



# Development and Selection of the Human $V\gamma 9V\delta 2^+$ T-Cell Repertoire

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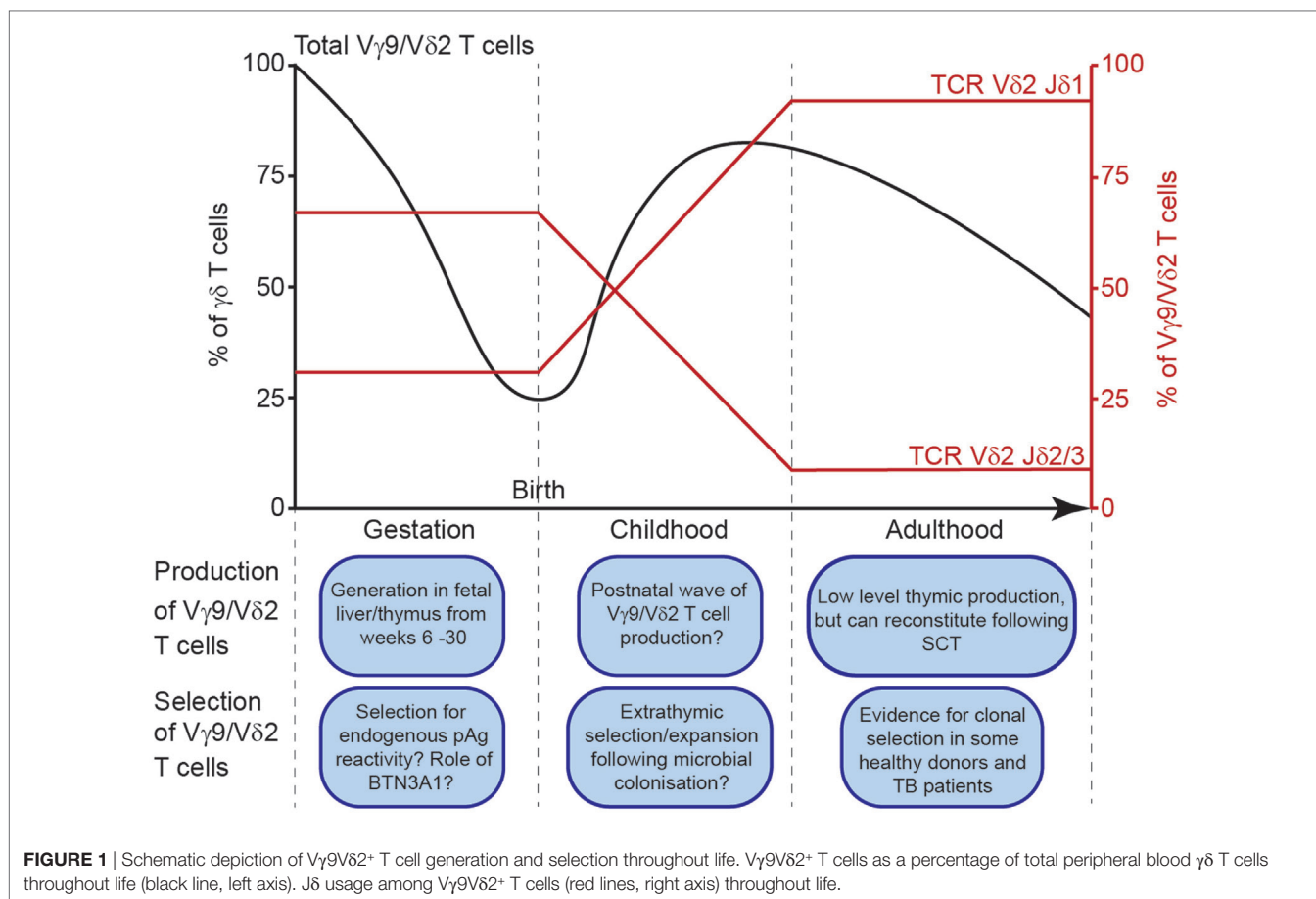
$V\gamma 9V\delta 2^+$  lymphocytes are among the first T-cells to develop in the human fetus and are the predominant peripheral blood  $\gamma\delta$  T-cell population in most adults. Capable of broad polyclonal responses to pyrophosphate antigens (pAg), they are implicated in immunity to a diverse range of infections. Previously  $V\gamma 9V\delta 2^+$  development was thought to involve postnatal selection and amplification of public  $V\gamma 9$  clonotypes in response to microbial stimuli. However, recent data indicate the  $V\gamma 9V\delta 2^+$  T-cell receptor (TCR) repertoire, which is generated early in gestation, is dominated by public  $V\gamma 9$  clonotypes from birth. These chains bear highly distinct features compared to  $V\gamma 9$  chains from  $V\delta 1^+$  T-cells, due either to temporal differences in recombination of each subset and/or potentially prenatal selection of pAg-reactive clonotypes. While these processes result in a semi-invariant repertoire featuring  $V\gamma 9$  sequences preconfigured for pAg recognition, alterations in TCR $\delta$  repertoires between neonate and adult suggest either peripheral selection of clonotypes responsive to microbial antigens or altered postnatal thymic output of  $V\gamma 9V\delta 2^+$  T-cells. Interestingly, some individuals demonstrate private  $V\gamma 9V\delta 2^+$  expansions with distinct effector phenotypes, suggestive of selective expansion in response to microbial stimulation. The  $V\gamma 9V\delta 2^+$  T-cell subset, therefore, exhibits many features common to mouse  $\gamma\delta$  T-cell subsets, including early development, a semi-invariant TCR repertoire, and a reliance on butyrophilin-like molecules in antigen recognition. However, importantly  $V\gamma 9V\delta 2^+$  T-cells retain TCR sensitivity after acquiring an effector phenotype. We outline a model for  $V\gamma 9V\delta 2^+$  T-cell development and selection involving innate prenatal repertoire focusing, followed by postnatal repertoire shifts driven by microbial infection and/or altered thymic output.

