

UNIVERSITY OF BIRMINGHAM

Research at Birmingham

The human V2+ T-cell compartment comprises distinct innate-like V9+ and adaptive V9- subsets

Willcox, Benjamin; Davey, Martin; Willcox, Carrie; Hunter, Stuart; Salim, Mahboob; Mohammed, Fiyaz; Oo, Ye Htun

DOI:

[10.1038/s41467-018-04076-0](https://doi.org/10.1038/s41467-018-04076-0)

License:

Creative Commons: Attribution (CC BY)

Document Version

Publisher's PDF, also known as Version of record

Citation for published version (Harvard):

Willcox, B, Davey, M, Willcox, C, Hunter, S, Salim, M, Mohammed, F & Oo, YH 2018, 'The human V2+ T-cell compartment comprises distinct innate-like V9+ and adaptive V9- subsets', Nature Communications, vol. 9, no. 1, 1760. <https://doi.org/10.1038/s41467-018-04076-0>

[Link to publication on Research at Birmingham portal](#)

Publisher Rights Statement:

Published in Nature Communications on 02/05/2018

DOI: 10.1038/s41467-018-04076-0

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

ARTICLE

DOI: 10.1038/s41467-018-04076-0

OPEN

The human $V\delta 2^+$ T-cell compartment comprises distinct innate-like $V\gamma 9^+$ and adaptive $V\gamma 9^-$ subsets

Martin S. Davey¹, Carrie R. Willcox¹, Stuart Hunter^{1,2}, Sofya A. Kasatskaya^{3,4}, Ester B.M. Remmerswaal⁵, Mahboob Salim¹, Fiyaz Mohammed¹, Frederike J. Bemelman⁶, Dmitriy M. Chudakov^{3,4,7,8}, Ye H. Oo² & Benjamin E. Willcox¹

$V\delta 2^+$ T cells form the predominant human $\gamma\delta$ T-cell population in peripheral blood and mediate T-cell receptor (TCR)-dependent anti-microbial and anti-tumour immunity. Here we show that the $V\delta 2^+$ compartment comprises both innate-like and adaptive subsets. $V\gamma 9^+$ $V\delta 2^+$ T cells display semi-invariant TCR repertoires, featuring public $V\gamma 9$ TCR sequences equivalent in cord and adult blood. By contrast, we also identify a separate, $V\gamma 9^-$ $V\delta 2^+$ T-cell subset that typically has a $CD27^{hi}CCR7^+CD28^+IL-7R\alpha^+$ naive-like phenotype and a diverse TCR repertoire, however in response to viral infection, undergoes clonal expansion and differentiation to a $CD27^{lo}CD45RA^+CX_3CR1^+granzymeA/B^+$ effector phenotype. Consistent with a function in solid tissue immunosurveillance, we detect human intrahepatic $V\gamma 9^-$ $V\delta 2^+$ T cells featuring dominant clonal expansions and an effector phenotype. These findings redefine human $\gamma\delta$ T-cell subsets by delineating the $V\delta 2^+$ T-cell compartment into innate-like ($V\gamma 9^+$) and adaptive ($V\gamma 9^-$) subsets, which have distinct functions in microbial immunosurveillance.

¹Cancer Immunology and Immunotherapy Centre, Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham B15 2TT, UK. ²Centre for Liver Research and NIHR Biomedical Research Unit in Liver Disease, Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham B15 2TT, UK. ³Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Science, Moscow 117997, Russia. ⁴Centre for Data-Intensive Biomedicine and Biotechnology, Skolkovo Institute of Science and Technology, Moscow 143026, Russia. ⁵Department of Experimental Immunology, Academic Medical Center, Amsterdam 1105 AZ, The Netherlands. ⁶Renal Transplant Unit, Division of Internal Medicine, Academic Medical Center, Amsterdam 1105 AZ, The Netherlands. ⁷Central European Institute of Technology, Masaryk University, Brno 625 00, Czech Republic. ⁸Pirogov Russian National Research Medical University, Moscow 117997, Russia. These authors contributed equally: Martin S. Davey, Carrie R. Willcox, Stuart Hunter. Correspondence and requests for materials should be addressed to M.S.D. (email: m.davey@bham.ac.uk) or to B.E.W. (email: b.willcox@bham.ac.uk)

$\gamma\delta$ T cells have coevolved alongside B cells and $\alpha\beta$ T cells in the vertebrate immune system for almost 450 million years¹. They provide anti-microbial² and anti-tumour immunity³, but whether they occupy an innate-like or adaptive immunological niche has remained unclear. Notably, $\alpha\beta$ T cells incorporate a group of unconventional T cells, including mucosal-associated invariant T (MAIT) cells and invariant natural killer T (iNKT) cells that recognise antigens in the context of single MHC-like proteins (MR1 and CD1d), and display a semi-invariant T-cell receptor (TCR) repertoire, suggestive of an innate-like biology whereby TCR sensitivity is retained but the $\gamma\delta$ TCR may arguably function as a surrogate pattern recognition receptor⁴. Notably, studies in mice have suggested that innate-like $\gamma\delta$ T-cell development in the thymus can occur via distinct pathways involving agonistic signals⁵. In addition, recently, Wencker et al.⁶ have suggested that following TCR triggering during development, mouse innate-like T cells may transition to a state of TCR hyporesponsiveness in which they preferentially respond to TCR-extrinsic stimuli such as cytokine exposure.

Human $\gamma\delta$ T cells are often delineated into $V\delta 2^+$ and $V\delta 2^-$ subsets⁷. $V\delta 2^-$ $\gamma\delta$ T cells have been directly implicated in anti-viral and anti-tumour immunity^{3,8} and utilise germline-encoded antigen receptors also present on innate-like lymphocytes, including NKG2D and NKP30^{9,10}. However, recent evidence has suggested that they may adopt a TCR-dependent adaptive immunobiology, based on highly clonotypically focused expansions alongside differentiation from a naive to effector phenotype¹¹ and perturbations in clonal expansion upon cytomegalovirus (CMV) infection in post-stem cell transplant patients¹². Conversely, $V\delta 2^+$ T cells are arguably the prototypic unconventional T cell, typically co-expressing $V\gamma 9$ TCR chains and representing the major $\gamma\delta$ subset in adult peripheral blood¹³. $V\gamma 9^+$ $V\delta 2^+$ T cells respond to prenyl pyrophosphate metabolites (phosphoantigens, or P-Ags) produced either by the host mevalonate pathway (isopentenyl pyrophosphate, IPP) or microbial non-mevalonate pathway (*(E)*-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate, HMB-PP)¹⁴, which are sensed in the context of butyrophilin 3A1 (BTN3A1)^{15–17}. They mount important anti-microbial immune responses, including against *Mycobacterium tuberculosis*¹³ and *Plasmodium falciparum*¹⁸, and drive $\alpha\beta$ T-cell responses^{19,20}.

Previously, the extrathymic expansion of $V\gamma 9^+$ $V\delta 2^+$ T cells observed in adult peripheral blood, which is proposed to result from exposure to P-Ag-producing microbes encountered after birth^{21,22} and thought to involve clonotypic expansion^{23,24}, arguably suggests an adaptive immunobiology. However, $V\gamma 9^+$ $V\delta 2^+$ T cells are highly enriched in foetal peripheral blood and display restricted complementarity-determining region (CDR) 3 $\gamma 9$ usage early in gestation²⁵, more consistent with an innate-like pre-natal repertoire of $V\gamma 9^+$ $V\delta 2^+$ T cells.

To better understand human $V\delta 2^+$ $\gamma\delta$ T-cell immunobiology we carried out a dedicated analysis of their clonotypic diversity and cellular phenotype. Our findings suggest $V\delta 2^+$ T cells can be delineated into two discrete subsets: $V\gamma 9^+$ $V\delta 2^+$ T cells adopt a predominantly innate-like biology originating in neonatal development and allowing a degree of clonotypic plasticity, whereas $V\gamma 9^-$ $V\delta 2^+$ T cells adopt a distinct adaptive immunobiology, including focused clonal expansions and differentiation evident both in peripheral blood and solid tissues, and generated in response to acute viral infection. These findings revise our understanding of human $\gamma\delta$ T cells, and prompt further investigation of the adaptive immunity provided by the $V\gamma 9^-$ $V\delta 2^+$ subset.

Results

Adult and neonatal $V\delta 2^+$ T cells have similar TCR diversity. Consistent with previous studies, we confirmed that human cord

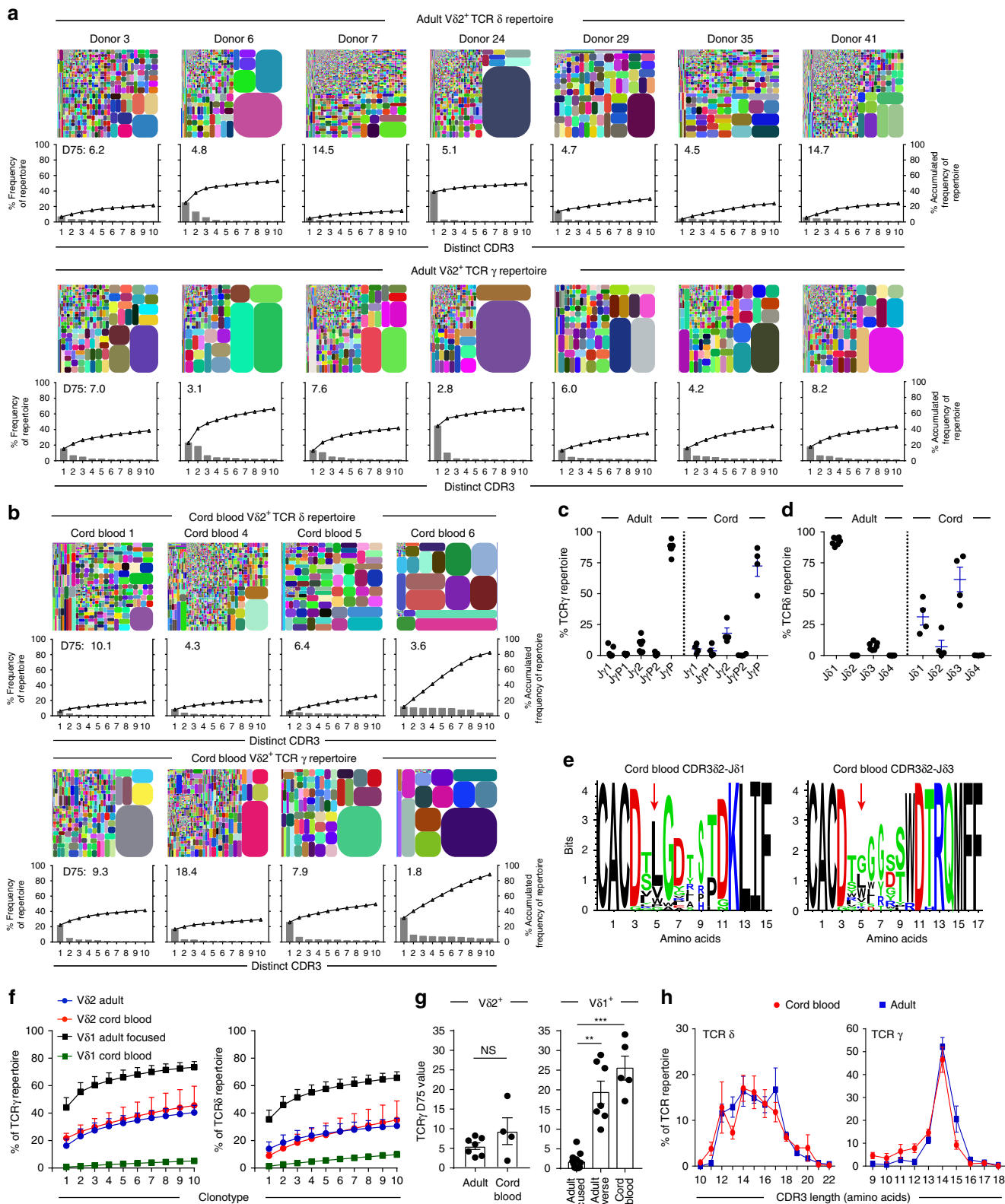
blood $V\delta 2^+$ $\gamma\delta$ T cells were present at relatively low levels, but were increased as a proportion of peripheral T cells by adulthood^{22,26} (Supplementary Fig. 1a), resulting in an adult human $V\delta 2^+$ T-cell repertoire that is uniformly responsive to P-Ag metabolites (Supplementary Fig. 1b). To address whether $V\delta 2^+$ T-cell expansion from the neonatal pool is highly clonally focused, as for $V\delta 1^+$ T cells¹¹, we performed a TCR repertoire analysis restricted to the $V\delta 2^+$ T-cell population (Fig. 1a, b). Indicative of successful $V\delta 2^+$ $\gamma\delta$ T-cell sorting, TCR δ repertoires entirely comprised $V\delta 2$ chain usage (Supplementary Fig. 1c). Consistent with previous findings, TCR γ repertoires were predominantly composed of $V\gamma 9$ chains (Supplementary Fig. 1d) and the joining region $J\gamma P$ (Fig. 1c), and neonatal $V\delta 2$ chains preferentially used joining region $J\delta 3^{27}$, whereas $J\delta 1$ was more commonly utilised in adults²⁸ (Fig. 1d). TCR δ -mediated responses to P-Ag have been associated with hydrophobic amino acid residues (LVW) at position 5 in CDR3 $\delta 2$ sequences²⁹, and this is observed in adult $V\delta 2$ CDR3 repertoires¹¹. We found that although neonatal $V\delta 2$ - $J\delta 3$ sequences generally lacked this motif, and overall were enriched relative to $V\delta 2$ - $J\delta 1$ sequences for neutral residues at position 5, particularly Gly, some $V\delta 2$ - $J\delta 3$ sequences contained the hydrophobic residue Leu, whereas $V\delta 2$ - $J\delta 1$ sequences, although rarer in neonates, were highly enriched for hydrophobic residues at position 5 (Fig. 1e).

