and non-infectious stressors. Natural killer (NK) cells share some characteristics with γδ T cells as both are usually considered constituents of innate immunity, recognize transformed cells, play a prominent role in antiviral protection and are cytolytic lymphocytes.[8–10] Like NK cells, human γδ T cells express activating receptors, such as NKG2D that recognizes stress-inducible MHC class I-related MICA/MICB molecules and the UL16-binding proteins that are upregulated on malignant or stressed cells and induce cytolysis,[11,12] and they are inhibited by the expression of killer Ig-like receptors, which bind certain MHC class I alleles expressed by non-malignant cells and trophoblasts.[13–15] However, the versatility of γδ T cells is further extended by their demonstrated ability to assume the role and appearance of professional antigen presenting cells.[16,17] This unusual functional plasticity has given the impression that these multitalented T cells are 'Jacks of all-trades, but masters of none', and they are sometimes erroneously delegated to a niche occupied by vestigial

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Table 1 Major differences between human αβ and γδ T cells

Feature	*αβ T cells*	*γδ T cells*
Frequency in blood	• 65%–75% of PBMC	• <10% of PBMC (25%–60% gut)
MHC restriction	• $CD4^+$: MHC class II • $CD8^+$: MHC class I	• No MHC restriction • Possible roles of CD1 and MICA/B
CD4/CD8 expression	• ~60% $CD4^+$; ~30% $CD8^+$; <1% double positive; • <1% double negative	• Majority (>70) double negative; <1% $CD4^+$; ~30% $CD8^+\alpha\alpha$ (as IELs in gut)
Antigen recognition	• Processed peptide/MHC	• Unprocessed, not peptides
TCR V gene germ line repertoire	• Large	• Small
TCR diversity	• Very diverse	• Relatively restricted expression despite high potential for junctional diversity; expression variance dictated by tissue localization
Function	• Adaptive immunity	• Immune regulation, surveillance and homeostasis

Abbreviations: IEL, intraepithelial lymphocyte; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cell; TCR, T cell receptor.

features of modern anatomy by some who are left confounded as to their real purpose.

This review provides a biographical sketch of human γδ T cells in an attempt to convey the essence of their fundamental nature and what may influence their life trajectory. Figure 1 illustrates the highly simplified paradigm for NK cells, αβ T cells and the emerging view of where in the continuum that encompasses immune homeostasis and protection γδ T cells take the throne. At opposing ends, NK and αβ T cells are particularly well equipped to respond to the 'missing self' and the 'dangerous non-self', respectively, or as Klas Kärre had so aptly put it—'to recognize a foreign submarine'.[18] Between these two extremes, we are chronically faced with the challenge of making peace with the 'safe non-self' and dealing with the inevitable 'distressed self', and it is within this more complex realm γδ T cells excel thanks to their highly empathetic nature. What hangs in the balance is the ability to differentiate the friends from the foe and the pathological from the benign—and adapt swiftly and efficiently to life's many stresses.

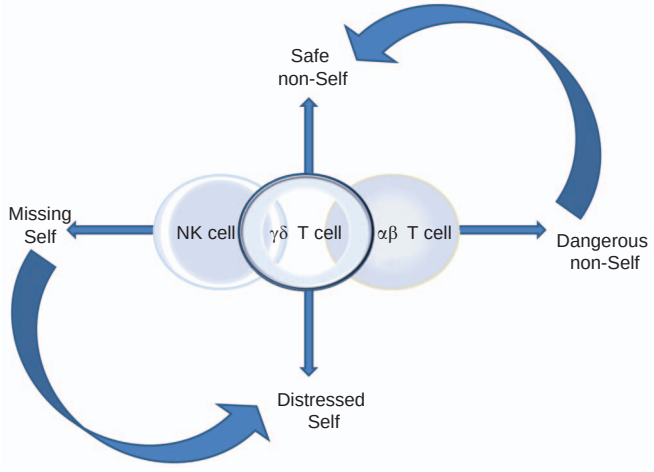

Figure 1 A simplified paradigm illustrating where in the continuum of immune protection and homeostasis γδ T cells fall in relation to innate NK cells and the adaptive αβ T cells. At the two extreme ends, NK and αβ T cells are particularly well equipped to respond to the 'missing self' and the 'dangerous non-self', respectively. Between these two extremes, we are chronically faced with the challenge of making peace with the 'safe non-self' and dealing with the inevitable 'distressed self', and it is within this more complex realm γδ T cells excel. It should be recognized that these different 'selves' and the immune response(s) that they trigger exist in a continuum and are modulated by the context in which they are presented. Both NK and αβ T cells work with γδ T cells to fill in the gaps of this spectrum—with NK cells contributing to responding to the 'distressed self' and αβ T cells having some regulatory training to temper the response to the 'safe non-self'. NK, natural killer.