**Keywords:** gamma/delta T-cell, T-cell receptor repertoire,  $V\gamma 9V\delta 2^+$  T-cell, phosphoantigen, HMBPP

## DEVELOPMENT OF THE $V\gamma 9V\delta 2^+$ T-CELL COMPARTMENT

$V\gamma 9V\delta 2^+$  lymphocytes are the predominant  $\gamma\delta$  T-cell subset in healthy adult peripheral blood. Essentially all  $V\gamma 9V\delta 2^+$  T-cells respond to small pyrophosphate antigens (pAg) (1) in a T-cell receptor (TCR)-dependent manner (2), a process dependent on target cell expression of the butyrophilin (BTN) family member BTN3A1 (3). The population expands during childhood (4), typically comprising ~1–10% of total peripheral blood T-cells in healthy adults.

The  $V\gamma 9$  and  $V\delta 2$  variable (V) gene segments are the first  $\gamma/\delta$  chains to undergo rearrangement in development, detected in fetal liver from as early as 5–6 weeks gestation (5), and in fetal thymus after 8 weeks gestation (6). By mid-gestation (20–30 weeks),  $V\gamma 9V\delta 2^+$  T-cells dominate the  $\gamma\delta$  repertoire (7) (**Figure 1**). However,  $V\delta 1^+$  T-cell generation increases later in gestation, and  $V\delta 1^+$  T-cells comprise



the majority of the  $\gamma\delta$  repertoire in cord blood (7, 8), and in pediatric thymus (9). It is unclear whether gestationally produced V $\gamma$ 9V $\delta$ 2<sup>+</sup> cells persist in fetal blood, and become outnumbered by subsequent V $\delta$ 1<sup>+</sup> T-cell production, or whether most V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells exit circulation and populate the tissues. However, the dramatic postnatal numerical expansion of V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells likely occurs following microbial exposure, with the V $\gamma$ 9V $\delta$ 2<sup>+</sup> subset ultimately dominating the circulating  $\gamma\delta$  T-cell repertoire during childhood (4, 10). Consistent with this, V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells mature in phenotype early after birth concomitant with their numerical expansion (4); moreover, several infections stimulate V $\gamma$ 9V $\delta$ 2<sup>+</sup> expansion, and tellingly, identical twins have different V $\gamma$ 9V $\delta$ 2<sup>+</sup> profiles (4).