We next analysed $V\delta 2^+$ T-cell repertoires using approaches we previously applied to the $V\delta 1^+$ compartment¹¹. Tree plot analysis revealed the presence of some relatively prominent clonotypes in adult $V\delta 2$ TCR γ (between 12 and 47%) and TCR δ (between 1.8 and 39%) repertoires (Fig. 1a). The ten most prevalent TCR γ clonotypes in each donor formed a substantially smaller portion ($P = 0.003$; Mann–Whitney) of the $V\delta 2^+$ T-cell repertoire (mean 40.5% of total $V\delta 2$ reads) than expanded $V\delta 1$ clonotypes (mean 73.42% of total $V\delta 1$ reads¹¹). Unlike the $V\delta 1$ compartment, similarly prominent clonotypes were also present in cord blood $V\delta 2^+$ TCR γ (between 18 and 34%) repertoires (Fig. 1a, b). Analysis of the cumulative frequency curves of the 10 most prevalent CDR3 sequences highlighted both adult and cord blood $V\delta 2^+$ TCR repertoires exhibit an intermediate degree of clonotypic focusing, relative to cord blood $V\delta 1$ (low focusing) and clonally expanded adult $V\delta 1$ repertoires (high focusing; Fig. 1f). Analysis of D75 diversity metrics (the percentage of clonotypes required to occupy 75% of TCR repertoire) highlighted that in $V\delta 2^+$ T-cell repertoires diversity did not generally differ between adult and cord blood $V\delta 2^+$ repertoires (Fig. 1g and Supplementary Fig. 1e). Moreover, $V\delta 2^+$ TCR repertoire D75 metrics were also independent of the level of diversity seen in matched $V\delta 1^+$ TCR repertoires (Supplementary Fig. 1f). Finally, and consistent with a broadly similar $V\delta 2^+$ repertoire in cord and adult blood, the CDR3 length profile of TCR δ and TCR γ repertoires were highly comparable between cord blood and adult (Fig. 1h). Therefore, while some variability in the level of focusing is observed in both settings, the neonatal and adult $V\delta 2^+$ repertoires are broadly similar in diversity, in terms of both accumulated clonotype frequency, D75, and CDR3 length analysis.

Public $V\gamma 9^+$ CDR3s span adult and neonatal $V\delta 2^+$ T cells. Due to the largely similar $V\delta 2^+$ repertoire characteristics found in cord blood and adult, we next assessed the proportion of CDR3 amino acid sequences that were “public”, i.e., detected in more than one donor. Consistent with a high degree of diversity within the TCR δ repertoire, adult TCR $\delta 2$ repertoires contained few shared CDR3 δ sequences (mean 8.7%), although this proportion was increased in cord blood CDR3 δ repertoires (mean 36.8%; Fig. 2a, Supplementary Table 1 and 2). However, a mean 79.3% of

the CDR3 γ amino acid sequences present in a given individual were shared with at least one other adult or cord blood donor (Fig. 2b). Moreover, the majority of sequences in cord blood were directly shared in adult repertoires (Fig. 2c), indicating a core set of CDR3 γ sequences in adult donors essentially indistinguishable from equivalent unrelated neonatal donor sequences.

To further explore this publicity, we examined the 10 most prevalent CDR3 γ sequences from our cohort (see Methods), representing a mean 38.9% of the total CDR3 γ repertoire (Fig. 2d). This highlighted a set of public CDR3 γ sequences that were prevalent in both adult and cord blood repertoires, and at similar frequencies (Fig. 2d). Notably, two simple CDR3 γ sequences were prevalent in all adult and neonatal repertoires



analysed: CALWEVQELGKKIKVF (mean 13.1% in adult, and mean 24.6% in neonates) and CALWEVRELGKKIKVF (mean 4.3% in adults and mean 5.4% in neonates). Two processes explain the high degree of sharing within the CDR3 γ repertoire. Firstly, single-cell TCR sequencing highlighted that CALWEVQELGKKIKVF could be generated by simple recombination of V γ 9 and J γ P gene segments containing no added N nucleotides ("germline") (Table 1). This sequence was common in foetal liver³⁰, and foetal blood²⁵ and observed to persist into adulthood³¹. While a high prevalence in repertoire data might indicate an expanded clonotype, single-cell TCR analysis also revealed that this germline-encoded CDR3 γ sequence in fact was comprised of multiple TCR "pseudoclonotypes", in which the same CDR3 γ was independently generated and paired with different CDR3 δ sequences in the same individual (Table 1). Secondly, CDR3 γ sequences such as CALWEVQELGKKIKVF and CALWEVRELGKKIKVF could also be generated by convergent recombination, whereby variable degrees of exonuclease activity and N nucleotide addition result in the generation of the same CDR3 γ amino acid sequences from different nucleotide sequences (Table 1). Both of these processes explain why CDR3 γ repertoires were less diverse compared to CDR3 δ repertoires (Fig. 2e), and are consistent with the generation of public TCR sequences³², but importantly, these mechanisms are not evident in V δ 1⁺ TCR repertoires¹¹.

Altered V γ 9⁺ V δ 2⁺ T-cell phenotype links to clonal expansion.

We evaluated the memory phenotype of V δ 2⁺ T cells from each donor to establish the relationship with TCR clonotype. Adult V γ 9⁺ V δ 2⁺ T cells from healthy donors (16/18 of total donors, 5/7 TCR repertoire study donors) regularly display a major CD27⁺ CD45RA⁻ phenotype (similar to Central Memory (CM) CD8⁺ $\alpha\beta$ T cells) and a minor CD27⁻ CD45RA⁻ phenotype (similar to Effector Memory (EM) CD8⁺ $\alpha\beta$ T cells) (Fig. 2f), consistent with other studies³³. Importantly, V γ 9⁺ V δ 2⁺ T cells differ from V δ 1⁺ T-cell populations in their expression levels of CD27 (Fig. 2g) and distinct naive and effector T-cell surface marker expression (Fig. 2h).

Although overall our adult cohort displayed similar V γ 9⁺ V δ 2⁺ TCR diversity to cord blood samples, two adult donors (6 and 24) appeared to exhibit a degree of clonotypic focusing, based on their low D75 values for both TCR γ (mean 2.9) and TCR δ (mean 5.0) combined with their higher cumulative frequency of the 10 most prevalent clonotypes (mean 60.5% of TCR γ and mean 50.3% of TCR δ) (Fig. 1a). Consistent with this, each donor displayed individual clonotypes with a similar amplified frequency in both TCR γ and δ repertoires (Fig. 1a). Both donor 6 and 24 each possessed a distinct uncommon but shared sequence, CALWEVRKELGKKIKVF (detected in donor 6 and

also in 2 other donors) or CALWEKMQELGKKIKVF (detected in donor 24 and in 4 other donors), usually outside the top 10 most prevalent shared clonotypes, but now representing 18.5% (donor 6) and 44.6% (donor 24) of the TCR γ repertoire, respectively (Fig. 2d). Also, donor 6 possessed a common sequence found in all donors, CALWETQELGKKIKVF, normally accounting for a mean 0.95% of the TCR γ repertoire, but increased to 22.9% of donor 6's TCR γ repertoire (Fig. 2d). Furthermore, these unusual clonal amplifications were linked to phenotypic changes in the total V γ 9⁺ V δ 2⁺ T-cell population, whereby these donor's cells adopted a predominant CD27⁻ CD45RA⁻ phenotype (Fig. 2i). These data suggest that despite the public repertoire and associated dominant phenotype (CD27⁺ CD45RA⁻) in the majority of donors, there is scope within the V γ 9⁺ V δ 2⁺ TCR repertoire for a degree of specific clonal amplification, alongside concomitant phenotypic changes.

A discrete V γ 9⁻ V δ 2⁺ subset persists from birth into adults.

TCR γ repertoire datasets from V δ 2⁺ T cells were dominated by V γ 9 TCR sequences, but we noted the presence of non-V γ 9 (i.e., V γ 2–8) sequences in adult TCR γ repertoires (mean 4.46%) (Fig. 3a). Although such chain usage could conceivably represent second productive TCR γ rearrangements in V γ 9⁺ V δ 2⁺ T cells, an alternative possibility was that they reflected the existence of an unusual V γ 9⁻ V δ 2⁺ T-cell subset that may persist in adulthood. As V γ 9⁻ TCR usage by neonatal V δ 2⁺ T cells has been previously reported³⁴, we established flow cytometry-based identification of V γ 9⁻ cells in cord blood V δ 2⁺ T cells (Fig. 3b) and quantified their relative frequency (mean 32.41%) (Fig. 3c). Importantly, adult V δ 2⁺ T-cell pools retained this V γ 9⁻ subset at low frequency (mean 4.82%) (Fig. 3c and Supplementary Fig. 2a), but on average occupying a similar frequency within total peripheral blood T cells to that of α -GalCer/CD1d reactive natural killer T cells (NKT) (Fig. 3d and Supplementary Fig. 2b). Notably, the identification of V γ 9⁻ V δ 2⁺ T cells was only possible with the use of an anti-V δ 2 TCR antibody clone, 123R3 (Miltenyi), while this population was undetectable using another TCR V δ 2 antibody clone, B6 (Biolegend), which is likely to be specific for the V γ 9⁺ V δ 2⁺ TCR pairing (Fig. 3e).

V γ 9⁻ V δ 2⁺ T cells and V γ 9⁺ V δ 2⁺ T cells are distinct. As adult peripheral blood V δ 2⁺ T cells are commonly responsive to host and microbial P-Ags, we assessed the proliferative capacity of V γ 9⁻ V δ 2⁺ T cells towards microbial P-Ag (HMB-PP). While CD8⁺, both V δ 2⁺ subsets and V δ 1⁺ T cells all proliferated in response to anti-CD3/CD28 stimulation, only V γ 9⁺ V δ 2⁺ T cells responded to HMB-PP (Fig. 3f). We then compared the memory phenotype of V γ 9⁺ V δ 2⁺ T cells to other human $\gamma\delta$ T-cell

Fig. 1 Adult and cord blood V δ 2⁺ TCR repertoires are broadly similar. **a** Tree maps show each adult donor's V δ 2⁺ TCR repertoire, with each CDR3 clonotype as a coloured segment (each coloured CDR3 segment is chosen randomly and does not match between plots) plotted in relation to the total repertoire size and accompanying clonotype frequency graphs showing the individual clone frequency (left y axis) and the accumulated frequency for the 10 most prevalent clonotypes (right y axis). Inset into each graph are D75 repertoire diversity metrics (measuring the percentage of clonotypes required to occupy 75% of the total TCR repertoire). **b** Tree maps showing TCR γ and δ CDR3 clonotypes, accumulated frequency graphs and D75 metric from cord blood V δ 2⁺ T cells. **c** J γ and **d** J δ segment usage in V δ 2⁺ TCR repertoires from adult peripheral blood ($n = 7$) and cord blood samples ($n = 4$). **e** Logo analysis of amino acid enrichment at each position in neonatal V δ 2–J δ 1 CDR3 δ (left) and V δ 2–J δ 3 CDR3 δ (right) sequences. Analysis was confined to the 10 most abundant CDR3 δ 2 sequences of 13–16 amino acid length. The different amino acids are coloured according to physicochemical properties (acidic (red); basic (blue); hydrophobic (black); and neutral (green)). Red arrows indicate position 5 in the CDR3 sequence (see Methods section). **f** Comparison of accumulated frequency curves generated from the 10 most prevalent TCR γ (left) and δ (right) clonotypes in V δ 2⁺ and V δ 1⁺ TCR repertoires (V δ 1 cohort data analysed from¹¹) from adult peripheral blood (V δ 2⁺, $n = 7$ and V δ 1⁺, $n = 13$) and cord blood (V δ 2⁺, $n = 4$ and V δ 1⁺, $n = 5$). **g** Comparison of TCR γ D75 metrics from adult peripheral blood and cord blood V δ 2⁺ (adult: $n = 7$; cord blood: $n = 4$) and V δ 1⁺ repertoires (adult focused: $n = 13$; adult diverse: $n = 7$; cord blood: $n = 5$). **h** Comparison of the CDR3 length profiles in V δ 2⁺ TCR δ and γ repertoires from adult peripheral blood ($n = 7$) and cord blood ($n = 4$). Error bars indicate means \pm SEM; ** $P < 0.01$; *** $P < 0.001$; p -values were determined by Student's t -test (**g**: left) and Kruskal-Wallis test (ANOVA) with Tukey's post hoc testing (**g**: right). NS not significant