SPECIES SPECIFICITY: THE BEST LAID PLANS OF MICE AND MEN OFT GO AWRY

Among the many fascinating and important conundrums that have yet to be solved for γδ T cells is the large phenotypic difference between species when it comes to their relative numbers, location and antigen recognition. In both mice and primates, γδ T cells comprise a small population of peripheral T cells (averaging 5% of the total peripheral T cell pool in Caucasians living in Western Europe and North America), and they are poorly represented within conventional T cell zones found in lymphoid tissue.[19] In contrast, sheep, cattle, rabbits and chickens have much higher levels of circulating γδ T cells.[20] The reason behind this disparity in the constitution of γδ T cells between and within species is currently unknown, but the discrepancy seen between 'low' and 'high' γδ T cell species has been correlated with the total level of TCR variable genes, which is represented largely by the variable potential of the αβ TCR, in conjunction with the diversity present in the Ig variable genes. Thus, species having a high number of variable TCR and Ig genes tend to have low numbers of peripheral γδ T cells (Table 2).[20] It can be speculated that the role of and exposure to microbes in the species' lifecycle may be associated with these immune parameters. For example, omnivorous rodents and primates may have less of a need to maintain peace with a diverse population of microbiota in comparison to cows, sheep, rabbits and chickens for which microbes play a more significant role in digestion and metabolism.[21–24] Therefore, the immune cell repertoire that recognize more variable 'non-self' as dangerous (i.e., the features that we depend on from the adaptive immune system) may be less advantageous when balancing the need to protect beneficial or non-threatening microbes that are needed for sustenance and/or to prevent chronic inflammation.

As shown in Table 3, the numbers of Vγ and Vδ gene segments in both mice and men are greatly limited in comparison to αβ T cells, and despite their relative low levels in peripheral blood, γδ T cells are found to be enriched at mucosal interfaces, such as the intestinal tract, that are heavily populated by largely non-pathogenic and symbiotic organisms.[19,25,26] In the murine system, distinct subsets of γδ T cells bearing specific pairs of the γ and δ TCR chains are targeted to particular epithelial locations during development such as the skin, lungs, uterus, vagina and tongue where they make up a significant portion of the local intraepithelial lymphocyte population and appear to respond to endogenous stress-induced antigens that have yet to be unambiguously identified.[27–31] For some of these special niche localized γδ T

Table 2 Comparative diversity and percent composition of peripheral γδ T cells between species (adapted from Su *et al.*, 1999[20])

	Percent peripheral γδ T cells	*Diversity of αβ T cell receptor variable genes*	*Diversity of immunoglobulin variable genes*
Human	~5% (low)	High	High
Mouse	~5% (low)	High	High
Chicken	~20% (high)	Low	Low
Rabbit	~20% (high)	Low	Low
Sheep	~30% (high)	Low	Low
Cattle	~30% (high)	Low	Low

cells, such as the invariant dendritic epithelial Vγ3Vδ1 T cells found in murine skin,[28] no human equivalent has been found. However, it is interesting to note that the phenomenon observed at the specie level is extended to niches found within an organism and that an abundance of certain subsets of γδ T cells are found where there is a vital need to guard against inappropriate immune responses to 'safe non-selves' at the same time as keeping in check the inevitable 'distressed self'—a combinatorial function that neither αβ T cells nor NK cells are especially designed to deal with alone.