## THE V $\gamma$ 9V $\delta$ 2<sup>+</sup> TCR REPERTOIRE IN HEALTHY ADULTS

Early studies identified V $\gamma$ 9V $\delta$ 2<sup>+</sup> TCR features required for pAg responsiveness. Interestingly, adult V $\delta$ 2 CDR3s were highly diverse, composed of V $\delta$ 2 joined to one (or occasionally two) diversity (D) segments (usually D $\delta$ 3), and typically used joining (J) segment J $\delta$ 1 (11, 12). A hydrophobic amino acid, typically Val/Leu/Ile at position 97 of the V $\delta$ 2 framework (position 5 of the CDR3, defined as the amino acids between the V $\delta$ 2 segment C-terminal Cys and the conserved Phe of the J segment), generated by N-nucleotide addition, was required for pAg recognition (12, 13).

Conversely, V $\gamma$ 9 gene segments were relatively restricted in CDR3 $\gamma$  sequence and length, and exclusively utilized J $\gamma$ P and constant region C $\gamma$ 1 (11, 14, 15). One clonotype (CALWEVQELGKKIKVF), generated by germline V $\gamma$ 9-J $\gamma$ P recombination with minimal nucleotide trimming and no N-nucleotide addition, was present in many healthy donors (15). Further low-throughput analyses detected many “public” V $\gamma$ 9 clonotypes in multiple individuals (16). Although peripheral blood  $\gamma\delta$  T-cell numbers vary widely between individuals and are influenced by age and sex (17), public clonotypes are conserved irrespective of age, sex, and race (16), and between cord blood and adult (18). Although the presence of such public V $\gamma$ 9 sequences was thought to reflect strong postnatal peripheral selection and amplification of specific clonotypes following microbial exposure (19), an improved understanding of the V $\gamma$ 9V $\delta$ 2<sup>+</sup> TCR repertoire suggests alternative possibilities.

## EVIDENCE FOR CONVERGENT RECOMBINATION IN THE V $\gamma$ 9 TCR REPERTOIRE

Deep sequencing analyses of V $\gamma$ 9V $\delta$ 2<sup>+</sup> TCR repertoires (20–23) have confirmed a high frequency of public V $\gamma$ 9 clonotypes in adult V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells, and reveal the basis for V $\gamma$ 9 TCR public-ity. The most prevalent of these, CALWEVQELGKKIKVF, highlighted in many previous studies (7, 11, 15, 16, 18), comprised

between 4 and 45% of the V $\gamma$ 9 repertoire (20–22). As noted (15), this amino acid sequence can be generated by near-germline recombination of V $\gamma$ 9 and J $\gamma$ P gene segments with minimal nucleotide trimming and no N-nucleotide addition. However, it can also result from several different nucleotide sequences: (1) involving trimming of nucleotides at the 3' end of the V region and/or 5' end of the J region, (2) incorporation of one or more palindromic (P)-nucleotides, and/or (3) addition of one or several non-templated (N)-nucleotides by terminal deoxynucleotidyl transferase (TdT), resulting in the same amino acid sequence (Table 1). Moreover, other public V $\gamma$ 9 clonotypes can be generated in multiple ways depending on the extent of V and J gene segment trimming, and N/P-nucleotide addition (Table 1) (23).

These features suggest the publicity of the V $\gamma$ 9 repertoire is due to convergent recombination, a phenomenon proposed for generation of public TCR $\beta$  repertoires (24), whereby distinct recombination events “converge” to generate the same nucleotide sequences, and multiple nucleotide sequences “converge” to encode the same amino acid sequence. Venturi et al. proposed that public TCR $\beta$  responses arise from clonotypes with a high precursor frequency in two ways. Public sequences could arise independently multiple times in each individual by convergent recombination. Alternatively, precursor frequency could be increased if a single TCR $\beta$  rearrangement, which undergoes several rounds

of proliferation after pre-TCR selection, could pair with many TCR $\alpha$  chains. Importantly,  $\gamma\delta$  T-cells do not undergo pre-TCR selection or proliferate after successful TCR $\gamma$  rearrangement (but before TCR $\delta$  rearrangement) during T-cell development. Public V $\gamma$ 9 sequences observed in adults must, therefore, result from convergent recombination.