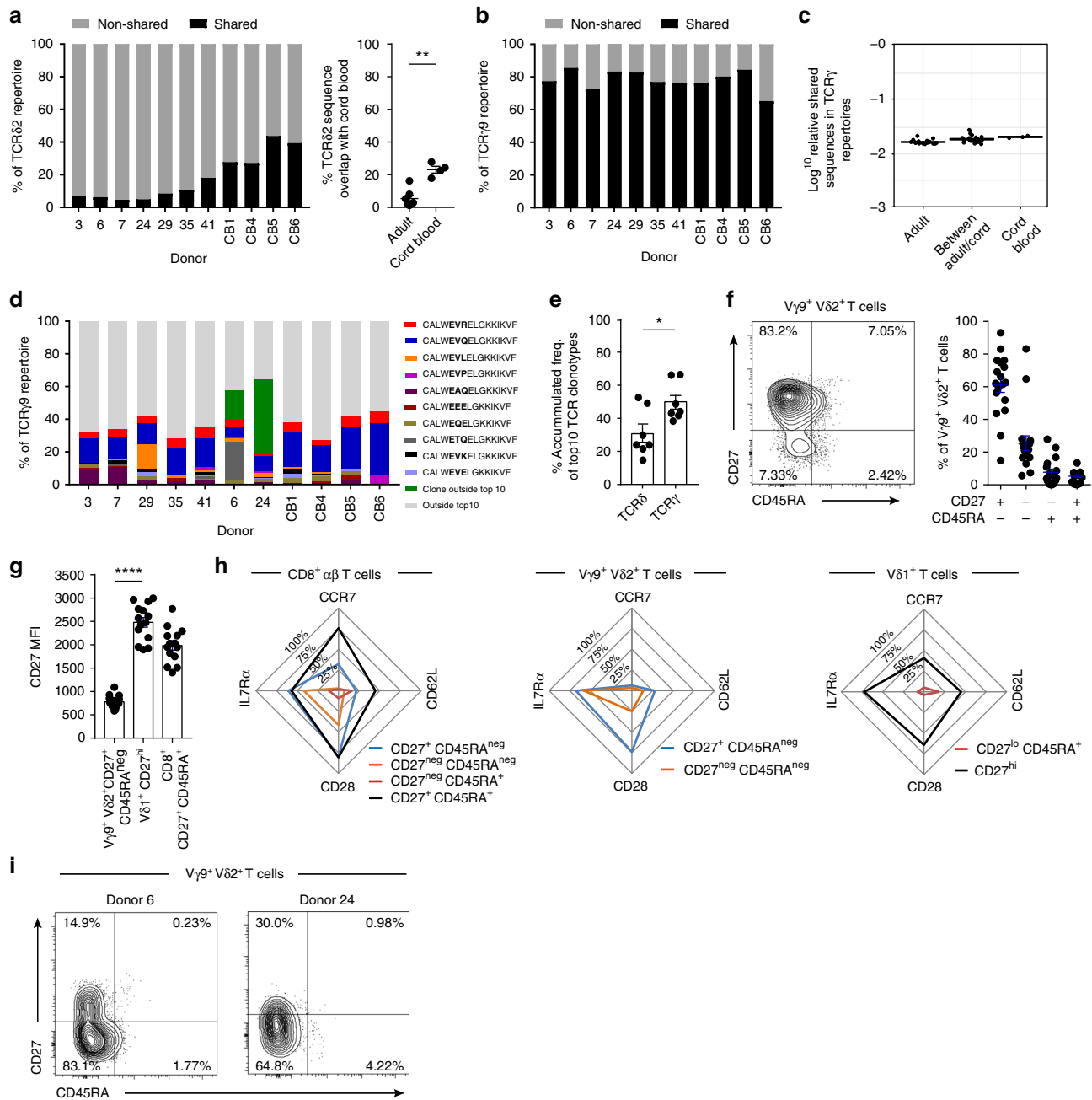


Fig. 2 Vδ2⁺ TCR repertoires are formed of public clonotypes. **a** Percentage of CDR3δ sequences (amino acid) shared between >2 donors within both adults and cord blood (left) and sequences shared with cord blood only (right). **b** Percentage of CDR3γ sequences (amino acid) shared between >2 donors (public sequences). **c** Comparison of the sequence overlap (relative publicity) of TCRγ9 repertoires in adult peripheral blood donors (*n* = 7) or cord blood samples (*n* = 3) and then between both groups. **d** Frequency of each of the 10 most common clonotypes in each donor's TCRγ9 repertoire, with the addition of exceptional expanded shared clonotypes usually found outside the top 10 (dark green). **e** Comparison of the accumulated repertoire frequency occupied by the first 10 clonotypes in Vδ2⁺ TCRδ and γ (*n* = 7). **f** CD27 and CD45RA T-cell memory marker expression by Vγ9⁺ Vδ2⁺ T cells from adult peripheral blood samples (*n* = 18). **g** Comparison of CD27 expression levels (MFI) on CD27⁺ CD45RA^{neg} Vγ9⁺ Vδ2⁺ T cells (*n* = 18), CD27^{hi} Vδ1⁺ (*n* = 14) and CD27⁺ CD45RA⁺ CD8⁺ T cells (*n* = 14) from adult peripheral blood samples. **h** Summary radar plot data detailing the mean % positive cells for each indicated T-cell marker analysed within each sub-population of Vγ9⁺ Vδ2⁺ (*n* = 18), Vδ1⁺ (*n* = 14) and CD8⁺ αβ T cells (*n* = 14). **i** Healthy adult peripheral blood donor 6 and 24's expression of CD27 and CD45RA T-cell memory markers on Vγ9⁺ Vδ2⁺ T cells. Error bars indicate means ± SEM; **P* < 0.05; ***P* < 0.01; *****P* < 0.0001; *p*-values were determined by Student's *t*-test (**a**, **e**) and one-way ANOVA with Tukey's post hoc testing (**g**)

populations. Cord blood Vγ9⁻ Vδ2⁺ T cells expressed a CD27^{hi} phenotype which extended to the adult population, remarkably similar to naive-like Vδ1⁺ T cells (Fig. 3g). We next assessed the expression of naive and effector T-cell markers in paired γδ T-cell populations from the same donors. Vγ9⁻ Vδ2⁺ T cells

commonly displayed lower levels of TCR Vδ2 expression and higher levels of CD27 expression than their Vγ9⁺ counterparts (Fig. 3h). Vγ9⁻ Vδ2⁺ T cells expressed the lymphoid homing receptor CCR7, the homoeostatic cytokine receptor IL7Rα (Fig. 3i) but lacked the cytotoxic effector molecule Granzyme

Table 1 Single-cell TCR sequencing of adult V γ 9⁺ V δ 2⁺ T cells

Donor	CDR3 γ 9 (amino acid)	CDR3 γ 9 nucleotide (nt) sequence	N nt	P nt	CDR3 δ 2 (amino acid)
3	CALWE VEVQ ELGKKIKVF	TGTGCCTTGTGGGAGGTGCAAGAGTTGGGCCAAAAAATCAAGGTATTT	Germline	0	CACDSLGLDTPNFDKLF
	CALWE VEVQ ELGKKIKVF	TGTGCCTTGTGGGAGGTGCAAGAGTTGGGCCAAAAAATCAAGGTATTT	Germline	0	CACDT GG AAQSWDRQMF
28	CALWE VEVQ ELGKKIKVF	TGTGCCTTGTGGGAGGT T CAAGAGTTGGGCCAAAAAATCAAGGTATTT	1	0	CACDSLGGTYTDKLF
	CALWE VEVQ ELGKKIKVF	TGTGCCTTGTGGGAGGTGCAAGAGTTGGGCCAAAAAATCAAGGTATTT	Germline	0	CACD IL GGQYTDKLF
35	CALWE VEVQ ELGKKIKVF	TGTGCCTTGTGGGAGGTGCA G AGTTGGGCCAAAAAATCAAGGTATTT	1	2	CACES LG PPTGGNPPSSDKLF
	CALWE VEVQ ELGKKIKVF	TGTGCCTTGTGGGAGGT T CAAGAGTTGGGCCAAAAAATCAAGGTATTT	1	0	CACDT TI HRTGGPQVTDKLF
42	CALWE VEVQ ELGKKIKVF	TGTGCCTTGTGGGAGGTGCAAGAGTTGGGCCAAAAAATCAAGGTATTT	Germline	0	CACD GL GGEYTDKLF
	CALWE VEVQ ELGKKIKVF	TGTGCCTTGTGGGAGGTGCAAGAGTTGGGCCAAAAAATCAAGGTATTT	Germline	0	CACD KV GYGSPWDRQMF
231	CALWE VEVQ ELGKKIKVF	TGTGCCTTGTGGGAGGTGCAAGAGTTGGGCCAAAAAATCAAGGTATTT	Germline	0	CACD TI PTGGHDPYTDKLF
	CALWE VEVQ ELGKKIKVF	TGTGCCTTGTGGGAGGT A CAAGAGTTGGGCCAAAAAATCAAGGTATTT	1	0	CACD TV TRDKGADKLF
1014	CALWE VEVQ ELGKKIKVF	TGTGCCTTGTGGGAGGT A CAAGAGTTGGGCCAAAAAATCAAGGTATTT	1	0	CACD TM GARHTDKLF
	CALWE VEVQ ELGKKIKVF	TGTGCCTTGTGGGAGGT A CAAGAGTTGGGCCAAAAAATCAAGGTATTT	1	0	CACD TF GLVQSHGPKRTDKLF
261	CALWE VEVQ ELGKKIKVF	TGTGCCTTGTGGGAGGTGCAAGAGTTGGGCCAAAAAATCAAGGTATTT	Germline	0	CACD RL GGNTDKLF
	CALWE VEVQ ELGKKIKVF	TGTGCCTTGTGGGAGGT C AGAGTTGGGCCAAAAAATCAAGGTATTT	3	0	CACD TV PSTGGPKD TD DKLF
	CALWE VEVQ ELGKKIKVF	TGTGCCTTGTGGGAGGT C AGAGTTGGGCCAAAAAATCAAGGTATTT	1	1	CACD TV GWGTPLYTDKLF

Public TCR γ 9 clonotypes and their paired TCR δ 2 sequence from seven donors. Sequences were analysed using IMGJ Junction Analysis, which identified V, D and J gene segments used and highlighted N nucleotide addition (bold and italic) and P nucleotide addition (underlined). The table depicts CDR3 γ 9 amino acid sequence, CDR3 γ 9 nucleotide (nt) sequence, germline or N nt addition, P nt addition, CDR3 δ 2 amino acid sequence. Hydrophobic amino acids at position 5 (see Methods section) in the CDR3 δ 2 sequence are highlighted in bold

(Grz) A or the endothelial homing receptor CX₃CR1 (Fig. 3j), with these expression patterns contrasting with V γ 9⁺ V δ 2⁺ T cells (V δ 2^{hi} CD27^{int} CCR7⁻ IL7R α ⁺ Grz A⁺ CX₃CR1⁺) but together highly similar to CD27^{hi} V δ 1⁺ T cells (Fig. 3h–j). Moreover, V γ 9⁻ V δ 2⁺ T cells were responsive to CD3/CD28 stimulation, but like V δ 1 T cells were unresponsive to IL-12/IL-18 (Fig. 3k), whereas V γ 9⁺ V δ 2⁺ T cells were responsive to both CD3/CD28 and IL-12/IL-18¹¹. CD27^{hi} V δ 1⁺ T-cell populations lack expanded clonotypes and possess a diverse and private TCR repertoire¹¹. Analysis of the publicity of adult V γ 9⁻ V δ 2⁺ TCR repertoires indicated a far more private TCR repertoire compared to V γ 9⁺ V δ 2⁺ T cells (Fig. 3l), but equivalent to the highly private V δ 1⁺ TCR repertoire¹¹. This was confirmed by single-cell TCR sequencing of adult V γ 9⁻ V δ 2⁺ T-cell populations, which contained diverse TCR sequences, comprising a range of V γ chains (Fig. 3m), and featuring TCR γ and δ chains that each lacked motifs previously linked to P-Ag reactivity (Table 2). In contrast, V γ 9⁺ V δ 2⁺ T cells sorted from the same donors displayed sequences that included prevalent shared TCR γ sequences (Fig. 3m and Table 1). Collectively, these data suggest that the V γ 9⁻ V δ 2⁺ subset is functionally, phenotypically and clonotypically distinct from its V γ 9⁺ counterparts, but may share a similar biology to that of V δ 1⁺ T cells.