'Non-Vδ2 T cells': the high art of non-self acceptance

In adult humans, two subsets of γδ T cells, defined by the usage of either the Vδ2 or Vδ1 TCR, predominate. The majority of the tissue-associated γδ T cells bear the Vδ1 TCR with Vδ3 and Vδ5 making up minor populations.[32,33] In the literature, these are often collectively called the 'non-Vδ2' γδ T cells. In addition to their role in maintaining immune homeostasis in the local microenvironment,[25] tissue-associated γδ T cells, in both mice and men, play an important function in wound healing, removing distressed or transformed epithelial cells and subduing excessive inflammation.[19,26,34–39] For example, in the gastrointestinal tract γδ T cells play a non-redundant role in maintaining tolerance to food antigens as well as the intestinal flora.[34,40] The Vγ chain pairing of tissue-associated Vδ1 T cells in humans is less stringent in comparison to the highly restricted Vγ9Vδ2 T cells found in peripheral blood—though they prefer pairing with Vγ4 and Vγ5 over Vγ9.[32,41] This greater diversity in pairing and structure is taken to imply that there exists a broader range of ligands that are recognized—but the recognition is thought to be largely introspective as it is usually in response to the changing states of self. The actual antigen specificity and mechanism of recognition of these non-Vδ2 T cells is still in the unraveling. The stress-induced molecules, MICA and MICB, have been found to be tumor antigens recognized by cancer infiltrating Vδ1 T cells.[42,43] The redundancy of this recognition is a point in the pondering since both Vδ1 and Vδ2 T cells express NKG2D, an activating C-type lectin that is the known receptor for MICA, MICB and related UL16-binding protein.[44,45] Nevertheless, the crystal structure analysis showed that the binding of Vδ1 T cells to MICA was mutually exclusive to NKG2D, although the complementary determining region (CDR) of the TCR appeared to have an uncharacteristically flat potential binding surface.[46] Extending this apparent tendency to respond to non-polymorphic MHC class I family-related proteins, CD1c, a molecule that is able to present both foreign and self lipid antigens,[47] is another candidate ligand for Vδ1 T cells.[48] It remains to be seen exactly how broadly based these recognition patterns are within the Vδ1 T cell family since many of these studies have relied on a limited number of γδ T cell clones or lines. Adding a new twist to this storyline, it was recently revealed by the working groups of Willcox and Dechanet-Merville that a 'non-Vδ2' T cell clone expressing Vγ4Vδ5 TCR was activated by the recognition of endothelial protein C receptor.[49] This interaction involved the CDR3 of Vγ4 and was similar in nature to the binding of antibody to antigen. Of particular note, recognition was highly dependent on the multimolecular stress signature that was induced by either infection by cytomegalovirus or malignant transformation.[49] Endothelial protein C receptor is another member of the CD1/MHC class I superfamily that is expressed on endothelium and trophoblasts[50] and can bind lipids similar to CD1 family members; however, binding of the Vγ4Vδ5 T cell clone was determined to be independent of the presence of any lipid antigen.[49]

In continuation of the theme of sometimes needing to accept the 'safe non-self', the immunological state of pregnancy is an example of a time when this requirement is essential. It is still an enigma how the maternal immune system in mammals is not only able to prevent rejection of the fetal semiallograft, but also successfully support its growth for 9 months. Human Vδ1 T cells have been observed to be recruited to the maternal/fetal interface during pregnancy where their activation is believed to play a role in the induction of tolerance to paternal antigens.[15,51–54] Furthermore, there is evidence suggesting that the early pregnancy human decidua is actually a site of extrathymic maturation of Vδ1 T cells.[55,56] This enrichment of γδ T cells at the maternal/fetal interface and their mysterious role in pregnancy is not unique to humans as the same has been observed in sheep[57] and mice[58], suggesting a central conserved role of γδ T cells involved in pregnancy-related immune changes. In women with healthy pregnancies, the changes are not only found at the maternal/fetal interface since the number of Vδ1 T cells in circulation have also been reported to be increased in relation to the Vδ2 subset.[59–61] Of note, some immune-related pregnancy complications where maternal immune tolerance is compromised and there is risk of premature pregnancy termination, a significant drop in the number of circulating Vδ1 T cells is observed.[62] The cognate pregnancy-associated γδ T cells in the murine system were found to directly recognize an unknown antigen on trophoblasts *via* their TCR, and this recognition was MHC-unrestricted and, intriguingly, not species-specific.[63] Although expression

Table 3 Comparative diversity of the α, β, δ and γ TCR chains in mice and men[32]

	Human	*Mice*
Vγ	~6 (5 from same family and 1 distantly related)	~6 (2 from same family and 4 broadly diverged)
Vδ	~8 functional genes (only 3 commonly used—VD1, VD2 and VD3)	~16 (6 homologous and 10 distinct)
Vα	~42	~75
Vβ	~47	~23

Abbreviation: TCR, T cell receptor.

of inhibitory non-classical MHC class I molecules, such as HLA-E and HLA-G, on trophoblasts appear to play a role in suppressing Vδ2 T cells during pregnancy, they are unlikely to be the candidates recognized by the Vδ1 T cells actively on guard.[15] Mammals require a reliable mechanism to maintain immune tolerance to 'safe non-selves', as a failure to do so would be extinction. In this regard, Vδ1 T cells seem to have a conserved acumen for brokering peace for the benefit of generations to come.

