Innate and adaptive γδ T cells: how, when, and why

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Running title:

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Abstract

γδ T cells comprise the third cell lineage of lymphocytes that use, like αβ T cells and B cells, V(D)J gene rearrangement with the potential to generate a highly diverse T cell receptor (TCR) repertoire. There is no obvious conservation of γδ T cell subsets (based on TCR repertoire and/or function) between mice and human, leading to the notion that human and mouse γδ T cells are highly different. In this review we focus on human γδ T cells, building on recent studies using high-throughput sequencing to analyze the TCR repertoire in various settings. We make then the comparison with mouse γδ T cell subsets highlighting the similarities and differences and describe the remarkable changes during lifespan of innate and adaptive γδ T cells. Finally, we propose mechanisms contributing to the generation of innate versus adaptive γδ T cells. We conclude that key elements related to the generation of the γδ TCR repertoire and γδ T cell activation/development are conserved between human and mice, highlighting the similarities between these two species.

Keywords: gammadelta; TCR repertoire; fetus; newborn; infant; human; short homology repeat; Lin28b

1. Introduction

T lymphocytes, αβ and γδ T cells, together with B lymphocytes, use V(D)J gene rearrangement with the potential to generate a set of highly diverse receptors to recognize antigens. These three cell lineages have been conserved seemingly since the emergence of jawed vertebrates, more than 450 million years ago (1), while a similar tripartite subdivision exists even in jawless vertebrates such as lamprey and hagfish (2,3). γδ T cells express somatically diversified T cell receptors (TCR, or TR according to LeFranc (4)) composed of a γ-chain (TRG) and a δ-chain (TRD), which were accidentally identified while characterizing the αβ TCR genes (5–7). Unlike αβ T cells, γδ T cells are not limited to recognition of peptides presented by MHC proteins, lipids presented by CD1, and metabolites presented by MR1, although this does not exclude such reactivities from their repertoire (8–11). A variety of valuable contributions to the immune system have been described for γδ T cells, including lysis of infected and stressed cells, cytokine production, dendritic cell maturation, priming of αβ T cells via antigen presentation, tissue regulation and B cell help (12).

γδ T cells are the first T cells to develop in all species studied to date(10). The TCR gene rearrangement usually occurs in the thymus where single V (variable), D (diversity; only for TRD), and J (joining) gene segments are joined to form a final chain. The variability created during the V(D)J recombination is significantly enhanced by the junctional diversity which comprises: 1) incorporation of palindromic sequences (“P nucleotides”); 2) the introduction of additional random nucleotides (“N additions”) in the junction by the terminal deoxynucleotidyl transferase (TdT enzyme); and 3) deletion of nucleotides (by exonuclease)(13). The pairing of a single TRG with a TRD chain will give rise to the final TCR expressed on the surface of the γδ T cell. The most variable domain, usually accountable for antigen recognition, is found in the complementarity determining region 3 (CDR3) and is the region most often analyzed.

What sets γδ T cells apart from conventional αβ T cells is their innate ability to respond rapidly to stress despite expressing a TCR, a hallmark of adaptive immunity. Such responsiveness permits those cells to contribute to lymphoid stress surveillance, with implications for tumor immunology, allergy and inflammation (14). The functional potential of γδ T cells is often developmentally pre-programmed rather than taking the time to differentiate anew in the periphery as for conventional αβ T cells (14,15). Together with other innate-like lymphocyte populations, such as invariant Natural Killer T (iNKT) and mucosal-associated invariant T (MAIT) cells, γδ T cells straddle innate and adaptive immunity harboring mainly restricted TCR repertoires. However, polyclonal TCR repertoires have been detected among γδ T cells and oligoclonal expansions have been observed, pointing towards an adaptive immune response in human (16,17).