V γ 9⁻ V δ 2⁺ T cells clonally expand into effectors. Within our healthy donor cohort, one individual (“donor X”) had a substantially increased V γ 9⁻ V δ 2⁺ T-cell population, comprising 1.2% of all CD3⁺ T cells (Fig. 4a), far higher than the 17 other healthy donors (mean 0.13% of CD3⁺ T cells). Importantly, and in contrast to the dominant CD27^{hi} phenotype displayed by V γ 9⁻ V δ 2⁺ T cells in all other healthy donors, “donor X’s” V γ 9⁻ V δ 2⁺ T-cell population had downregulated CD27 and retained CD45RA expression (CD27^{lo/neg} phenotype), now accounting for 91.4% of the V γ 9⁻ V δ 2⁺ T-cell population (Fig. 4a). We next carried out single-cell TCR sequencing to determine if any particular $\gamma\delta$ TCR clonotypes correlated with this phenotypic change. Strikingly, a single clone now dominated the V γ 9⁻ V δ 2⁺ T-cell population (35/36 single cells sequenced), composed of a V δ 2 CDR3 (CACGSWWGTYYTDKLF) paired with a V γ 8–J γ P1 chain (Fig. 4b), and now represented the single most dominant clonotype within the V δ 2⁺ T-cell repertoire (Fig. 4c). As this dominant clonotype occurred in conjunction with a CD27^{lo/neg} phenotype, we assessed effector T-cell marker expression. “Donor

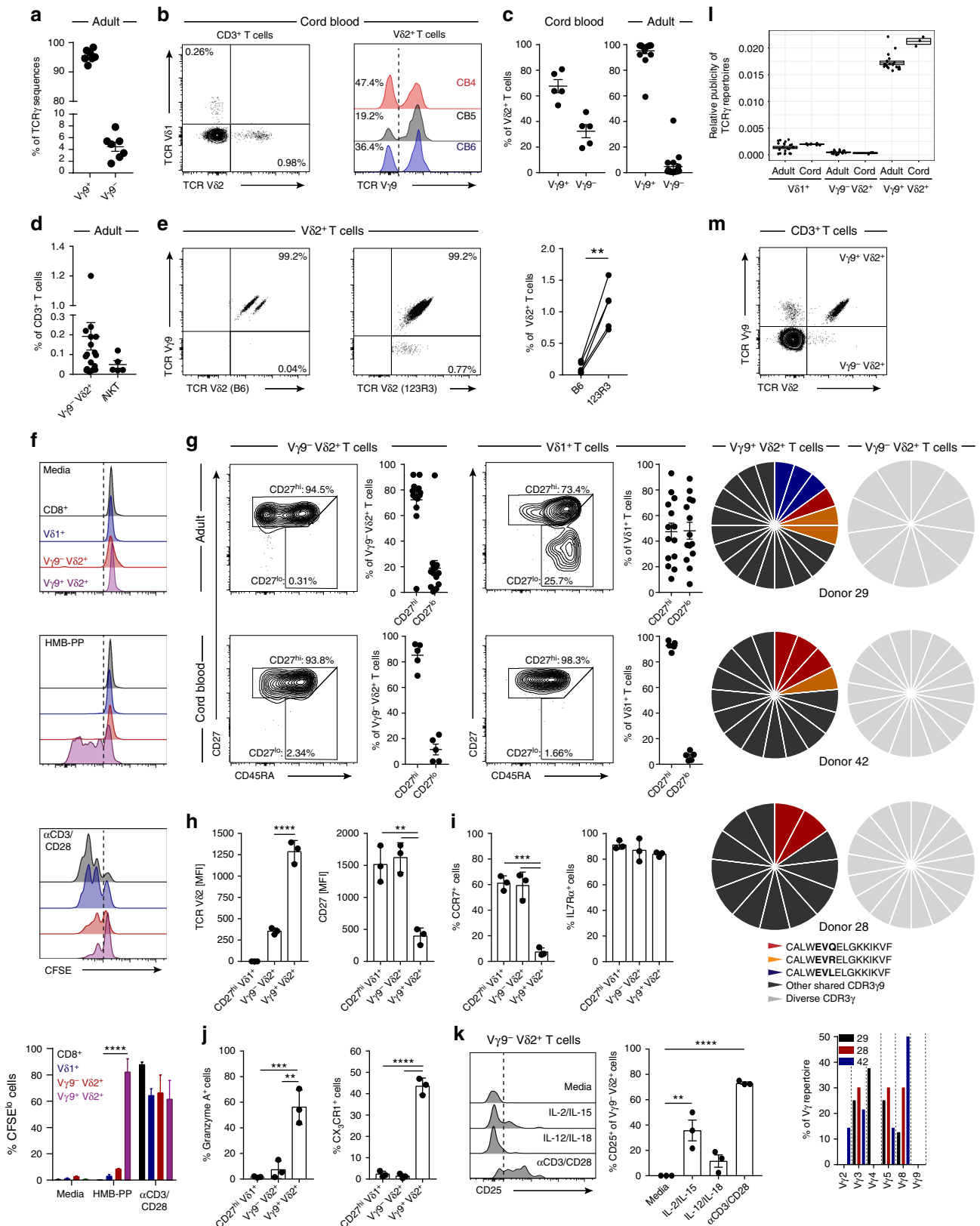
X’s” V γ 9⁻ V δ 2⁺ T-cell population expressed the antibody-mediated cytotoxicity receptor CD16 (Fc γ RIIIa), CX₃CR1, Grz A and had downregulated CCR7, in contrast to naive CD8 T cells, but closely matching CD27^{lo/neg} V δ 1⁺ T cells (Fig. 4d), which also comprised dominant clonotypes¹¹. These results highlight the potential for the V γ 9⁻ V δ 2⁺ T-cell compartment to undergo clonal selection and differentiation, mimicking the transition to clonality and effector status previously observed in V δ 1⁺ T cells¹¹.

Clonally focused V γ 9⁻ V δ 2⁺ T cells infiltrate human liver. V δ 1⁺ T cells are predominantly associated with a role in tissue immunity^{35,36}. To address whether the V γ 9⁻ V δ 2⁺ T-cell population was also represented in solid tissues, we analysed human liver samples for the presence of intrahepatic V γ 9⁻ V δ 2⁺ T cells (Fig. 5a). While the V δ 2⁺ T cells in the liver comprised a lower proportion of total CD3⁺ T cells than in peripheral blood, intrahepatic V δ 2⁺ T cells generally contained a higher fraction of V γ 9⁻ cells (Fig. 5b). Phenotypic analysis of intrahepatic V γ 9⁻ V δ 2⁺ T cells revealed these cells to consistently display a CD27^{lo/neg} phenotype (Fig. 5c, d). Single-cell TCR sequencing analysis, highlighted that a majority of intrahepatic V γ 9⁻ V δ 2⁺ T cells were comprised of a limited set of expanded clonotypes (Fig. 5e and Table 2). Moreover, clonally expanded intrahepatic V γ 9⁻ V δ 2⁺ T cells expressed effector markers CX₃CR1 and Grz A but had downregulated IL7R α (Fig. 5f).

Clonal expansion of V γ 9⁻ V δ 2⁺ T cells in acute CMV infection. The clonotypic and phenotypic parallels between V γ 9⁻ V δ 2⁺ and V δ 1⁺ $\gamma\delta$ T cells, combined with their strong distinction from P-Ag sensing V γ 9⁺ V δ 2⁺ T cells, prompted us to investigate if this subset was reactive to acute cytomegalovirus (CMV) infection, previously shown to drive the numeric³⁷ and clonal¹² expansion of V δ 1⁺ T cells. We examined a cohort of five CMV-seronegative (CMV⁻) patients receiving CMV-seropositive (CMV⁺) kidney transplants who then went on to either develop post-operative acute CMV infection (patient 261, 231 and 1014), or who remained CMV⁻ (patient 279 and 282)³⁸. We analysed peripheral blood samples for V γ 9⁻ V δ 2⁺ T cells before and after post-transplant CMV infection. Prior to CMV infection, at 0–4 weeks post-transplant, V γ 9⁻ V δ 2⁺ T cells represented low frequency populations in all donors (Fig. 6a and Supplementary Fig. 3). However, following CMV infection, occurring between 5–8 weeks post-transplant, we observed the substantial expansion

of the $V\gamma 9^- V\delta 2^+$ T-cell population in donor 261 and 231 (Fig. 6a). Conversely, patients 282 and 279 who did not become infected with CMV, displayed no expansion of $V\gamma 9^- V\delta 2^+$ T cells over a 5-year period after transplantation (Supplementary Fig. 3a). Of note is patient 1014, who became infected with CMV but did not display an expanded $V\gamma 9^- V\delta 2^+$ T-cell population

(Supplementary Fig. 3b), suggesting inter-individual differences in the $V\gamma 9^- V\delta 2^+$ T-cell response to CMV infection consistent with observations in $V\delta 1^+$ T cells from persistently infected CMV⁺ healthy adult donors¹¹. Phenotypic analysis of $V\gamma 9^- V\delta 2^+$ T cells from CMV⁻ patients 279 (Supplementary Fig. 3c) and 261 immediately after transplant (Fig. 6b), indicated a



CD27^{hi} phenotype. Conversely after CMV infection, the expanded V γ 9⁻ V δ 2⁺ T-cell populations in patients 261 and 231 acquired a dominant CD27^{lo/neg} phenotype (Fig. 6b). This prompted us to perform single-cell TCR sequencing of expanded V γ 9⁻ V δ 2⁺ T-cell populations in donors 261 and 231, which identified the selective expansion of dominant clonotypes after CMV infection and evidence of continued clonotypic selection 5 years later (Fig. 6c and Table 2). Finally, we assessed functional T-cell marker expression in V γ 9⁻ V δ 2⁺ T cells from patient 261 and 231 following CMV infection. V γ 9⁻ V δ 2⁺ T cells from healthy donors expressed CCR7, IL7R α and the co-stimulatory molecule CD28, while patient 261 and 231's V γ 9⁻ V δ 2⁺ T cells had downregulated these markers and upregulated CX₃CR1 and Grz A and B (Fig. 6d). Expression of these effector markers occurred concurrently with V γ 9⁻ V δ 2⁺ T-cell clonal expansion at ~1 year (patient 261) and were stable for over 5 years (patient 231) (Fig. 6c, d). These data reveal that as for V δ 1⁺ T cells, V γ 9⁻ V δ 2⁺ T cells exhibit a strong relationship between clonotype and phenotype and are suggestive of a role in adaptive antiviral immunosurveillance.

Discussion

This study sheds light on the biology of V δ 2⁺ T cells, highlighting divergent V γ 9⁺ and V γ 9⁻ subsets. Despite the ~ten-fold increase in V γ 9⁺ V δ 2⁺ T-cell numbers in the first year of life, our data indicate that the extent of clonotypic focusing within the V δ 2-associated V γ 9⁺ repertoire is broadly similar between neonatal cord blood and adulthood. This finding contrasts with the V δ 1⁺ TCR repertoire, which typically displays pronounced clonotypic focusing in adults relative to a highly unfocused neonatal repertoire¹¹. In addition, our analyses reveal an underappreciated degree of public amino acid sequences in the V γ 9 repertoire. This degree of V γ publicity, which relates to both recombination of simple V γ 9 sequences (either germline encoded or with limited N nucleotide addition) and convergent recombination, underlines the semi-invariant nature of the V γ 9⁺ V δ 2⁺ TCR repertoire. Importantly, using single-cell approaches public V γ clonotypes were shown frequently to pair with diverse V δ 2 chains within single individuals, and, therefore, do not usually represent expanded clonotypes. The innate-like biology of the semi-invariant V γ 9⁺ V δ 2⁺ T-cell subset contrasts markedly with the V δ 1⁺ subset, which displays clear hallmarks of adaptive immunity such as clonal amplification and differentiation from an initially unfocused, private TCR repertoire.