Vδ2 T cells: to know the complex burden of being human

In the peripheral blood of most healthy adult humans and some other primates such as the rhesus monkey, the vast majority of γδ T cells bear the Vδ2 TCR—usually paired with the Vγ9 chain.[64] These special canonical Vγ9Vδ2 T cells recognize non-peptidic phosphorylated antigens, such as isopentyl pyrophosphate (IPP), that are metabolites in the essential isoprenoid biosynthesis pathway present in virtually all living organisms.[6] Endogenously, IPP and its stereoisomer dimethylallyl diphosphate are substrates produced in the mevalonate pathway for cholesterol metabolism; however, they can also be produced by the deoxyxylulose pathway commonly used by organisms, such as *Escherichia coli* and certain plant cells, that lack the critical HMG-CoA reductase enzyme of the mevalonate pathway.[65,66] It was later determined that the stimulatory capacity of IPP and dimethylallyl diphosphate is actually fairly weak in comparison to some of the upstream intermediates of the alternative pathway of isoprenoid biosynthesis, such as (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate,[67] and it is usually in the context of malignancy or distress that cellular endogenous levels are able to awaken the effector functions of Vγ9Vδ2 T cells.[68] A class of drugs called aminobisphosphonates used for the prevention of bone fragility fractures and certain alkylamine compounds that are ubiquitously dispersed in everything from plants to amniotic fluid are also able to stimulate Vγ9Vδ2 T cells[69,70] by virtue of their ability to induce cellular accumulation of IPP. Both these compounds modulate IPP levels by blocking the metabolic activity of farnesyl pyrophosphate synthase, a key enzyme in the mevalonate pathway.[71,72]