Apart from human studies, γδ T cells have been studied extensively in animal models including mice, chicken, dogs, pigs, sheep, cattle, alpaca, dolphins, monkeys (18–23). The γδ TCR repertoire and function is not universal among the different species (18,24,25). Here we focus on human γδ T cells, building on recent studies that used high-throughput sequencing (HTS) to analyze the TCR repertoire in various settings. We make then the comparison with mouse γδ T cell subsets highlighting the similarities and differences that distance or bridge the biology of the most studied experimental species to that of human. We review the γδ TCR repertoire and function of γδ T cells, highlight the remarkable changes during lifespan and propose potential mechanisms contributing to the generation of innate versus adaptive γδ T cells.

2. γδ T cell subsets

Not long after their discovery, γδ T cells have been divided into subsets based on the type of Vγ (TRG) and/or Vδ (TRD) chain they express. Human γδ T cells have been traditionally divided into Vδ2+ and Vδ2- cells, with the Vδ1+ cells being the main contributor to the Vδ2- subset. More recently however, as detailed below, it became clear that a division between Vγ9Vδ2 and nonVγ9Vδ2 γδ T

cells may be more appropriate. Mouse γδ T cells in contrast are usually separated on the type of Vγ chain they express in their TCR. The division according to V usage is usually associated with enrichment in different tissue locations and/or certain effector functions. Since the human and mouse V gene segment are not showing clear homology (24,26), the human and mouse γδ subset division is discrete, and we discuss human and mouse γδ T cells separately in this section. While such a division strengthens the notion that human and mouse γδ T cells are highly distinct (27), we will discuss in the following sections (sections 3, 4 and 5) that key elements in γδ T cell biology are highly conserved between mice and humans. Note that we use the TRG and TRD nomenclature from the international ImMunoGeneTics information system (IMGT), the global reference in immunogenetics and immunoinformatics, throughout this paper (4,25).

When it comes to describing the TCR repertoire of the different γδ subsets several terms are broadly used. We include here a list of characterizations (Figure 1), where we briefly explain the meaning of each term. Germline-encoded sequences are sequences lacking N additions, where all nucleotides transcribed are template, coming from the initial pre-arranged DNA.

2.1 Human γδ T cell subsets

Human blood γδ T cells represent a minority compared to αβ T cells, accounting usually for <10% of circulating T lymphocytes (28–30). Similar levels of γδ T cells are found in the organized lymphoid tissues while higher frequencies have been observed in tissues such as gut and liver (1,31–33) (Table 1). Substantial expansions of blood γδ T cells have been described in response to invading pathogens such as tuberculosis and toxoplasma (34–37). In human, the TRD locus (14q11.2) contains eight variable genes (TRDV1, TRDV2, TRDV3, TRAV14/DV4, TRAV23/DV6, TRAV29/DV5, TRAV36/DV7, TRAV38-2/DV8), three diversity genes (TRDD1–3), four joining genes (TRDJ1–4) and one constant TRDC gene. The TRG locus (7p14) contains six functional variable genes (TRGV2–5, TRGV8, and TRGV9), five joining genes (TRGJ1, J2, JP, JP1, JP2), and two constant genes (TRGC1–2)(4). Each locus

rearranges using a single constant, variable, joining and (sometimes multiple) diversity gene segments to give rise to the TRD and TRG chains that form the final γδ TCR.

The pairing of the TRD chain containing a TRDV2 gene segment with the TRG chain containing the TRGV9 gene segment creates the Vγ9Vδ2 T cell subset, the main γδ population in the adult peripheral blood. These cells are reactive to phosphoantigens (phosphorylated metabolites of eukaryotic cells and microbes) and are strong IFNγ and granzyme producers (28,29). (E)-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate (HMBPP), produced by (pathogenic) bacteria and parasites of the phylum Apicomplexa (*Toxoplasma gondii, Plasmodium*), is the most potent natural phosphoantigen known (38). Of note, HMBPP is also generated in plant cells, more particularly in the plastids, that are likely derived from once free-living bacteria by endosymbiosis (39). This may contribute to the Vγ9Vδ2 T cell-activation potential of herbal drugs (40). Vγ9Vδ2 T cells are known for their antimicrobial activity and their anticancer potential is being exploited in several clinical trials (28,41,42).