High throughput V $\delta$ 2 TCR repertoire sequencing analyses provide corroborating evidence for convergent V $\gamma$ 9 recombination. CDR3 $\delta$ 2 repertoires are more diverse than CDR3 $\gamma$ 9 repertoires derived from V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells from most adults (21, 23). Therefore, prevalent V $\gamma$ 9 clonotypes (e.g., CALWEVQELGKKIKVF) do not reflect clonal expansion (if so equally large V $\delta$ 2 clonotypes would also be observed), but are likely recombined independently multiple times and pair with distinct V $\delta$ 2 chains. Single cell PCR in several individuals has substantiated the feasibility of this hypothesis, establishing unequivocally that public V $\gamma$ 9 CDR3 clonotypes each paired with multiple V $\delta$ 2 clonotypes (23), confirming that public V $\gamma$ 9 sequences arise frequently and independently. These findings prove that “convergent recombination” is an inherent feature of the V $\gamma$ 9 repertoire, in keeping with public sequences exhibiting high precursor frequency because they have arisen *via* many independent recombination events in each donor. They also raise the question of whether, rather than requiring selective postnatal clonotypic expansion, the prevalence of public V $\gamma$ 9 sequences may be preconfigured since birth.

**TABLE 1** | Common public V $\gamma$ 9-J $\gamma$ P sequences can be generated by convergent recombination.

V $\gamma$ 9	P N P J $\gamma$ P																P nt	N nt		
	Germline	TGT	GCC	TTG	TGG	GAG	GTG	T	GGG	CAA	GAG	TTG	GGC	AAA	AAA	ATC			AAG	GTA
CALWEVQELGKKIKVF																				
	TGT	GCC	TTG	TGG	GAG	GTG			CAA	GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	0	0
	TGT	GCC	TTG	TGG	GAG	GT			CAA	GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	0	1
	TGT	GCC	TTG	TGG	GAG	GT			CAA	GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	0	1
	TGT	GCC	TTG	TGG	GAG	GT			CAA	GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	0	1
	TGT	GCC	TTG	TGG	GAG	GTG				GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	2	1
CALWEVRELGKKIKVF																				
	TGT	GCC	TTG	TGG	GAG	GTG			A	GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	1	1
	TGT	GCC	TTG	TGG	GAG	GTG			A	GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	0	2
	TGT	GCC	TTG	TGG	GAG	GTG				GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	1	2
	TGT	GCC	TTG	TGG	GAG	GTG				GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	1	2
	TGT	GCC	TTG	TGG	GAG	GTG				GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	1	2
CALWEAQELGKKIKVF																				
	TGT	GCC	TTG	TGG	GAG	G			CAA	GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	0	2
	TGT	GCC	TTG	TGG	GAG	G			CAA	GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	0	2
	TGT	GCC	TTG	TGG	GAG	G			CAA	GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	0	2
	TGT	GCC	TTG	TGG	GAG	G			CAA	GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	0	2
CALWEVLELGKKIKVF																				
	TGT	GCC	TTG	TGG	GAG	GTG			A	GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	1	1
	TGT	GCC	TTG	TGG	GAG	GTG				GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	1	2
	TGT	GCC	TTG	TGG	GAG	GTG				GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	1	2
	TGT	GCC	TTG	TGG	GAG	GTG				GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	1	2
CALWEQELGKKIKVF																				
	TGT	GCC	TTG	TGG	GAG				CAA	GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	0	0
	TGT	GCC	TTG	TGG	GA				CAA	GAG	TTG	GGC	AAA	AAA	ATC	AAG	GTA	TTT	0	1

V $\gamma$ 9 and J $\gamma$ P gene segments are subject to nuclease activity, non-templated (N) nucleotide addition, and incorporation of palindromic (P) nucleotides, during recombination. Above are shown some of the possible different nucleotide sequences observed that generate the same CDR3 amino acid sequences, for five of the most common public V $\gamma$ 9 sequences. N-nucleotides are shown in red and P-nucleotides are shown in blue.

## SHAPING OF THE ADULT V $\gamma$ 9V $\delta$ 2 TCR REPERTOIRE: POSTNATAL SELECTION

An intriguing question is whether V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells expand *en masse* following microbial exposure during early childhood, concurrent with phenotypic maturation (4, 10), or whether dominant clonotypic selection operates, resulting in prevalent public V $\gamma$ 9 clonotypes in adults (19). Of relevance, a recent study has compared adult peripheral blood with cord blood V $\gamma$ 9V $\delta$ 2<sup>+</sup> TCR repertoires (23). Importantly, the most prevalent public V $\gamma$ 9 clonotype (CALWEVQELGKKIKVF) in the fetus (7) was also prevalent in cord (18, 23) and remains dominant in most adults (18, 20, 21). Moreover, other public V $\gamma$ 9 clonotypes are frequently found in all these populations (16, 23). Also, the CDR3 $\delta$  lengths in cord blood and adult peripheral blood are similar (23). Therefore, the public V $\gamma$ 9 clonotypes present in adult peripheral blood V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells are present at similar relative frequencies in cord blood V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells. Furthermore, there were relatively subtle changes in the diversity of V $\delta$ 2-associated V $\gamma$ 9 TCR repertoire from neonate to adult (23).