It is unclear whether these 'phospho-antigens' (pAgs) require presentation *via* some cell-surface accessory molecule; however, the fact that contact with cells of human origin is necessary for their ability to fully activate Vγ9Vδ2 T cells seems to suggest that some kind of assistance is necessary.[73] Recent developments in regards to alternative potential endogenous stress-induced ligands for Vγ9Vδ2 T cells provide new venues that may reveal how these cellular short-lived substrates can be potentially stabilized and presented extracellularly to the stress surveillance capabilities inherent in these lymphocytes. Scotet and colleagues[74] first reported that tumor recognition by Vγ9Vδ2 T cells could be mediated by the ectopic expression of ATP synthase/F1-ATPase, which is normally expressed on the internal membrane of mitochondria, and this interaction was enhanced by the cobinding of apolipoprotein A-I that is usually present in serum. Literally along the same vein, it was later observed that shear stress experienced by endothelial cells also led to the translocation of the ATP synthase β chain to the cell surface which resulted in the binding and activation of Vγ9Vδ2 T cells.[75] Reminiscent of the previous findings with F1-ATPase, the response of γδ T cells to endothelial cells expressing ATP synthase β was significantly potentiated by the coincident accumulation of cholesterol in the cell membrane, and this interaction led to the secretion of inflammatory cytokines by γδ T cells and upregulation of vascular cell adhesion molecules on endothelial cells.[75] This phenomenon was taken to suggest that endothelial dysfunction, characterized by the disturbed flow created by shear stress, and hypercholesterolemia work synergistically to activate γδ T cells and the endothelium[75]—providing a novel mechanism contributing to cardiovascular pathology. In an attempt to reconcile the established reactivity of Vγ9Vδ2 T cells to non-peptidic pAgs and these new observations of alternative stress-induced ligands, experiments were carried out to demonstrate that ATP synthase can also potentially bind to pAgs, and it was concluded that it seemingly has the appropriate features to function as a possible pAg presenting molecule for Vγ9Vδ2 T cells.[76] More recently, another putative Vγ9Vδ2 T cell tumor antigen, human MutS homologue 2 (MSH2), was identified using a peptide-based affinity screening system for the CDR3 of the δ chain.[77] MSH2 is normally located in the nucleus where it functions as a DNA mismatch repair gene, but it is often mutated in a number of different types of epithelial cancers and can be ectopically expressed.[78] Transformation of normal human B cells by Epstein–Barr virus or subjecting renal carcinoma cell lines to oxidative stress also led to an increased surface expression of MSH2 and rendered them susceptible to Vγ9Vδ2 T cell-mediated cell lysis that could be blocked by the use of anti-MSH2 antibodies or downregulation of its gene expression.[78,79] These observations were collectively taken to suggest that MSH2 may be yet another damage-associated molecular pattern recognized by Vγ9Vδ2 T cells. An overlooked point worth considering is the fact that MSH2 also has an intrinsic ability to bind and hydrolyze ATP,[80] which may be the property providing the common mechanism leading to the mobilization of human peripheral blood γδ T cells. Both ATP synthase/F1-ATPase and MSH2 are evolutionarily conserved molecules that serve essential stress-sensitive homeostatic functions in prokaryotes and eukaryotes and can bind nucleotide derivatives, and this may confer the potential to present low molecular weight pAgs to Vγ9Vδ2 T cells' clever immunosurveillance strategy. This is still in large part conjecture, and much of the story is still in the telling given that neither F1-ATPase nor MSH2 are absolutely indispensible for pAg recognition. Exogenously added pyrophosphate antigens readily stimulate Vγ9Vδ2 T cells in the presence of human accessory cells, yet both these molecules require some stress-induced event for their ectopic expression—which argues for there existing some other more widely expressed candidate(s) that would be more exclusive to the primate lineage. Harly and colleagues,[81] from the working groups of Scotet and Bonneville, may have uncovered at least one of the missing pieces of the story in the form of a molecule known generically as CD277—or BTN3A. This unassuming molecule is a member of the butyrophilin/B7-like group of proteins belonging to the Ig superfamily that are found clustered at the extended end of the classical MHC I genes on human chromosome 6.[82] In a series of elegant experiments involving specific activating and inhibitory antibodies to CD277 as well as domain-swapping and knockdown experiments, the Scotet–Bonneville group shows convincing evidence that this widely expressed molecule provides the necessary support that pAgs need to awaken Vγ9Vδ2 T cell effector functions.[81] The fact that no functional orthologue of CD277 exists in rodents adds further credence to its purported role as the long-awaited missing link—or what may very well turn out to be a series of interconnected links.

Given the fact that these unique pAg-reactive Vγ9Vδ2 T cells have no known equivalent in either rodents or ruminants, it is perplexing why their effective stress surveillance tactic appears to be solely a primate advantage. It is of considerable interest to determine where along our common evolutionary lineage they first made their appearance. With the emergence of the peripheral Vγ9Vδ2 T cell subset, primates seem to have done away with the functional niches of a

number of other various γδ T cell subsets and chiseled it down to mostly two highly specialized arsenals—the one in the periphery often found fanning the flames at the first site of trouble and the one entrenched in the tissues often putting out the fire before it starts.

RITES OF PASSAGE

First there were γδ T cells

Befitting their deeper evolutionary roots, the ontogeny of γδ T cells precedes αβ T cells in expression. γδ TCR gene rearrangement can be detected by embryonic day 14 in the murine thymus[83] and week 8 in humans,[32] and canonical subsets can also be detected extrathymically during fetal development in both species.[83,84] The most striking feature is the change from having fairly diverse pairs of γδ T cells (of which Vδ1$^+$ serve as the majority in cord blood at birth) to increasingly restricted pairings, with Vγ9Vδ2 T cells becoming the major subset with very limited receptor diversity by adulthood (Figure 2).[32] By 1 year of life, the majority of peripheral blood Vγ9Vδ2 T cells are non-naive, readily produce IFNγ upon stimulation and have cytolytic granules.[85] It was concluded that this phenomenon was evidence of 'the earliest immunological maturation of any lymphocyte compartment in humans.'[85] In contrast, the Vδ1 T cells remaining in the periphery in early life were found to be still naive—similar to αβ T cells.[85] This could be because the antigens that 'non-Vδ2' T cells recognize are more abundantly expressed in the tissue microenvironment where they are targeted, and an analysis of Vδ1 T cells in their true niches, such as the intestine, in this early period may very well find that they too have a mature phenotype. The absolute number of γδ T cells in the periphery increases from birth to about 10 years of age, which is congruent with the expansion of the Vγ9Vδ2 T cell subset going from a minor population at birth to usually >75% of circulating γδ T cells.[32] Although this clonal expansion has been seen as evidence of the vital role that these T cells play in responding to environmental challenges in early life, it has not been demonstrated that this phenomenon is in fact primarily in response to environmental challenges and not, at least in part, in response to endogenous stimuli as an extension of the adaptive changes taking place within the newborn as the immune system is being initiated.