Vγ9Vδ2 T cells appear to be the prototypic innate γδ T cell subset in human expressing a semi-invariant TCR which is activated by phosphoantigens. Different members of the butyrophilin family, most notably BTN3A1, BTN3A2 and BTN2A, play a crucial role in this reactivity towards phosphoantigens, with BTN3A1 acting as a phosphoantigen sensor (43–50). The 'innate' character of Vγ9Vδ2 T cells in the adult is highlighted by the presence of a public (shared among individuals) CDR3 TRGV9 sequence with multiple individuals showing broad overlap in their repertoire and a highly restricted TRGV9 repertoire length distribution (51,52). The CDR3 TRDV2 chain has a much more private repertoire compared to the CDR3 TRGV9 and shows a broader length distribution. This difference between TRG and TRD chain in the Vγ9Vδ2 TCR is maybe designed for distinct roles in their phosphoantigen-induced reactivity. Indeed, two groups have recently identified BTN2A as a ligand for the Vγ9 chain, independent from the Vδ2 chain (44,45). The role of the CDR3 of the Vγ9

chain in the BTN-interactions is unclear while the germline-encoded region between CDR2 and CDR3 of the Vγ9 chain is important for the interaction between the Vγ9Vδ2 TCR and BTN2A1 (45,46). Although the CDR3 TRGV9 is not involved in the BTN2A1 interaction, the striking length restrictions of the CDR3γ of the phosphoantigen-reactive Vγ9Vδ2 TCR might allow the correct positioning of the different domains and loops of the TCR in a spatial manner in order to interact with the complex formed by the BTN proteins together with the hitherto unidentified TRGV9 CDR3 interaction partner (44–46). Also the CDR3 TRDV2 chain is crucial but its interaction partner is not yet defined (44–46,51). Based on what we know so far on the activation mode of Vγ9Vδ2 T cells by phosphoantigens, it seems that a single TCR would provide both innate and adaptive recognition based on the TCR region involved, which has been termed 'adaptate' recognition (10).

The difference in CDR3 length and increased diversity of the CDR3 Vδ2 between early and later life (see section 3) leaves open the possibility of higher or lower affinity with optimal and suboptimal CDR3s regarding phosphoantigen reactivity (29,52).

Human γδ T cells other than the phosphoantigen-reactive Vγ9Vδ2 subset, can be grouped together as 'nonVγ9Vδ2 T cells'. In contrast to adult Vγ9Vδ2 T cells, it appears that a common feature of adult nonVγ9Vδ2 T cells is their adaptive characteristics in their TCR repertoire (16,17). NonVγ9Vδ2 T cells consist mainly of Vδ1 and Vδ3 chains pairing with various Vγ chains. These subsets thus do not recognize phosphoantigens and for some γδ T cell subsets or clones antigen-reactivities have been identified (for recent extensive reviews on γδ T cell ligands see (53,54)). Vδ1 T cells have shown specific expansions following infections, such as CMV, responding to cellular dysregulation (16,55,56). They are also employed in anti-cancer strategies especially after targeted polyclonal expansions (DOT cells)(57–59). Blood Vδ1 γδ T cells show adaptive characteristics with a diverse and private repertoire (different in each individual) which becomes focused after preferential expansion of reactive clonotypes (16,17).

Vδ2 pairing with Vγ chains other than Vγ9 is quite rare in the adult blood and have been shown to possess a highly private TRG repertoire (29,60–62). Very recently, Kaminski and colleagues showed a

direct, specific, and TCR-dependent recognition of CMV-infected cells by Vγ9-Vδ2+ γδ T cells in kidney transplant recipients with CMV seropositivity (63). Thus, these cells seem to offer adaptive responses reminiscing of Vδ1 γδ T cells.

Apart from the difference in the adult blood TCR repertoire, the distinct function of Vδ2 (Vγ9Vδ2) versus Vδ1 γδ T cells was assessed at single cell level with Vδ1 resembling more NK cells while Vδ2 resembling CD8 αβ T cells (64).