Collectively, our repertoire data suggest a model involving a multi-layered selection of V γ 9⁺ V δ 2⁺ T cells from foetal development into adulthood. First, as previously suggested by Dimova et al.²⁵, we propose gestational selection of the V γ 9⁺ V δ 2⁺ subset, potentially via exposure to endogenous P-Ag (e.g., IPP) most likely mediated by BTN3A1^{15–17,39,40}, allowing pre-

programming of a P-Ag reactive V γ 9⁺ V δ 2⁺ TCR repertoire. In keeping with this, we observed that V γ 9 chains are heavily enriched for the J γ P segment, which is not generally present in V γ 9 chains present in V δ 1⁺ TCR repertoires¹¹; similarly the J δ 1⁺ CDR3 δ 2 repertoire in cord blood is enriched for a hydrophobic amino acid at position 5 required for P-Ag recognition^{21,29}, but not in V δ 2 sequences in V γ 9⁻ V δ 2⁺ T cells (see below). Second, in the context of the reported lack of thymic output of V δ 2⁺ T cells after birth⁴¹, our observation that adult and cord blood repertoires contain overlapping canonical P-Ag-sensing V γ 9 sequences strongly indicates there is polyclonal post-natal expansion of this pre-selected repertoire, most likely in response to microbial P-Ag exposure¹¹. However, our observation that cord blood V δ 2 sequences generally utilised J δ 3 segments (also observed in foetal $\gamma\delta$ T cells,⁴²) whereas adult V δ 2 sequences preferentially used J δ 1 segments, suggests that post-natal microbial exposure may impose some constraints on the TCR repertoire that differ from those required in gestation. Of note, V δ 2–J δ 1 sequences typically contain a hydrophobic amino acid previously highlighted as important for V γ 9⁺ V δ 2⁺ T-cell reactivity to microbially-derived P-Ag^{21,29} whereas this is less conserved in V δ 2–J δ 3. This might imply V γ 9⁺ V δ 2⁺ T cells bearing J δ 1 clonotypes preferentially respond to microbial P-Ag after birth compared to J δ 3 clonotypes, a possibility which future studies can address. Finally, although most individuals' V γ 9⁺ V δ 2⁺ T cells were dominated by public V γ 9 sequences and associated with a common central memory phenotype, our observation that a minority of individuals exhibit amplification of unusual public clonotypes, concomitant with adoption of an effector memory phenotype, most likely reflects a third level of selection that imparts a degree of plasticity in this fundamentally innate-like paradigm. This observation extends previous work by Ryan et al.³³ indicating that further functional and transcriptional changes are linked to an effector memory phenotype in V γ 9⁺ V δ 2⁺ T cells. Therefore, the semi-invariant V γ 9⁺ V δ 2⁺ repertoire may provide scope for selective TCR-mediated responses to microbial P-Ag. Importantly, our model shares a number of features with MAIT cells, a semi-invariant population that is also subject to both gestational selection (through interactions with MR1) and post-natal expansion in response to microbial infection/colonisation⁴³, and that displays clonotype-specific responses to metabolite ligands⁴⁴ and microbial species⁴⁵.

Our analyses also highlight a population of V γ 9⁻ V δ 2⁺ T cells that is highly distinct from the V γ 9⁺ V δ 2⁺ P-Ag-reactive subset, and present in all adult peripheral blood and cord blood, where they commonly exist as a CD27^{hi}, TCR diverse subset phenotypically equivalent to naive-like V δ 1⁺ T cells. We note this distinction based on V γ 9 chain usage cannot be applied to the $\gamma\delta$ T-cell compartment as a whole, as the V δ 1⁺ T-cell subset, which appears to exhibit an adaptive immunobiology, contains a

Fig. 3 V γ 9⁻ V δ 2⁺ T cells are clonally and phenotypically distinct from V γ 9⁺ V δ 2⁺ T cells. **a** Frequency of V γ 9 chain usage in V δ 2⁺ TCR repertoire sequencing data from adult peripheral blood ($n = 7$). **b** Identification of V δ 2⁺ T cells in CD3⁺ T cells (left) and V γ 9⁻ cells within V δ 2⁺ T cells (right) from cord blood ($n = 5$). **c** Frequency of V γ 9⁻ and V γ 9⁺ cells in V δ 2⁺ T cells from cord blood (left; $n = 5$) and adult peripheral blood (right; $n = 18$). **d** Frequency of V γ 9⁻ V δ 2⁺ T cells ($n = 18$) and NKT cells (α Galcer/CD1d⁺; $n = 5$) in adult peripheral blood. **e** V γ 9⁻ V δ 2⁺ T cells identified by TCR V δ 2 antibody clones (B6 and 123R3) in matched adult peripheral blood donors ($n = 5$). **f** Proliferation by CFSE dilution of CD8⁺ $\alpha\beta$ and $\gamma\delta$ T-cell populations from PBMC treated with medium alone, 10 nM HMB-PP or 5 μ g/ml anti-CD3/CD28 ($n = 5$). **g** CD27 and CD45RA T-cell memory marker expression profiles on V γ 9⁻ V δ 2⁺ and V δ 1⁺ T cells from adult peripheral blood (top row: V δ 2, $n = 14$; V δ 1, $n = 14$) and cord blood (bottom row: V δ 2 and V δ 1, $n = 5$). **h** V δ 2⁺ TCR and CD27 expression levels, **i** naive and **j** effector T-cell marker expression on donor matched $\gamma\delta$ T-cell populations from adult peripheral blood ($n = 3$). **k** Sorted CD3⁺ T cells were incubated for 72 h with cytokines or anti-CD3/CD28 beads. V γ 9⁻ V δ 2⁺ T cells were then assessed for the upregulation of CD25 ($n = 3$). **l** Comparison of CDR3 γ sequence sharing in $\gamma\delta$ T-cell repertoires from adult peripheral blood (V δ 1, $n = 20$; V δ 2, $n = 7$) and cord blood (V δ 1, $n = 5$; V δ 2, $n = 3$). **m** CDR3 γ sequence analysis of single cell sorted V γ 9⁺ and V γ 9⁻ V δ 2⁺ T cells, public sequences are coloured (black, shared sequences from deep sequencing); graph shows V γ usage of V γ 9⁻ V δ 2⁺ TCR sequences from each donor. Error bars indicate means \pm SEM; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; p -values were determined by paired t -test (**e**), RM two-way ANOVA with Tukey's post hoc testing (**f**); one-way ANOVA with Tukey's post hoc testing (**h**, **i**) or Dunnett's post hoc testing (**j**, **k**)

Table 2 Single-cell TCR sequencing of Vγ9⁻ Vδ2⁺ T cells

Donor	CDR3δ2	Dδ	Jδ	CDR3δ length	Vγ	Jγ	CDR3γ length
3	CACGS W WGTYTDKLI F	1,3	1	14	8	P1	11
231	CACDT I VGDTLTDKLI F	3	1	15	8	P1	9
	CACV R GGGYPRGADKLI F	3	1	16	8	1/2	14
	CACDT E GEVNTDKLI F	3	1	14	4	P1	11
261	CACDT R FRPGRSARVVRLTAQL F	1	2	22	3	P1	14
Liver 1	CACSR E QAHTDKLI F	3	1	13	3	1/2	10
	CACDT G WGIRGRYTDKLI F	3	1	17	4	1/2	11
Liver 2	CACDR S GARWDKLI F	—	1	13	5	1/2	10
42	CACD A RHYWGISTDKLI F	3	1	16	2	1/2	12
	CACD A RLGEHTDKLI F	3	1	14	ND	ND	ND
	CACDR M GDLPSWDRQMF F	3	3	18	3	1/2	13
	CACGS S WGVSHYTDKLI F	3	1	16	5	1/2	10
	CACD L LGDFTDKLI F	3	1	14	8	1/2	8
	CACSV R GHWGRSTDKLI F	3	1	16	8	1/2	14
	CACDT R GIGDTLPDKLI F	3	1	16	8	P1	12
	CACA H YWGTPWDTDKLI F	3	1	17	3	P1	11
	CACDT G DTDTDKLI F	3	1	13	8	P1	13
	CACD R LLLGDTDKLI F	3	1	14	ND	ND	ND
	CACDT R TGGRDKLI F	3	1	14	5	1/2	9
	CACD K IRWGNPYTDKLI F	3	1	17	8	P2	10
	CACEV P SYEPYWGTKKYTDKLI F	2,3	1	21	3	1/2	11
	CACDT G DWGINTDKLI F	3	1	15	8	P1	14
	CACD Q KYWGGSTDKLI F	3	1	16	2	P	13

TCRδ sequences of clonally expanded Vγ9⁻ Vδ2⁺ TCRs from donors expressing a CD27^{lo/neg} phenotype (top section). All TCRδ2 sequences from a representative healthy donor with a CD27^{hi} phenotype (bottom section). Sequences were analysed using IMGT Junction Analysis, which identified V, D and J gene segments used. The table depicts CDR3δ sequences, Dδ usage, Jδ usage, CDR3δ length, Vγ usage, Jγ usage and CDR3γ length. Amino acids (aa) at position 5 of the CDR3 sequence (see Methods) are highlighted in bold. ND not determined

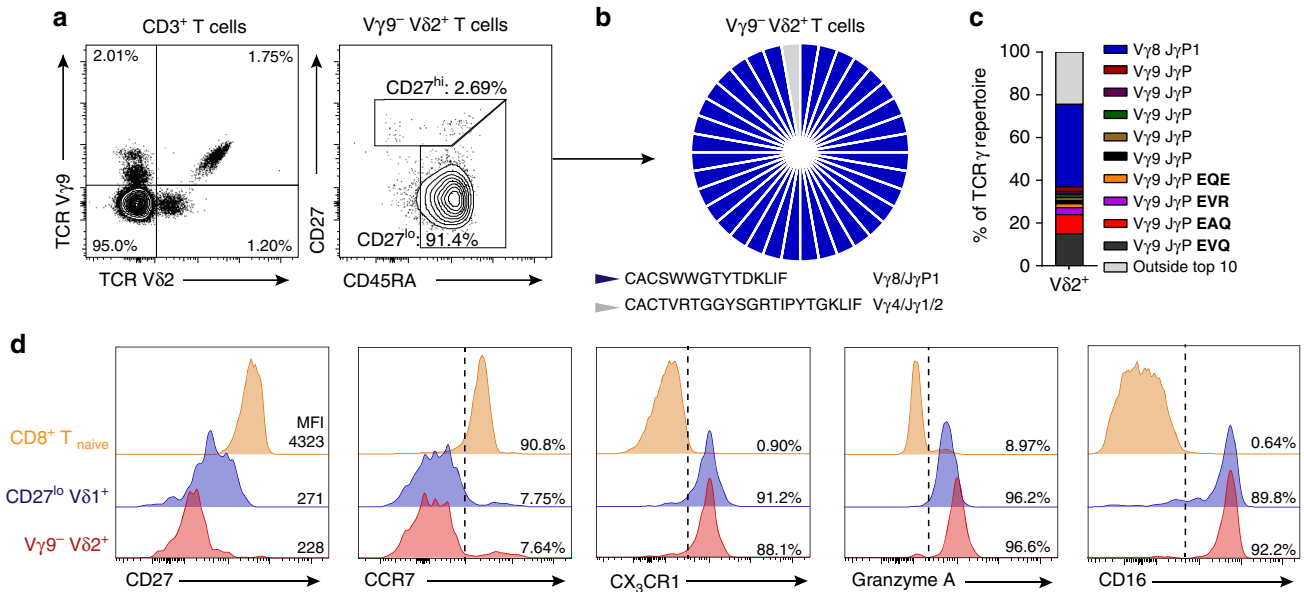


Fig. 4 Vγ9⁻ Vδ2⁺ T cells undergo clonal expansion. **a** Identification of Vγ9⁻ Vδ2⁺ T cells (left) and CD27/CD45RA T-cell memory marker expression on Vγ9⁻ Vδ2⁺ T cells (right) in a peripheral blood sample from “donor X”. **b** Single-cell CDR3δ sequence analysis and Vγ usage by Vγ9⁻ Vδ2⁺ T cells sorted from a peripheral blood sample from “donor X”. **c** TCRγ repertoire analysis of Vδ2⁺ T cells from “donor X”, highlighting the frequency of “donor X’s” Vγ9⁻ Vδ2⁺ T-cell clone (from **b**) within the top 10 most prevalent clonotypes, highlighted are prevalent Vγ9⁺ clonotypes (EVQ, EAQ, EVR and EQE). **d** Analysis of naive and effector T-cell marker expression by Vγ9⁻ Vδ2⁺, CD8⁺ CD27^{hi} CD45RA^{hi} (CD8⁺ T_{naive}) and CD27^{lo/neg} Vδ1⁺ T-cell populations from a peripheral blood sample from “donor X”

sizeable portion of Vγ9⁺ T cells¹¹. Moreover, consistent with a distinct biology for Vγ9 chains in these two contexts, the Vγ9 CDR3 sequences associated with Vδ1 chains did not utilise JγP sequences and were highly distinct from those associated with Vδ2 chains¹¹. Consistent with expression of TCRγ and TCRδ sequences divergent from the Vγ9⁺ Vδ2⁺ subset, the Vγ9⁻ Vδ2⁺

population was not P-Ag responsive. Thus, Vγ9⁻ Vδ2⁺ T cells represent a universal γδ subset that is clonotypically, phenotypically and functionally divergent from Vγ9⁺ Vδ2⁺ T cells. Moreover, as evidenced from a single unusual peripheral blood donor and several liver samples, Vγ9⁻ Vδ2⁺ T cells appeared capable of undergoing an adaptive-like programme of highly