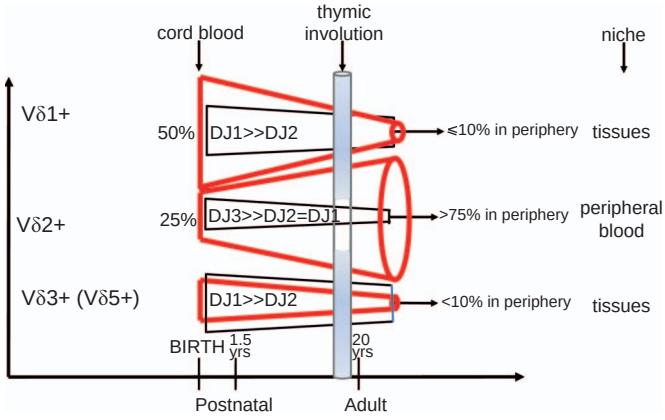

Figure 2 Schematic diagram of the changes in the diversity of γδ T cell repertoire through development in humans. At birth, γδ T cells show high junctional diversity, which is generated through the addition of 'P' (palindromic) and 'N' (non-templated) nucleotides while combining the V-D-J segments of their TCR. Despite the limited variable genes available for γδ T cells, their potential for junctional diversity rivals that of αβ T cells. However, with age, the diversity that is observed at birth gets noticeably restricted, especially for the main pool of circulating γδ T cells, the Vγ9Vδ2 T cell subset, which are a minor population in cord blood but become the predominant subset in circulation by 1 year of life. TCR, T cell receptor.[32]

Defined by experiences tainted by innate tendencies

It should be noted that what has been described for the early life and developmental trajectories of γδ T cells is based largely on studies conducted in North America and Western Europe, and it is now appreciated that there are great interindividual and regional differences in the make-up of one's γδ T cell repertoire. A small study looking at ethnic differences in peripheral γδ T cell numbers found Turkish and 'non-Japanese Asians' (which comprised of a handful of people from Iran, India, Thailand and China) had significantly higher levels of γδ T cells (a median of 9.3% and 9.2%, respectively, of the total T cell pool) in comparison to Swedes (4.2%) and Japanese Asians (4.5%).[86] A childhood history of tuberculosis in one Japanese man was associated with an unusually high percentage of γδ T cells (23.5%), which was similar to some of those sampled from Turkey and non-Japanese Asian countries. Thus, it was concluded that environmental factors, rather than genetic, are likely to be the driving force behind these ethnic and regional disparities in the proportion of γδ T cells found in peripheral blood. It is interesting to note the regional and interindividual differences in the distribution and number of γδ T cells is in line with the 'hygiene hypothesis' which purports that the highly sterilized environment of industrialized or 'Westernized' nations impacts the developmental trajectory of the immune system that is partly shaped by its reciprocal interaction with the microbial world at an early age.[87] This is speculated to be a major contributive factor for the higher prevalence of immune and inflammatory disorders present in industrialized countries.[87,88]