The γδ TCR repertoire in human tissues is often different from that of blood which we summarize here for adult liver, gut, skin, lung, breast, and decidua tissues (Table 1). The human adult liver is enriched for γδ T cells compared to blood with an average of around 12% of total lymphocytes expressing the γδ TCR (33). It mainly harbors nonVγ9Vδ2 γδ T cell populations (Vδ1, Vδ3) with both circulating and tissue-resident TCRs with expanded and private repertoires(33). Similarly, Vδ1 and Vδ3 are the main populations in the human adult gut with private repertoires containing high levels of N additions (65–67). γδ T cells are especially enriched in the intestinal epithelium (ranging from 8 to 38% of total lymphocytes depending on the study (32,68–70)) while in the lamina propria their frequency is significantly lower (68,70) (Table 1). Both liver and gut γδ TCR repertoires have been described as oligoclonal and private possibly indicating expansion upon antigen-encounter (33,65,71). The main Vγ chain used in healthy human intestine is the Vγ4 followed by Vγ3 and Vγ8, all members of the subgroup 1 of Vγ chains (Vγ9 is the only member of the Vγ 2 subgroup)(71,72). Like Vγ9Vδ2 T cells, the Vγ4+ TCR response is dependent on a BTN-butyrophilin-like (BTNL) protein complex, this time on the dimer formed by BTNL3 and BTNL8 (73). Regions outside the CDR3 of the Vγ4 chain (HV4) bind to BTNL3, while the CDR3 itself can interact with a clonally restricted autoantigen (EPCR), thus reflecting a dual character of the γδ TCR with either innate or adaptive properties depending on spatially distinct regions of the TCR (45,72,74).

Daniels et al recently performed an analysis of the γδ TCR repertoire in the different parts of the human skin under steady-state conditions (75) (Table 1). The outer layer of healthy skin (epidermis

and dermis) is dominated by nonVγ9Vδ2 γδ T cells, with more than half of γδ T cells being Vδ1 (around 54% in dermis; 85% in epidermis are Vδ1+) while Vδ2 T cells are absent. However, in the subcutaneous adipose tissue around 80% of γδ T cells are Vδ2+ and the remaining are Vδ1-. Interestingly, none of the Vδ2+ γδ T cells paired with the Vγ9 chain as it broadly occurs in the periphery (75) and thus could represent enrichment of the above described Vγ9-Vδ2+ γδ T cells. In the case of cutaneous lymphomas (not studied in healthy skin), both Vδ1 and Vδ2 subsets showed preferential pairings with the Vγ3 chain, which might be the counterpart of the intestinal Vγ4 chain (71,73,75). Previous spectratyping and Sanger nucleotide sequencing on healthy human skin showed clonally expanded γδ T cells (Vδ1 and Vδ2) broadly distributed and distinct from the paired peripheral blood (76). The dedicated epidermal and dermal skin Vδ1 TCR repertoire might be linked to wound healing properties of skin-resident Vδ1 γδ T cells(77).

The abundance of γδ T cells in the human lung under steady-state conditions is not clear, while in tuberculosis lesions there is an enrichment for Vδ1 γδ T cells (78). Based on Sanger nucleotype sequences, the CDR3 of TRGV9 containing sequences appears less diverse than that of the blood with more identical junctional regions (79).

In healthy human breast, the majority of γδ T cells are tissue-resident Vδ1+ with cytolytic properties and cytokine potential (IFNγ, TNFα etc.)(80). This innate-like population has a restricted repertoire with a few expanded clones in normal tissue (80). Finally, during pregnancy, human decidua is relatively enriched for γδ T cells (10-16% of T cells) mainly expressing a diverse Vδ1 TCR repertoire as analyzed by TCR spectratyping (81). Of note, the obtainment and characterization of lymphocytes from healthy human tissues can be confounded by irreproducible yields and low cell viability, explaining the sometimes variable results between different studies (Table 1). Wu et al has used a "grid" explant culture to overcome this in analyzing γδ T cells from healthy breast (80). Alternatively, the frequency of γδ T cells within healthy human tissues can be analyzed by immunohistochemistry, confirming the highest frequency in gut (colon), while the breast showed intermediate frequencies; ovarian and pancreatic tissue sections showed only very low (or absent) γδ T cell frequencies (82).