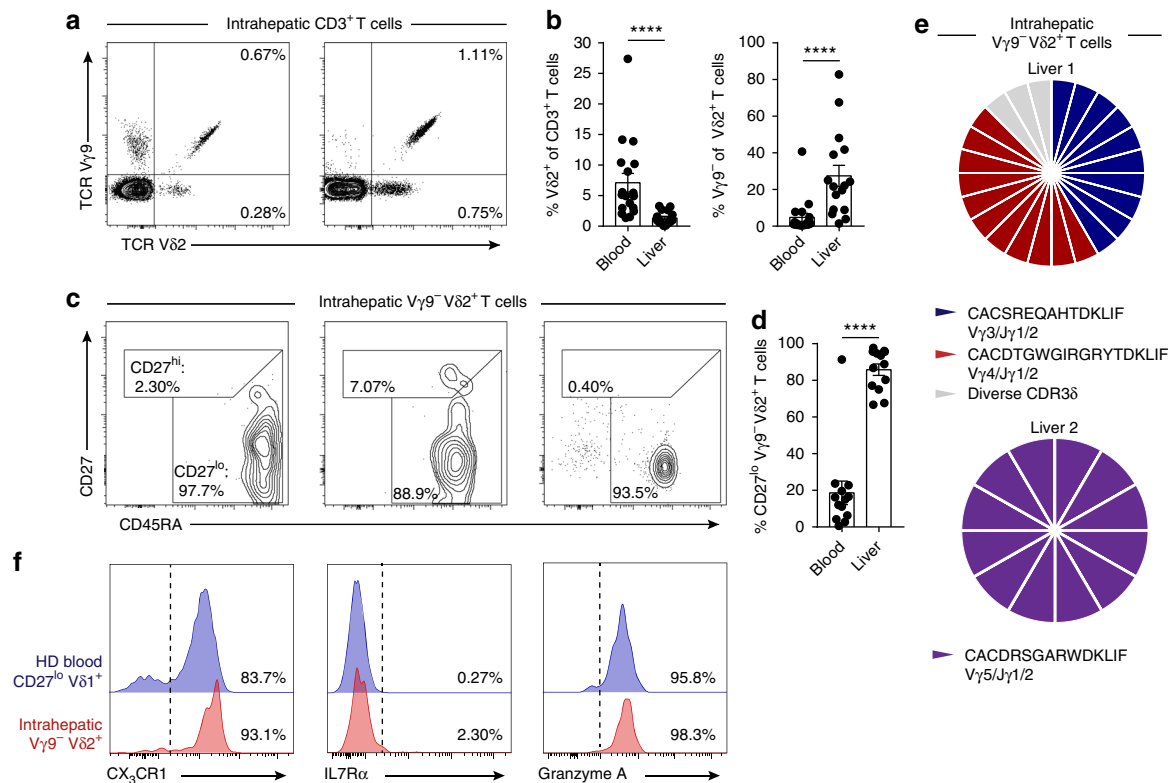


Fig. 5 Clonally expanded $V\gamma 9^- V\delta 2^+$ T cells infiltrate human liver tissue. **a** Representative identification of intrahepatic $V\gamma 9^- V\delta 2^+$ T cells in $CD3^+$ lymphocytes obtained from liver tissue and **b** summary data of the frequency of $V\delta 2^+$ T cells in $CD3^+$ T cells (left) or $V\gamma 9^- V\delta 2^+$ T cells in $V\delta 2^+$ T cells (right) from peripheral blood ($n = 18$) and liver tissue ($n = 16$). **c** Representative intrahepatic $V\gamma 9^- V\delta 2^+$ T-cell expression profiles of CD27 and CD45RA T-cell memory markers and **d** summary data from peripheral blood ($n = 18$) and liver tissue ($n = 16$). **e** Single-cell CDR3 δ sequence analysis and $V\gamma$ usage by intrahepatic $V\gamma 9^- V\delta 2^+$ T cells sorted from two independent liver tissue samples. **f** Representative analysis of CX_3CR1 , $IL7R\alpha$ and Granzyme A expression by intrahepatic $V\gamma 9^- V\delta 2^+$ T cells ($n = 3$) and a healthy donor's (HD) $CD27^{lo/neg} V\delta 1^+$ T-cell population ($n = 8$). Error bars indicate means \pm SEM; **** $P < 0.0001$; p -values were determined by Mann-Whitney t -test (**b, d**)

focused clonotypic expansion and concomitant phenotypic differentiation that closely matches that of $V\delta 1^+$ T cells but is not observed in $V\gamma 9^+ V\delta 2^+$ T cells. This considerably extends the findings of Ravens et al.¹², who detected unusual $V\gamma 9^- V\delta 2^+$ clonotypes in the peripheral blood of one healthy donor and one HSCT recipient.

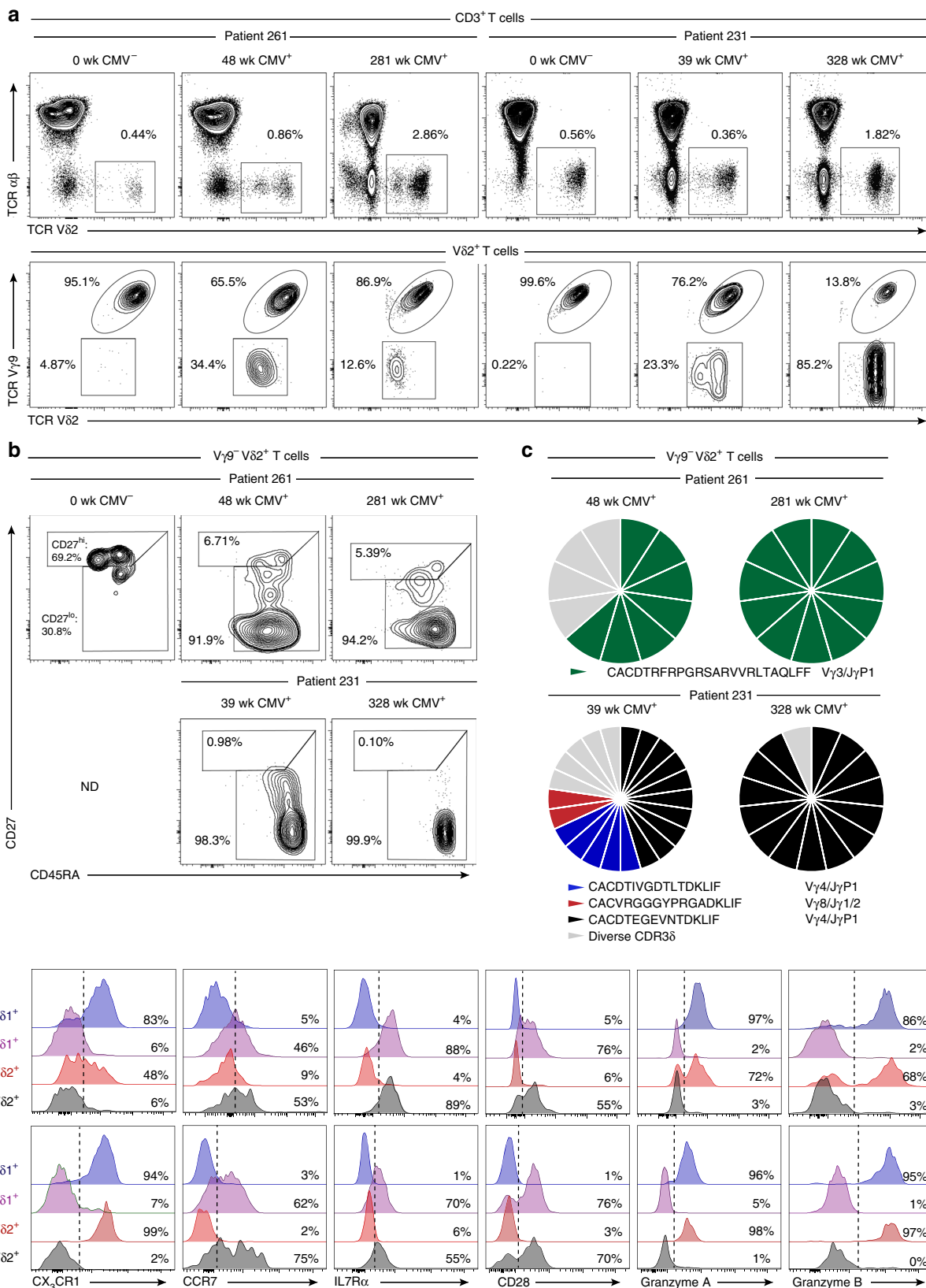
This adaptive biology was confirmed by our analysis of patients following acute CMV infection, which highlighted a striking expansion of the $V\gamma 9^- V\delta 2^+$ subset after infection, linked to the selection of dominant clonotypes, which occurred concomitantly with differentiation from a naive $CD27^{hi}$ to a $CD27^{lo/neg}$ phenotype, and acquisition of CX_3CR1 and cytotoxic granzymes, similar to CMV-specific cytotoxic $CD8^+$ T cells^{46,47}. Interestingly, the single healthy donor who exhibited a clonally expanded $V\gamma 9^- V\delta 2^+$ T-cell subset ("donor X") was CMV⁺ and unusually, displayed elevated IgG titres, raising the possibility of recent CMV reactivation. Our findings therefore suggest a role for the $V\gamma 9^- V\delta 2^+$ T-cell subset in unconventional immunosurveillance against viral infection, and provide a clear indication that this subset assumes an adaptive immunobiology, highly similar to that suggested for $V\delta 1^+$ T cells^{11,12}. These data suggest clonal selection from a $CCR7^+$, potentially lymphoid tissue homing, naive-like $V\gamma 9^- V\delta 2^+$ T-cell population that expresses a pool of TCRs, enabling amplification of responses to microbial challenges, via differentiation of cells bearing biologically relevant clonotypes to an effector phenotype, resulting in long-lived, functional $\gamma\delta$ T-cell memory. The liver provided an attractive human model in which to examine $V\gamma 9^- V\delta 2^+$ T cells in solid tissues, as it functions as an important site for clearance of both commensals and

pathogens from portal circulation, and has a high proportion of unconventional T cells, such as MAIT cells⁴⁸. Our finding that clonally expanded effector $V\gamma 9^- V\delta 2^+$ T cells can be recruited to the liver suggests this subset may complement the innate-like recognition provided by such hepatic semi-invariant T cells with an unconventional adaptive memory response. Unfortunately, we were unable to obtain CMV serostatus for the patients from whom explanted liver samples were derived, and therefore unable to conclude if the presence of such hepatic $V\gamma 9^- V\delta 2^+$ T cells solely reflected CMV seropositivity or alternatively a wider set of immune challenges. Of relevance, a monoclonally expanded myocytotoxic T-cell population bearing a $V\gamma 3^+ V\delta 2^+$ TCR detected in a patient with polymyositis highlights the potential of the $V\gamma 9^- V\delta 2^+$ T-cell subset to trigger autoimmunity^{49,50}. Further studies are required to define additional microbial and non-microbial challenges that stimulate this unconventional T-cell subset.

In addition to clonal expansion in response to CMV, the finding that peripheral blood $V\gamma 9^- V\delta 2^+$ T cells were TCR responsive but unresponsive to IL-12/IL-18 is another feature that distinguishes them from $V\gamma 9^+ V\delta 2^+$ T cells, and one that again aligns them closely with the $V\delta 1^+$ T-cell subset, which has also been highlighted to display adaptive features^{11,12}. Further studies on such adaptive subsets ($V\delta 1^+$ and $V\gamma 9^- V\delta 2^+$ T cells), ideally ultimately in the context of physiological TCR ligands, are required to explore the full range of functional differences with innate-like subsets. Of note, $V\gamma 9^+ V\delta 2^+$ T cells have previously been demonstrated to exert pleiotropic effector functions in response to cognate P-Ag ligands and different cytokine stimulation⁵¹. Moreover, recent studies in mice highlight that $\gamma\delta$ TCR

triggering of innate-like lymphocytes induced a state of TCR hyporesponsiveness in which T-cell activation could be induced by TCR-extrinsic stimuli⁶. Unfortunately, a systematic comparison of the TCR responsiveness of naive vs. effector V γ 9⁻ V δ 2⁺ T cells within one tissue was not possible, due to the strong dominance of naive V γ 9⁻ V δ 2⁺ T cells in peripheral blood and

effector V γ 9⁻ V δ 2⁺ T cells in liver. However, importantly within the V δ 1⁺ T-cell compartment, CD27^{lo/neg} effectors display a far quicker response to TCR stimulation than CD27^{hi} naive cells¹¹. This observation emphasises the strong distinction between innate-like and adaptive subsets within the $\gamma\delta$ T-cell compartment.



Human $V\delta^+$ T cells therefore comprise two separate subsets, which reflect distinct paradigms in human $\gamma\delta$ T-cell biology and also have distinct roles in antimicrobial immunity. $V\gamma^+ V\delta^+$ T cells appear to represent an innate-like subset, most likely originating via gestational selection, which is 'pre-armed' from birth with a semi-invariant TCR repertoire that includes a high level of public $V\gamma$ TCR chains, permitting polyclonal TCR-mediated responses to microbial P-Ag, as well as to innate cytokines independently of the TCR. However, the $V\gamma^+ V\delta^+$ TCR repertoire diversity may provide the potential for clonotype-specific responses potentially targeted at specific microbial antigenic challenges. In contrast, $V\gamma^+ V\delta^+$ T cells, which are P-Ag-unreactive, represent a previously ill-defined unconventional T-cell compartment that exhibits many of the key hallmarks of an adaptive immunobiology, including clonal expansion and differentiation to effector lymphocytes from an initially naive-like and TCR diverse T-cell pool, including in response to acute viral infection. In doing so, $V\gamma^+ V\delta^+$ T cells most likely contribute to both peripheral blood and tissue immunosurveillance.