Although a thorough study of potential genetic determinants is still lacking, its contribution to the observed phenotype of γδ T cells should not be ignored. An analysis of the repertoire of peripheral blood γδ T cells in the West African region of Ghana found that healthy people there had a five-fold higher level of circulating Vδ1 T cells in comparison to their European counterparts.[89] This reversed ratio of Vδ1/Vδ2 T cells showed no age dependency nor did it appear to be an antigen-driven event.[89] This region is highly endemic for *Plasmodium falciparum*, the causative agent of malaria, for which the Vδ1 T cell subset seems to have a special immunoregulatory function.[90,91] It is therefore feasible that this change in γδ T cell distribution observed in never-infected individuals from this West African population stems from evolutionary selection based on an immune advantage by those having a high proportion of Vδ1 T cells—which would again align with the association of there being some adaptive influence of microbial exposure on the make-up of γδ T cells in an organism. Other infectious diseases that are associated with the recruitment and expansion of specifically the Vδ1 T cell subset in the periphery are cytomegalovirus,[33,92] Epstein–Barr virus[93,94] and HIV.[95,96] In most of these cases, the specific activation and expansion appears to be driven by the presentation of endogenous molecules rather than virally derived antigens—keeping with the notion that these T cells are particularly well adapted to sensing the turmoil within rather than relying exclusively on external validation.

Tales told by battle scars and tree rings

A number of chronic inflammatory diseases are also associated with perturbed γδ T cell distributions.[97–101] In some cases, such as systemic sclerosis, rheumatoid arthritis, Takayasu's arteritis and Wegner's granulomatosis, an inverse ratio of peripheral blood γδ T cells is seen again such that the Vδ1 T cell subset outnumber the Vδ2 T

cells.[100,101](and unpublished data) This occurrence is often associated with the concomitant loss of peripheral Vγ9Vδ2 T cells—possibly due to their tendency to undergo activation-induced cell death *via* FAS-mediated apoptosis which occurs following repeated stimulation of their TCR.[102–104] Death by exhaustion appears to be a consequence for the highly empathetic in an environment subjected to chronic distress. An example of this is seen in people with Crohn's disease who were found to have a global deficit in the number of γδ T cells present.[98] Evidence that this may be a result of activation-induced cell death as opposed to an inherent immune anomaly of the disease comes from the observation that treatment with infliximab, an anti-TNFα agent, resulted in clonal expansion of γδ T cells in Crohn's patients on treatment[105], highlighting the potential *in vivo* role of TNFα signalling in γδ T cell self-modulation. However, *in vitro* studies looking at the functional effects of infliximab on γδ T cells derived from patients with Behçet's disease or rheumatoid arthritis, in contrast, found that the expansion and reactivity of these excitable T cells was notably impaired,[106,107] underscoring again the often discrepant outcomes when attempting to extrapolate findings from manipulated artificial settings *in vitro* to what occurs *in vivo*. This has been a particularly challenging hurdle when trying to implement the remarkable anti-cancer arsenal of γδ T cells for cancer immunotherapy.[10,108–110] Despite the ready expansion and cytolytic effector functions of γδ T cells demonstrated at the bench, attempts to harness this potential in the clinical setting have been disappointing due to the inevitable loss of these cells that is observed in many cancer patients.[111,112] It is therefore of great interest to find ways to keep these cells from collapsing from the often overwhelming task of meeting the many needs of the distressed self.

Of course the cumulative stressors experienced over a lifetime also take their toll, and a gradual loss of peripheral γδ T cell pool is found to occur with age in the industrialized nations studied.[113–115] The life events that affect γδ T cell function are not limited to infectious and physiological insults, but also include psychological stress that especially mobilize terminally differentiated 'effector memory' cells characterized as being negative for cell surface marker CD27 but expressing CD45RA.[116] This phenotype represents γδ T cells that have already tackled some hurdles in their life and have reduced proliferative capacity, but are still able to pull the trigger given their highly cytolytic potential.[117] Thus, differences in γδ T cell numbers in relation to parameters such as gender and race that are inconsistent across the various populations studied[89,113–115] may partly reflect the discrepant burdens that contribute to overall life stress experienced by members of these groups. For example, African Americans in Baltimore were found to have significantly lower peripheral blood γδ T cells in comparison to their Caucasian counterparts;[113] however, Africans from Ghana have significantly more γδ T cells (of both the Vδ1 and Vδ2 T cell variety) in comparison to Europeans.[89] This observation makes it highly suspect that the racial difference observed in the North American cohort is due to an intrinsic property of African Americans to have fewer γδ T cells, and it may reflect the different cumulative life stresses they face that take a toll on immune function.