Thus overall, human γδ T cells in the adult can be divided into the phosphoantigen-reactive innate-like Vγ9Vδ2 T cells that are enriched in peripheral blood and the adaptive-like nonVγ9Vδ2 γδ T cells that are mainly enriched in solid tissues. The human intestinal epithelium appears to possess the highest percentage of γδ T cells, followed by decidua and liver.

2.2 Mouse γδ T cell subsets

γδ T cells have been extensively studied in mouse models comprising innate-like lymphocytes with pre-programmed functions. There are no obvious homologies between mouse and human TCR genes (24,26). For example, the well-studied innate-like γδ T cells found in the mouse skin epidermis (Vγ5Vδ4 in the IMGT nomenclature (4), commonly known as Vγ5Vδ1 cells according to Heilig and Tonegawa (83)) do not exist in human while the abundant human phosphoantigen-reactive Vγ9Vδ2 T cell population does not exist in rodents (28,84). In mouse, the TRA/TRD locus (14C2) contains fifteen (to sixteen) variable genes (TRDV1, TRDV2-1, TRDV2-2, TRDV4, TRDV5, TRAV15D-1/DV6D-1, TRAV15-1/DV6-1, TRAV15D-2/DV6D-2, TRAV15-2/DV6-2, TRAV15N-1, TRAV13-4/DV7; TRAV14D-3/DV8, TRAV6-7/DV9, TRAV4-4/DV10, TRAV16D/DV11, TRAV21/DV12), two diversity genes (TRDD1, TRDD2), two joining genes (TRDJ1, TRDJ2) and one constant TRDC gene that are used in the γδ TCR (25,85). The TRG locus (13A2) contains seven variable genes (TRGV1–7), four joining genes (TRGJ1-4), and three functional constant genes (TRGC1–2, TRGC4)(4). Mouse γδ T cells are usually divided into subsets based in the Vγ chain distribution which coincides with their tissue localization (Table 1) and function.

There are characteristic tissue-resident populations acting as barriers forming a first line of defense such as the epidermis. The dendritic epidermal T cells (DETCs) are intraepithelial γδ T cells expressing an invariant Vγ5Vδ4 TCR (IMGT nomenclature, previously described as Vγ5Vδ1; Table 2) and displaying a critical role in regulation of cutaneous malignancy (e.g. via the production of IL13) and in wound repair (via the production of keratinocyte growth factor) (86–89).

Another invariant γδ T cell population in the mouse is the Vγ6Vδ4 (IMGT nomenclature, previously described as Vγ6Vδ1) T cell subset which reside in the dermis, lung and uterus, and are known for their efficient IL17 production (28,90,91). Of note, the TRGV6-containining CDR3 sequences contain exactly the same CDR3 sequences present in the epidermal-resident Vγ5Vδ4 subset (Table 2). Furthermore, like in Vγ5Vδ4 T cells the TRDV4 gene segment is used and the same CDR3 is formed in the TRD chain as the one generated in Vγ5Vδ4 T cells (Table 2). Thus, despite these high similarities between these two TCRs, they are located at different tissue sites in the body and possess different effector functions (92–94). Vγ4 T cells are found in the lung, blood, spleen, and lymph nodes with overall polyclonal TCR repertoires and containing small subsets with several (semi-)invariant TCRs (Table 2) (92,95). Finally, Vγ1 T cells are found in the blood, spleen and lymph nodes with diverse TCR repertoires as the gut-resident Vγ7 γδ T cells (95–97).

3. When: Development of the γδ TCR repertoire in waves

γδ T cells are the first T lymphocytes generated in human and mouse and, as detailed below, in both species a pattern is observed of (semi-)invariant γδ T cell generation in early life (as a 'wave') followed by the production of a polyclonal γδ TR repertoire.