Despite these observations, postnatal changes in the V $\delta$ 2 repertoire are ultimately inconsistent with the concept of V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cell expansion *en masse*. Crucially, most V $\gamma$ 9V $\delta$ 2<sup>+</sup> cells in adult peripheral blood express V $\delta$ 2 recombined with J $\delta$ 1 (12), whereas in the cord blood most V $\delta$ 2 rearrangements use J $\delta$ 3, and to a lesser degree J $\delta$ 2 (12, 23) (Figure 1). This difference could be explained in two ways. One possibility is that extrathymic selection of specific clonotypes may occur in response to microbial exposure. Of relevance, it is currently unclear whether cord blood V $\gamma$ 9V $\delta$ 2-J $\delta$ 3 cells are reactive to common pAg. While most V $\delta$ 2-J $\delta$ 1<sup>+</sup> sequences in cord blood do generally contain a hydrophobic amino acid at position 5 (a motif previously linked to pAg reactivity) (23), fewer V $\delta$ 2-J $\delta$ 3<sup>+</sup> sequences contain this motif (23). Consistent with this, V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells from cord blood are generally less responsive to pAg than adult V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells (10, 18, 25), however, the V $\delta$ 2 repertoire of responsive cells has not been reported, and conceivably only V $\delta$ 2-J $\delta$ 1 TCRs were responding in these assays.

A second possibility that could explain postnatal alterations in the V $\delta$ 2 TCR repertoire is a second wave of V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cell production after birth. Thymic V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cell output is thought to decrease after birth, based on failure to detect V $\gamma$ 9 or V $\delta$ 2 gene expression in pediatric thymus samples (26), or detection of <10% of thymocytes expressing V $\delta$ 2 in thymi from children (4, 9). Surprisingly, V $\gamma$ 9 expression was not detected in the thymus during childhood, despite its co-expression by V $\delta$ 1<sup>+</sup> cells (21), which continue to be generated after birth (4, 26). Conceivably this issue warrants reinvestigation, and perhaps postnatal thymic V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cell generation has been underappreciated. Consistent with this, Ravens (22) and others (27, 28) have shown V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cell reconstitution following stem cell transplantation. Newly generated V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells presumably originate in the recipient's thymus (22). Detailed comparison of V $\delta$ 2-J $\delta$ 1 sequences in cord blood and adult repertoires (23) also hints at postnatal V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cell production. Although V $\delta$ 2-J $\delta$ 1 clonotypes are relatively uncommon in cord blood (most use V $\delta$ 2-J $\delta$ 3 at that time), those present often have shorter CDR3s, incorporating fewer N-nucleotides [as observed in fetal liver (5)] in comparison to

the longer, more private V $\delta$ 2-J $\delta$ 1 clonotypes observed in adults. However, if the V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells that predominate in adults are indeed generated in the postnatal thymus, we have observed no obvious differences in the V $\gamma$ 9 repertoire of these cells, suggesting that the thymus continues to generate V $\gamma$ 9-J $\gamma$ P rearrangements with low diversity even when TdT is expressed and when V $\gamma$ 9 CDR3s found in V $\delta$ 1<sup>+</sup> cells are highly diverse (21).