'*While there is tea, there is hope.*'

Fortunately, there is a good amount of evidence suggesting that the adaptogenic properties of γδ T cells can be protected and enhanced in those who need it most, namely those burdened with chronic stress. γδ T cells seem particularly responsive to certain nutrient properties found in various food and supplement products, especially those with high antioxidant values (Table 4).[69,118–127] In a double-blind placebo-controlled randomized control trial, capsules containing fruit and vegetable concentrate (containing, among other compounds, standardized levels of β-carotene, vitamin C, vitamin E, folate and calcium) were given to law school students, taken as a representative of young adults living with modern day stresses, over a period of 11 weeks.[128] γδ T cells were found to be specifically increased (by about 30%; no change in circulating αβ T cells) in parallel to an increase in the antioxidant capacity of the study subjects, and *in vitro* analyses showed that this effect was associated with a more tempered inflammatory response upon phorbal myristate acetate stimulation.[128] For some of these compounds with high antioxidant properties, their impact on γδ T cells may be partly derived from their direct effect on the distress levels of cells that γδ T cells are sensitive to. For example, treating renal carcinoma cells subjected to oxidative stress with the strong antioxidant, *N*-acetylcysteine, blunted the inflammatory response and activation of Vγ9Vδ2 T cells by downregulating the ectopic expression of the stress-induced molecule, MSH2.[79] Therefore, some of these immunomodulatory bioactive compounds, which include diverse items like procyanidins from unripe apple peels[129] and polysaccharide components of the Acai berry,[119] their noted influence on γδ T cells may be through the modulation of the type of stress signals displayed by cells that mobilize their activity. In contrast, other compounds, such as the aforementioned alkylamines (e.g. ethylamine, propylamine, buylamine and amylamine),[69] L-theonine from tea[69] and heat-treated mistletoe extracts,[125] the effect may be more direct and specific to human peripheral blood Vγ9Vδ2 T cells through the modulation of isoprenoid synthesis and the generation of pAgs. Understanding the mechanism by which γδ T cell function is affected by various immunomodulatory compounds is central to formulating specific effective protocols for the targeted management of the various ailments these unique cells have the capacity to help treat and/or influence, including cancer, immunodeficiency and autoimmunity. Importantly, antioxidants could play an essential role in providing a buffer to prevent the untimely burn-out of these often over-worked but highly empathetic unconventional lymphocytes.

Table 4 Bioactive compounds that have been studied to enhance γδ T cell function

Compound	*Reference*
Tea (L-theonine)	69,123,127
Alkylamines	69,72,118
Heat-treated mistletoe	125
Plant tannins; procyanidins (e.g. from unripe apple peels)	121,122,129
Polysaccharides from Acai berry and Yamoa	119,120
Concord grape juice and fruit/vegetable concentrate	126,128
Aged garlic	124

EPILOGUE

In light of their story, it can be appreciated that the difficulty in accurately defining γδ T cells within existing prototypes stems from the fact that these T cells operate according to their own exclusive paradigm, as illustrated in Figure 1. In many ways they are the true 'adaptive' T cells as they are able to sense changes within the self that have the potential to disturb equilibrium, and they help the 'self' adapt as efficiently as possible to life's many challenges. However, unlike αβ T cells, their own multifaceted but sensitive character remains relatively invariant in the face of the diverse stressors to which they help the self acclimatize. One strategy that γδ T cells use to fulfill the complex nature of their task is to creatively merge the instincts of

the innate with the diversity of the adaptive immune systems. Thus, some of the antigens that they recognize are evolutionarily shared by the self and non-self and their subsequent response is contextually guided by the level of distress that they sense from within—often in the context of the contingent presence of other damage-associated molecular patterns. Given that different organisms are subjected to different types of stressors that impact their livelihood, it is not surprising that the phenotypic repertoire and distribution of γδ T cells between species is quite diverse and tailored for the specific needs that they help meet. In fact, it was noted that substantial differences can be observed in the γδ T cell fingerprint born between individuals even of the same species depending on the nature and extent of their life stress exposures—somewhat like the story held within the rings of a tree trunk. It still remains to be seen how these interindividual differences in γδ T cell profiles are associated with health outcomes. For diseases such as cancer, a relationship between higher levels of circulating responsive γδ T cells and a more favorable outcome has been observed;[108] however, there is still much to be learned about the potential contribution of γδ T cells in providing general resiliency to malaise. An important challenge is to determine how to best support these intuitive T cells with their burden—some food for thought while destressing over a cup of afternoon tea.

COMPETING FINANCIAL INTERESTS

The authors have no conflict of interest to disclose.

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