In human, γδ T cells are the most abundant lymphoid population in the embryonic thymus in early gestation, with a shift around gestation week 11 when they decrease significantly and the αβ T cells take the lead (98). The very first γδ T cell population to arise in human is the Vγ9Vδ2 subset, detected in embryonic (pre-thymic) liver from as early as 5–6 weeks gestation(99), and in fetal thymus after 8 weeks of gestation (52,100). These Vγ9Vδ2 T cells likely exit the fetal thymus to the peripheral blood, as the fetal blood at 20-30 wk of gestation is dominated by Vγ9Vδ2 T cells possessing a semi-invariant TCR (Table 2)(52,101). In the fetal intestine (around 20 wk of gestation), Vδ2 γδ T cells with a limited TCR diversity are the major population in contrast to adult intestine which is enriched for

Vδ1 (and Vδ3) γδ T cells with a diverse TRD repertoire (65,70). Some TCR sequences detected in early fetal liver and thymus are shared with those from the fetal intestine suggesting that the latter is inhabited by an early wave of fetal semi-invariant TRDV2 γδ TCRs to be later stepped away by post-natal diverse TRDV1 TCRs (65,99,102,103). This hypothesis could be verified by HTS of the TCR repertoires of fetal and infant/adult intestine-derived γδ T cells. In a similar fashion, the first wave of γδ T cells in the mouse is the invariant Vγ5Vδ4 population (Table 2), that arises at embryonic day 13.5 and home to skin epidermis to form the DETC (104–106). While Vγ9Vδ2 T cells and Vγ5Vδ4 T cells are the first T cells generated in human and mouse respectively, there appear to be clear differences in their maintenance in later life. Mouse Vγ5Vδ4 cells are only generated in the fetal thymus and are maintained in the skin epidermis as DETC until adulthood by clonal self-renewal (93,107). In striking contrast, human Vγ9Vδ2 T cells are generated anew later in life possessing more variable TCRs than the fetal counterparts and are the main source of Vγ9Vδ2 T cells in the adult peripheral blood (Figure 2) (52). Indeed, we have recently shown that fetal and adult Vγ9Vδ2 T cells are generated by the thymus at different timepoints in life (52). We identified key differences in the γδ T cell repertoire of fetal and adult blood Vγ9Vδ2 T cells, including in features important for phosphoantigen-reactivity (52). Fetal Vγ9Vδ2 TCRs contained very few N additions and were widely public among individuals at the fetal stage (Figure2). This was in contrast to a private adult TRD repertoire, with high number of N additions and characteristic usage of TRDJ region (J1 instead of the fetal-like J2 and J3). The highly prevalent fetal nucleotype (Table 2), encoding CALWEVQELGKKIKVF by short homology repeat recombination (see also section 5), represents around 40% of the fetal Vγ9Vδ2 T cell repertoire while in the adult this nucleotype represents only around 8% of the sorted Vγ9Vδ2 T cells (52). Notwithstanding, in the adult there are other nucleotypes, with N additions, encoding this clonotype as well as other public TRGV9 clonotypes by convergent recombination (52,108). Next, we saw that expansion of phosphoantigen-reactive fetal blood Vγ9Vδ2 T cells does not lead to an adult-type TCR repertoire. Consequently, we analyzed Vγ9Vδ2 thymocytes from both fetal and post-natal biopsies and observed that most of these key adult Vγ9Vδ2 TCR features were

already present in the postnatal thymus and were further enhanced upon selection by the microbial-derived phosphoantigen HMBPP. Thus, even though the human post-natal thymus generates mainly Vδ1 γδ T cells, Vγ9Vδ2 T cells are still newly made with high variability in the junctions (52).

This post-natal production of innate Vγ9Vδ2 T cells maybe be related to the much longer lifespan of human compared to mouse. The difference in gestation may also influence findings related to the functional thymic output during development when comparing humans to other species, even though thymus organogenesis is in general comparable (109).