## EVIDENCE FOR PRENATAL SHAPING OF THE V $\gamma$ 9V $\delta$ 2<sup>+</sup> TCR REPERTOIRE

Postnatal processes clearly strongly influence the V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cell compartment. However, other events may also shape the prenatal V $\gamma$ 9V $\delta$ 2<sup>+</sup> repertoire (Figure 1). The V $\gamma$ 9 repertoire is already highly restricted in CDR3 length during gestation, with public clonotypes evident (7), consistent with the cord blood V $\gamma$ 9 repertoire (23). This indicates postnatal pAg exposure is not required for the selection of these features. However, the possibility that there might be some selection for pAg-reactive semi-invariant V $\gamma$ 9V $\delta$ 2<sup>+</sup> T cells before postnatal microbial exposure has been suggested previously (7), which potentially could operate intra- or extra-thymically. Conceivably, this could involve elevated levels of endogenous pAgs such as IPP derived from fetal isoprenoid metabolism, or pAg derived from placental microbiota; in addition, a specific selecting element, such as one or more of the BTN3 gene products could be involved (7). Bearing these possibilities in mind, enrichment of J $\delta$ 3 within cord blood V $\delta$ 2 sequences relative to adult peripheral blood could relate to more permissive positive selection of clonotypes responding to such fetal-specific selection events relative to postnatal responsiveness to exogenous microbially derived pAg. However, alternatively, genetic processes may explain the restricted nature of the V $\gamma$ 9 repertoire in fetal and cord blood V $\delta$ 2<sup>+</sup> cells. Consistent with this suggestion, the mouse OP9-DL1 thymic organ culture system can support V $\gamma$ 9V $\delta$ 2<sup>+</sup> T cell generation (9), arguing against a stringent positive selection step involving BTN3A1/pAg-mediated events. Of relevance to inherent genetic bias in V $\gamma$ 9 chain recombination, whereas V $\delta$ 1-associated V $\gamma$ 9 chains are diverse in length and rarely use J $\gamma$ P, V $\delta$ 2-associated V $\gamma$ 9 CDR3 sequences are restricted in length, and exclusively utilize J $\gamma$ P, including in adults. These differences could merely reflect changes in gene segment accessibility during V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cell generation in early gestation, or regulation of V $\gamma$ 9 chain recombination that favor simpler public V $\gamma$ 9 rearrangements during the earlier timescale of fetal V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cell generation, before TdT is expressed (i.e., before 20 weeks of gestation) (29).

## COMPARISONS BETWEEN V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-CELLS AND SEMI-INVARIANT MOUSE $\gamma$ $\delta$ T-CELL SUBSETS

Several features of the V $\gamma$ 9V $\delta$ 2<sup>+</sup> compartment suggest similarities to mouse  $\gamma$  $\delta$  T-cell subsets (30). The early fetal wave of V $\gamma$ 9V $\delta$ 2<sup>+</sup> production, combined with the semi-invariant V $\gamma$ 9V $\delta$ 2<sup>+</sup> TCR repertoire, mirrors early waves of semi-invariant mouse  $\gamma$  $\delta$  T-cells. The first T-cells to develop in mouse fetal thymus are V $\gamma$ 5V $\delta$ 1<sup>+</sup> dendritic epidermal T-cells, which have limited junctional

diversity in both TCR chains (31). This is followed by production of V $\gamma$ 6V $\delta$ 1 TCRs, also of limited diversity, then postnatal production of more diverse  $\gamma\delta$  T-cell populations using V $\gamma$ 4, V $\gamma$ 1, and V $\gamma$ 7 chains (32). Some of these  $\gamma\delta$  populations undergo intrathymic or extrathymic selection events. DETC cells undergo intrathymic selection involving the BTN family member Skint1 (33, 34); the V $\gamma$ 7 repertoire requires the presence of BTNL1/6 for extrathymic intestinal selection (35). Another semi-invariant mouse population expresses V $\gamma$ 4 sequences of restricted length and diversity (analogous to public human V $\gamma$ 9 sequences) with a germline-encoded V $\delta$ 5-D $\delta$ 2-J $\delta$ 1 sequence (36, 37), although its role and the signals that drive selection are unknown. The presence of  $\gamma\delta$  T-cells expressing semi-invariant TCRs in both mice and humans suggests this may reflect a shared paradigm for generation of T-cell populations with uniform reactivity to particular antigenic epitopes. Consistent with a related immunobiology, both BTN3A1 and BTN3A2/3 are important for V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cell recognition (38). However, while some semi-invariant mouse  $\gamma\delta$  T-cell populations can become hyporesponsive to TCR triggering following initial strong TCR signaling during development (39), this does not apparently apply to human V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells. Notably V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells remain responsive to both pAg and anti-CD3 stimulation, a feature which underlies their potential use in several cancer immunotherapy applications (40), and they also exhibit the potential for further TCR-mediated plasticity (41–44).