Methods

Ethical approval and samples. Peripheral blood samples were obtained from healthy donors who had provided written informed consent for sample collection and subsequent analysis; project approval for this aspect of the study was granted by the NRES Committee West Midlands ethical board (REC reference 14/WM/1254). Samples from patients undergoing renal transplantation were obtained at the Academic Medical Centre, Amsterdam; the medical ethics committee of the Academic Medical Center, Amsterdam, approved this arm of the study and all subjects provided written informed consent in accordance with the Declaration of Helsinki. Longitudinal heparinized blood samples were collected from CMV-seronegative patients who developed primary CMV infection after receiving a renal transplant from a CMV-seropositive donor. As controls, longitudinal PBMC from age matched CMV-seronegative patients who remained CMV-seronegative during the first 5 years following renal transplantation were used. All patients were EBV-seropositive. The course of CMV infection was followed by longitudinal 2-weekly PCR for CMV viral load, and seroconversion was confirmed by detection of CMV-specific IgM and IgG. The patients did not receive CMV prophylaxis, but once the CMV load reached 10^4 copies/ml, the MMF dose was halved and Valcyte was given. Only patient 1014 experienced CMV-related symptoms: CMV gastritis. All but one patient were treated with a basic immunosuppressive regimen comprising of CD25 mAb (basiliximab) induction treatment, prednisolone, cyclosporine A and MMF. Patient 261, however, was treated with one dose of prednisolone upon transplantation with a kidney from an identical twin, whereafter all immunosuppression was ceased. Liver infiltrating T cells were isolated from explanted diseased human liver tissues from patients undergoing liver transplantation for inflammatory liver diseases, including primary biliary cholangitis, primary sclerosing cholangitis, alcoholic liver disease, and autoimmune hepatitis (Local Research Ethics Committee reference no. 98/CA5192) or normal liver samples from donor liver tissue surplus to clinical requirements (Local Research Ethics Committee reference no. 06/Q2708/11). Umbilical cord blood units were obtained from the Anthony Nolan Cell Therapy Centre Nottingham (ANCTC) under generic tissue bank ethics held by ANCTC and extended to the researchers under a material transfer agreement (MTA).

T-cell isolation, culture and activation. PBMC were isolated from heparinized venous blood by lymphoprep® (Stem Cell Technologies) density gradient centrifugation as per the manufacturer's instructions. For proliferation of T cells, PBMC were labelled with 0.3 μ M carboxyfluorescein succinimidyl ester (CFSE) (eBioscience) and cultured with 10 nM HMB-PP (Sigma) or CD3/CD28 T activator beads (Invitrogen) for 7 days in RPMI-1640 medium (Invitrogen) supplemented with 2 mM L-glutamine, 1% sodium pyruvate, 50 μ g/ml penicillin/streptomycin (Invitrogen) and 10% fetal calf serum (Sigma).

Antibodies and flow cytometry. For total and single-cell sorting of $V\delta^+$ populations, PBMC were labelled with anti-CD3 (UCHT1; Biologend; 1:100), TCR $\alpha\beta$ (BW242/412; Miltenyi; 1:200), TCR $V\delta$ (123R3; Miltenyi; 1:200), CD27 (M-T271; 1:200), CD45RA (HI100; 1:200); both Biologend, and populations were sorted on an ARIA III Fusion (BD) (Supplementary Fig. 4a). For repertoire analysis, $V\delta^+$ T-cell populations were sorted directly into RNeasy (Qiagen). For phenotypic analysis, freshly isolated or frozen PBMC, or cultured cells were labelled with Zombie Aqua viability dye (Biolegend), and then subsequently stained (Supplementary Fig. 4b) for cell surface antigens with antibodies directed against CD3 (UCHT1 or HIT3a; 1:100), CD8 (SK1; 1:200), CD45RA (HI100; 1:200), CD27 (M-T271; 1:200), CCR7 (G043H7; 1:50), IL7R α (A019D5; 1:100), CD28 (28.2; 1:100), CX₃CR1 (2A9-1; 1:100), CD16 (3G8; 1:150), CD69 (FN50; 1:100), TCR $V\delta$ (B6; 1:100), TCR $\alpha\beta$ (IP26; 1:50); all Biologend. TCR $V\gamma$ 9 (IMMU360; 1:400); Beckman Coulter. TCR $V\delta$ 1 (REA173; 1:200) and TCR $V\delta$ (123R3; 1:200); Miltenyi, or α GalCer loaded CD1d dextramers (ProImmune; 1:20). For intracellular staining, after surface antibody staining cells were fixed in IC Fixation buffer (eBioscience) and stained in Permeabilisation Buffer (eBioscience) with antibodies directed against Granzyme A (CBO9; 1:100) and Granzyme B (GB11; 1:100); Biologend). Cells were acquired on an LSR Fortessa X20 (BD) and data analysed with FlowJo V10.2 (TreeStar).

TCR repertoire analysis. RNA was purified from sorted cells (adult $V\delta^+$: 25,000 cells; cord blood $V\delta^+$: 2,400 - 8,800 cells) using an RNAmicro plus kit (Qiagen) according to the manufacturer's instructions. For high throughput deep sequencing of $\gamma\delta$ TCRs, we used amplicon rescued multiplex (ARM)-PCR and a MiSeq (Illumina) next-generation sequencer (NGS)⁵² to analyse all sorted $V\delta^+$ T-cell populations. As detailed in patent WO2009137255A2, a modified version of a protocol devised by Han et al.⁵³ was used, involving initial first-round RT-PCR using high concentrations of gene-specific primers, followed by use of universal primers for the exponential phase of amplification, allowing deep, quantitative and non-biased amplification of TCR γ and TCR δ sequences. All cDNA synthesis, amplification, NGS library preparation and sequencing were performed by iRepertoire, Inc. (Huntsville, USA). We analysed positively sorted $\alpha\beta$ TCR- $V\delta^+$ $\gamma\delta$ T cells from 7 healthy donors and 4 umbilical cord blood units (Anthony Nolan Trust, Nottingham).

Single-cell TCR sequencing. PBMC were labelled as above and $V\delta^+$ T cells were single cell sorted directly into individual wells in a 96-well plate containing 2 μ l of Superscript VILO cDNA synthesis kit reaction mix (ThermoFisher) containing 0.1% Triton X-100, and incubated according to manufacturer's instructions. TCR γ and TCR δ cDNAs were amplified by two rounds of nested PCR using GoTaq mastermix (Promega) and primers for $V\delta$, TCTGGCAGGAGTTCATGT (external) and GAAAGGAGAAGCGATCGGTAAC (internal); for C δ GCAGGA TCAAACCTCTGTATCTTC (external) and TCCTTCACCAGACAAGCGAC (internal); for $V\gamma$ 1-8 CTGGTACCTACACCAGAGGGGAAGG (external) and TGTGTTGGAATCAGGAVTCAG (internal); for $V\gamma$ 9 AGAGAGACCTGGTG AAGTCATACA (external) and GGTGGATAGGATACCTGAAACG (internal) and for C γ CTGACGATACATCTGTGTTCTTTG (external) and AATCGTGTG TCTCTTCTTTCTT (internal). PCR products were separated on 1.2% agarose gels, and products of successful reactions were incubated with ExoSAP-IT PCR cleanup enzyme (Affymetrix) before sequencing with BigDye Terminator v3.1 (Applied Biosystems) following manufacturer's instructions and running on an ABI 3730 capillary sequencer (Functional Genomics Facility, University of Birmingham).

TCR repertoire data analysis. V, D and J gene usage and CDR3 sequences were identified and assigned and tree maps generated using iRweb tools (iRepertoire, Inc, Huntsville, AL, USA)⁵⁴. Tree maps show each unique CDR3 as a coloured rectangle, the size of each rectangle corresponds to each CDR3s abundance within the repertoire and the positioning is determined by the V region usage. To determine the 10 most prevalent shared CDR3 γ 9 sequences, the first 10 most dominant sequences by frequency were filtered from each donor's CDR3 γ 9 protein lists. These sequences were then ordered by frequency for sequences that were shared between >2 donors (to create a hierarchy of 10 common sequences) and uncommon highly amplified sequences were included in the final analysis. For more detailed analysis of the TCR repertoire, datasets were processed using the MiXCR software package⁵⁵ to further correct for PCR and sequencing errors. Diversity metrics, clonotype overlap and gene usage were plotted in R, by VDJTools⁵⁶.

Fig. 6 $V\gamma^+ V\delta^+$ T cells exhibit TCR-specific clonal expansion in response to viral infection. **a** Identification of $V\delta^+$ T cells in CD3⁺ T cells (top row) and $V\gamma^+$ cells within $V\delta^+$ T cells (bottom row) in longitudinal peripheral blood samples from two CMV-seronegative kidney transplant patients developing post-operative acute CMV infection (CMV-seroconversion: patient 231, 8 weeks; patient 261, 7 weeks). **b** CD27 and CD45RA T-cell memory marker expression profiles by detectable $V\gamma^+ V\delta^+$ T cells populations in longitudinal peripheral blood samples from patients 231 and 261. Cytometry data from patient 261 at 281 weeks was acquired on an alternative flow cytometer (**a** and **b**). **c** Single-cell CDR3 δ sequence analysis and $V\gamma$ usage by $V\gamma^+ V\delta^+$ T cells sorted from patients 231 and 261 peripheral blood samples between 39 and 328 weeks after transplantation. **d** Analysis of the indicated naive and effector T-cell markers by $V\gamma^+ V\delta^+$ T cells from patients 261 (week 48) and 231 (week 328) and in comparison with CD27^{hi}, CD27^{lo/neg} V δ 1⁺ and $V\gamma^+ V\delta^+$ T cells from the peripheral blood of two healthy donors analysed in parallel. ND no detectable population

TCR sequence analyses. The CDR3 sequence was defined as the amino acids between the second Cysteine of the V region and the conserved Phenylalanine of the J region, according to IMGT. CDR3 sequences shown in tables include the conserved Cysteine and Phenylalanine, but only the amino acids between these residues are counted for CDR3 length analysis and for analysis of residues at position 5. N and P nucleotides were identified using the IMGT Junction Analysis tool^{57,58}. Neonatal V δ 2 sequence logos were generated on the Seq2Logo server⁵⁹ in Shannon format without the use of pseudo counts, and give a visual representation of amino acids enriched at different positions in the observed CDR3 δ 2 sequences. The different amino acids are coloured according to physicochemical properties (acidic (DE), red; basic (RKH), blue; hydrophobic (ACFILMPVW), black; and neutral (NSGTY), green). For TCR δ 2 sequences, the ten most abundant clonotypes of 14–16 amino acids using either J δ 3 or J δ 1 from each donor were aligned using Clustal Omega52 with default parameters, before logo generation. Narrower bars in the sequence logo correspond to gaps in the sequences.

Statistical analysis. Each data set was assessed for normality using Shapiro–Wilk normality test. Differences between columns were analysed by two-tailed Student's *t*-tests for normally distributed data and Mann–Whitney for non-parametric data. Differences between groups were analysed using one-way ANOVA with Dunnett's or Tukey's post tests for normally distributed data or with Kruskal–Wallis with Tukey's post tests for non-parametric data and RM two-way ANOVA with Tukey's post tests was used when comparing groups with independent variables. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001. Correlation was assessed for non-parametric data by Spearman correlation. Tabulated data were analysed in Graphpad PRISM 7 (Graphpad Software, Inc.).

Data availability. All data are available from the authors upon request. The sequence data that support the findings of this study have been deposited in the NIH NCBI sequence read archive (SRA) database with the primary accession code SRP113556 and SRP096009, for V δ 2⁺ and V δ 1⁺ TCR repertoires respectively.