## POTENTIAL FOR CLONAL FOCUSING IN RESPONSE TO INFECTIOUS/STRESS CHALLENGE

Although clear evidence supports a broad polyclonal V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cell response to pAg, the extent to which clonotype-specific responses occur remains unclear. V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells expand in various infections (1) but TCR clonality is uncharacterized in most scenarios. While most healthy donors have similar V $\gamma$ 9 repertoires composed of up to 80% public V $\gamma$ 9 clonotypes and diverse V $\delta$ 2 clonotypes (23), a minority of healthy donors have one or several expanded V $\gamma$ 9 and V $\delta$ 2 clonotypes reminiscent of V $\delta$ 1 expansions (21), with the top clone comprising 20–40% of all V $\gamma$ 9 and V $\delta$ 2 CDR3s (23). These clones express V $\gamma$ 9 clonotypes shared less frequently between adult donors, often with longer or more complex CDR3s containing more added N-nucleotides. In these donors, a V $\delta$ 2 clonotype of similar frequency is detected, and pairing of the top V $\gamma$ 9 and V $\delta$ 2 clonotypes can be confirmed by single cell PCR. This clonal expansion correlated with a change in V $\delta$ 2<sup>+</sup> T-cell phenotype to CD45RA<sup>neg</sup>CD27<sup>neg</sup> (23), distinct from the CD45RA<sup>neg</sup>CD45RO<sup>+</sup>CD27<sup>+</sup> phenotype observed in most healthy donors (45). The factors driving this clonal expansion and phenotypic maturation in these seemingly healthy donors are unclear. Ryan et al. (46) have also observed healthy donors with V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells of differing effector phenotypes, although the clonality of V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells was not examined. Expansion of particular V $\delta$ 2 clonotypes has also been noted in tuberculosis (47, 48), human leprosy (49), and in a macaque tuberculosis model (50). Public V $\gamma$ 9 clonotypes were not shown to change during BCG infection in macaques (51), however, a

lack of V $\delta$  TCR clonotype data could have obscured the presence of clonotypic expansions with distinct V $\delta$ 2 chains. Conceivably clonal expansion may occur after Epstein–Barr virus or other common viral infections, and may underlie clonal expansions observed in otherwise healthy donors. Moreover, it is unclear how expansion of particular V $\gamma$ 9V $\delta$ 2<sup>+</sup> clonotypes helps protect the host, given the polyclonal response of V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cells to pAg. Conceivably expanded clones could respond with higher avidity, or alternatively could be reactive to different pathogen-specific stimuli, such as chemically diverse antigens. Additional work will no doubt address these questions.

## CONCLUSION

In summary, we suggest V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cell development is shaped by both prenatal and postnatal events (Figure 1), which impact TCR repertoire and pAg reactivity. Importantly, the human V $\gamma$ 9V $\delta$ 2<sup>+</sup> TCR repertoire is composed of highly public V $\gamma$ 9 chains produced by frequent recombination events that occur in every individual, resulting in a semi-invariant repertoire largely preconfigured from birth for pAg reactivity. These V $\gamma$ 9 chains may undergo prenatal selection based on pAg reactivity, or unknown factors may constrain V $\gamma$ 9-J $\gamma$ P rearrangements. Alongside public V $\gamma$ 9 sequences, the V $\delta$ 2 repertoire is very diverse and private, and changes between neonatal and adult V $\delta$ 2 TCR repertoires suggest several selection events throughout life. V $\delta$ 2-J $\delta$ 3 TCRs are prevalent in cord blood and these may be positively selected in fetal development for recognition of host pAgs, or these rearrangements may be preferentially generated in early gestation. V $\delta$ 2-J $\delta$ 1 chains with longer CDR3 and hydrophobic amino acids at position 5 ultimately dominate the V $\delta$ 2 repertoire in adults, and these may be selected from rare rearrangements in cord blood following microbial pAg exposure, or further V $\gamma$ 9V $\delta$ 2<sup>+</sup> T-cell generation may occur in the postnatal thymus. Nevertheless, these selection events produce a repertoire that exploits the somatically recombined V $\gamma$ 9V $\delta$ 2<sup>+</sup> TCR as a surrogate pattern recognition receptor to sense pAgs. Further clonal selection appears to occur in some healthy adults and during some infections, however, exactly what protection such favored clonotypes provide that are not provided already by the broad V $\gamma$ 9V $\delta$ 2<sup>+</sup> TCR repertoire is an intriguing question future studies can address.

## AUTHOR CONTRIBUTIONS

CW, MD, and BW jointly conceived the concepts presented in this review. CW analyzed data, prepared figures, and wrote the first draft; MD prepared figures and helped finalize the manuscript; BW helped plan and write the final manuscript.

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