Received: 16 September 2017 Accepted: 3 April 2018

Published online: 02 May 2018

References

- Rast, J. P. et al. alpha, beta, gamma, and delta T cell antigen receptor genes arose early in vertebrate phylogeny. *Immunity* **6**, 1–11 (1997).
- Zheng, J., Liu, Y., Lau, Y. L. & Tu, W. gammadelta-T cells: an unpolished sword in human anti-infection immunity. *Cell. Mol. Immunol.* **10**, 50–57 (2013).
- Silva-Santos, B., Serre, K. & Norell, H. gammadelta T cells in cancer. *Nat. Rev. Immunol.* **15**, 683–691 (2015).
- Godfrey, D. I., Uldrich, A. P., McCluskey, J., Rossjohn, J. & Moody, D. B. The burgeoning family of unconventional T cells. *Nat. Immunol.* **16**, 1114–1123 (2015).
- Kisielow, J., Tortola, L., Weber, J., Karjalainen, K. & Kopf, M. Evidence for the divergence of innate and adaptive T-cell precursors before commitment to the alphabeta and gammadelta lineages. *Blood* **118**, 6591–6600 (2011).
- Wencker, M. et al. Innate-like T cells straddle innate and adaptive immunity by altering antigen-receptor responsiveness. *Nat. Immunol.* **15**, 80–87 (2014).
- Halary, F. et al. Shared reactivity of V{delta}2(neg) {gamma}{delta} T cells against cytomegalovirus-infected cells and tumor intestinal epithelial cells. *J. Exp. Med.* **201**, 1567–1578 (2005).
- Willcox, C. R. et al. Cytomegalovirus and tumor stress surveillance by binding of a human gammadelta T cell antigen receptor to endothelial protein C receptor. *Nat. Immunol.* **13**, 872–879 (2012).
- Hudspeth, K. et al. Engagement of NKp30 on Vdelta1 T cells induces the production of CCL3, CCL4, and CCL5 and suppresses HIV-1 replication. *Blood* **119**, 4013–4016 (2012).
- Correia, D. V. et al. Differentiation of human peripheral blood Vdelta1 +T cells expressing the natural cytotoxicity receptor NKp30 for recognition of lymphoid leukemia cells. *Blood* **118**, 992–1001 (2011).
- Davey, M. S. et al. Clonal selection in the human Vdelta1 T cell repertoire indicates gammadelta TCR-dependent adaptive immune surveillance. *Nat. Commun.* **8**, 14760 (2017).
- Ravens, S. et al. Human gammadelta T cells are quickly reconstituted after stem-cell transplantation and show adaptive clonal expansion in response to viral infection. *Nat. Immunol.* **18**, 393–401 (2017).
- Morita, C. T., Jin, C., Sarikonda, G. & Wang, H. Nonpeptide antigens, presentation mechanisms, and immunological memory of human Vgamma2Vdelta2 T cells: discriminating friend from foe through the recognition of prenyl pyrophosphate antigens. *Immunol. Rev.* **215**, 59–76 (2007).
- Riganti, C., Massaia, M., Davey, M. S. & Eberl, M. Human gammadelta T-cell responses in infection and immunotherapy: common mechanisms, common mediators? *Eur. J. Immunol.* **42**, 1668–1676 (2012).
- Rhodes, D. A. et al. Activation of human gammadelta T cells by cytosolic interactions of BTN3A1 with soluble phosphoantigens and the cytoskeletal adaptor periplakin. *J. Immunol.* **194**, 2390–2398 (2015).
- Sandstrom, A. et al. The intracellular B30.2 domain of butyrophilin 3A1 binds phosphoantigens to mediate activation of human Vgamma9Vdelta2 T cells. *Immunity* **40**, 490–500 (2014).
- Harly, C. et al. Key implication of CD277/butyrophilin-3 (BTN3A) in cellular stress sensing by a major human gammadelta T-cell subset. *Blood* **120**, 2269–2279 (2012).
- Costa, G. et al. Control of Plasmodium falciparum erythrocytic cycle: gammadelta T cells target the red blood cell-invasive merozoites. *Blood* **118**, 6952–6962 (2011).
- Davey, M. S. et al. Microbe-specific unconventional T cells induce human neutrophil differentiation into antigen cross-presenting cells. *J. Immunol.* **193**, 3704–3716 (2014).
- Tyler, C. J., Doherty, D. G., Moser, B. & Eberl, M. Human Vgamma9/Vdelta2 T cells: Innate adaptors of the immune system. *Cell. Immunol.* **296**, 10–21 (2015).
- Davodeau, F. et al. Peripheral selection of antigen receptor junctional features in a major human gamma delta subset. *Eur. J. Immunol.* **23**, 804–808 (1993).
- Parker, C. M. et al. Evidence for extrathymic changes in the T cell receptor gamma/delta repertoire. *J. Exp. Med.* **171**, 1597–1612 (1990).
- Carding, S. R. & Egan, P. J. Gammadelta T cells: functional plasticity and heterogeneity. *Nat. Rev. Immunol.* **2**, 336–345 (2002).
- Pauza, C. D. & Cairo, C. Evolution and function of the TCR Vgamma9 chain repertoire: It's good to be public. *Cell Immunol.* **296**, 22–30 (2015).
- Dimova, T. et al. Effector Vgamma9Vdelta2 T cells dominate the human fetal gammadelta T-cell repertoire. *Proc. Natl Acad. Sci. USA* **112**, E556–E565 (2015).
- De Rosa, S. C. et al. Ontogeny of gamma delta T cells in humans. *J. Immunol.* **172**, 1637–1645 (2004).
- McVay, L. D., Carding, S. R., Bottomly, K. & Hayday, A. C. Regulated expression and structure of T cell receptor gamma/delta transcripts in human thymic ontogeny. *EMBO J.* **10**, 83–91 (1991).
- Tamura, N. et al. Diversity in junctional sequences associated with the common human V gamma 9 and V delta 2 gene segments in normal blood and lung compared with the limited diversity in a granulomatous disease. *J. Exp. Med.* **172**, 169–181 (1990).
- Wang, H., Fang, Z. & Morita, C. T. Vgamma2Vdelta2 T Cell Receptor recognition of prenyl pyrophosphates is dependent on all CDRs. *J. Immunol.* **184**, 6209–6222 (2010).
- McVay, L. D., Jaswal, S. S., Kennedy, C., Hayday, A. & Carding, S. R. The generation of human gammadelta T cell repertoires during fetal development. *J. Immunol.* **160**, 5851–5860 (1998).
- Sherwood, A. M. et al. Deep sequencing of the human TCRgamma and TCRbeta repertoires suggests that TCRbeta rearranges after alphabeta and gammadelta T cell commitment. *Sci. Transl. Med.* **3**, 90ra61 (2011).
- Venturi, V., Price, D. A., Douek, D. C. & Davenport, M. P. The molecular basis for public T-cell responses? *Nat. Rev. Immunol.* **8**, 231–238 (2008).
- Ryan, P. L. et al. Heterogeneous yet stable Vdelta2(+) T-cell profiles define distinct cytotoxic effector potentials in healthy human individuals. *Proc. Natl Acad. Sci. USA* **113**, 14378–14383 (2016).
- Vermijlen, D. et al. Human cytomegalovirus elicits fetal gammadelta T cell responses in utero. *J. Exp. Med.* **207**, 807–821 (2010).
- Chien, Y. H., Meyer, C. & Bonneville, M. gammadelta T cells: first line of defense and beyond. *Annu. Rev. Immunol.* **32**, 121–155 (2014).
- Bonneville, M., O'Brien, R. L. & Born, W. K. Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity. *Nat. Rev. Immunol.* **10**, 467–478 (2010).
- Pitard, V. et al. Long-term expansion of effector/memory Vdelta2-gammadelta T cells is a specific blood signature of CMV infection. *Blood* **112**, 1317–1324 (2008).
- Klarenbeek, P. L. et al. Deep sequencing of antiviral T-cell responses to HCMV and EBV in humans reveals a stable repertoire that is maintained for many years. *PLoS Pathog.* **8**, e1002889 (2012).
- Palakodeti, A. et al. The molecular basis for modulation of human Vgamma9Vdelta2 T cell responses by CD277/butyrophilin-3 (BTN3A)-specific antibodies. *J. Biol. Chem.* **287**, 32780–32790 (2012).
- Salim, M. et al. BTN3A1 Discriminates gammadelta T cell phosphoantigens from nonantigenic small molecules via a conformational sensor in Its B30.2 domain. *ACS Chem. Biol.* **12**, 2631–2643 (2017).
- McVay, L. D., Hayday, A. C., Bottomly, K. & Carding, S. R. Thymic and extrathymic development of human gamma/delta T cells. *Curr. Top. Microbiol. Immunol.* **173**, 57–63 (1991).

42. McVay, L. D. & Carding, S. R. Extrathymic origin of human gamma delta T cells during fetal development. *J. Immunol.* **157**, 2873–2882 (1996).
43. Koay, H. F. et al. A three-stage intrathymic development pathway for the mucosal-associated invariant T cell lineage. *Nat. Immunol.* **17**, 1300–1311 (2016).
44. Keller, A. N. et al. Drugs and drug-like molecules can modulate the function of mucosal-associated invariant T cells. *Nat. Immunol.* **18**, 402–411 (2017).
45. Gold, M. C. et al. MR1-restricted MAIT cells display ligand discrimination and pathogen selectivity through distinct T cell receptor usage. *J. Exp. Med.* **211**, 1601–1610 (2014).
46. Remmerswaal, E. B. et al. Human virus-specific effector-type T cells accumulate in blood but not in lymph nodes. *Blood* **119**, 1702–1712 (2012).
47. Hertoghs, K. M. et al. Molecular profiling of cytomegalovirus-induced human CD8+T cell differentiation. *J. Clin. Invest.* **120**, 4077–4090 (2010).
48. Jeffery, H. C. et al. Biliary epithelium and liver B cells exposed to bacteria activate intrahepatic MAIT cells through MR1. *J. Hepatol.* **64**, 1118–1127 (2016).
49. Pluschke, G., Ruegg, D., Hohlfeld, R. & Engel, A. G. Autoaggressive myocytotoxic T lymphocytes expressing an unusual gamma/delta T cell receptor. *J. Exp. Med.* **176**, 1785–1789 (1992).
50. Hohlfeld, R., Engel, A. G., Ii, K. & Harper, M. C. Polymyositis mediated by T lymphocytes that express the gamma/delta receptor. *N. Engl. J. Med.* **324**, 877–881 (1991).
51. Vermijlen, D. et al. Distinct cytokine-driven responses of activated blood gammadelta T cells: insights into unconventional T cell pleiotropy. *J. Immunol.* **178**, 4304–4314 (2007).
52. Wang, C. et al. High throughput sequencing reveals a complex pattern of dynamic interrelationships among human T cell subsets. *Proc. Natl Acad. Sci. USA* **107**, 1518–1523 (2010).
53. Han, J. et al. Simultaneous amplification and identification of 25 human papillomavirus types with Tempex technology. *J. Clin. Microbiol.* **44**, 4157–4162 (2006).
54. Yang, Y. et al. Distinct mechanisms define murine B cell lineage immunoglobulin heavy chain (IgH) repertoires. *Elife* **4**, e09083 (2015).
55. Bolotin, D. A. et al. MiXCR: software for comprehensive adaptive immunity profiling. *Nat. Methods* **12**, 380–381 (2015).
56. Shugay, M. et al. VDJtools: unifying post-analysis of T cell receptor repertoires. *PLoS Comput. Biol.* **11**, e1004503 (2015).
57. Giudicelli, V. & Lefranc, M. P. IMGT/junction analysis: IMGT standardized analysis of the V-J and V-D-J junctions of the rearranged immunoglobulins (IG) and T cell receptors (TR). *Cold Spring Harb. Protoc.* **2011**, 716–725 (2011).
58. Yousfi Monod, M., Giudicelli, V., Chaume, D. & Lefranc, M. P. IMGT/Junction analysis: the first tool for the analysis of the immunoglobulin and T cell receptor complex V-J and V-D-J junctions. *Bioinformatics* **20**, i379–i385 (2004).
59. Thomsen, M. C. & Nielsen, M. Seq2Logo: a method for construction and visualization of amino acid binding motifs and sequence profiles including sequence weighting, pseudo counts and two-sided representation of amino acid enrichment and depletion. *Nucleic Acids Res.* **40**, W281–W287 (2012).

Acknowledgements

We thank all donors and patients who participated in this study, clinical staff at UHB NHS Foundation Trust for recruitment and provision of liver samples, the AMC biobank staff for provision of renal transplant patient samples and the Anthony Nolan Cell Therapy Centre for provision of cord blood samples. We also thank Dr Matthew McKenzie and the University of Birmingham CMDS Cell Sorting Facility for isolation of $\gamma\delta$ T cells and the University of Birmingham Protein Expression Facility for use of their facilities. The work was supported by a Medical Research Council Clinician Scientist award (G1002552 to Y.H.O.), Russian Foundation for Basic Research grant (17-04-01994 to S.A.K. and 17-54-10018 KO to D.M.C.), Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601 to D.M.C.) and Wellcome Trust Investigator award funding, supporting M.S.D., C.R.W., F.M. and M.S. (099266/Z/12/Z to B.E.W.).

Author contributions

M.S.D., C.R.W., B.E.W. conceived and designed experiments; M.S.D. and C.R.W. performed experiments and analysed data; S.A.K. and D.M.C. analysed deep sequencing data; S.H. and Y.H.O. provided liver tissue samples and data analysis; E.B.R. and F.J.B. provided kidney transplant patient samples and critical discussions; M.S. and F.M. provided assistance with single-cell TCR sequencing and critical discussions; B.E.W. supervised the project; M.S.D., C.R.W. and B.E.W. wrote the manuscript and all authors provided critical review.

Additional information

Supplementary Information accompanies this paper at <https://doi.org/10.1038/s41467-018-04076-0>.

Competing interests: The authors declare no competing interests.

Reprints and permission information is available online at <http://npg.nature.com/reprintsandpermissions/>

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2018