What drives the generation of distinct Vγ9Vδ2 T cells populations has been intriguing. The nature of the precursor cell (see also section 5) seems to define the type of TCR of the thymocyte rather than the intrathymic exposure to phosphoantigens (52). However, a potential role of the BTN3A1 and BTN2A1 molecules is not excluded, as they are conserved in vertebrates, but not rodents, concomitant to the TRGV9 and TRDV2 genes (84). Interestingly, the number of N additions used by TRDV2 CDR3s is lower than that of TRDV1, in all stages of life (29,110). This might be linked to some predisposition to innateness for the Vγ9Vδ2 T cells compared to the adaptive-like Vδ1 γδ T cells.

The second wave of γδ T cells in human are the Vδ2+Vγ9- γδ T cells (pairing with other Vγ chains, not reactive to phosphoantigens) which is the major population generated in mid-gestation thymus and, in contrast to the (small subset of) post-natal Vδ2+Vγ9- thymocytes, is enriched for certain germline-encoded TRDV2- and TRGV8-containing CDR3 sequences (Table 2) and contain programmed effector functions (110). While it is a minor population in human adult blood (29,62), recently Daniels and co showed that the subcutaneous fat adipose tissue is highly enriched for Vδ2+Vγ9- T cells (75), and are thus a possible tissue target for this second thymic wave. The second wave in the mouse consists of the invariant Vγ6Vδ4 population appearing at embryonic day 16 and seed fat tissue, reproductive tract, lung, tongue and skin dermis, and are known for their potent IL17 production (91,111,112).

Another wave has been described in the mouse that is generated around birth that contains a semi-invariant Vγ1Vδ6 TCR (Vδ6: TRAV15-1-DV6-1 according to IMGT, Table 2), described as NKT-like γδ

cells as they are capable of producing both IL4 and IFNγ like NKT αβ cells (113–115). Such NKT-like γδ cells have not been described in human.

Together with the Vγ9-Vδ2+ T cells, moderate levels of innate Vδ1+ are generated in the human fetal thymus that differ also from the adaptive ones which are massively generated post-partum (110). At birth, blood nonVγ9Vδ2 T cells are phenotypically naïve while in the adult they show phenotypical (and functional) maturation (17,61). However, when looking at the population in the fetus and its generation in the fetal thymus, we see that they exert programmed functions, a feature unique to the fetal-like γδ T cells and absent in the post-natal counterparts (110). So, the nonVγ9Vδ2 T cells show an innate behavior before birth which fades with advancing age.

In later developmental stages, variable TCRs start to appear with higher usage of N additions. In human cord blood, Vδ1 T cells are highly present with relative variability which increases postnatally (17). The human post-natal thymus is dominated by a diverse TRDV1 repertoire while the smaller population of TRDV8 increases compared to fetal thymus (110,116). In mouse, the Vγ1 and Vγ4 appear with variable repertoires which can be generated during neonatal as well as adult life (93).

4. Why: Role of innate and adaptive γδ T cells

We have previously shown the abundance and programmed function of Vγ9Vδ2 T cells in the fetal blood highlighting the innateness of this γδ T cell subset in human (101). Despite the cytokine production observed by the fetal blood Vγ9Vδ2, they remain hyporesponsive compared to adult Vγ9Vδ2 T cells with an increased phosphoantigen-activation thresholds in vitro (101). Towards term gestation, neonatal Vγ9Vδ2 T cells become less abundant in the fetal blood circulation (52,101). Interestingly, early after birth, following imminent environmental exposure, fetal-derived Vγ9Vδ2 T

cells expand and gain crucial cytotoxic potential consisting of a ready-to-fight army, very much needed in early post-natal life (Figure 2)(29). The increase of the Vγ9Vδ2 population in the first months of life compared to birth is not due to recent thymic immigrants but to fetal-derived cells that have now encountered various microbial cues (29,117). These microbial cues may include phosphoantigen-producing commensal microbiota (38,118) which might be activating the neonatal Vγ9Vδ2 T cells in preparation for the host to rapidly mount immune responses upon pathogen encounter. The polyclonal response of Vγ9Vδ2 T cells to phosphoantigens goes in line with the innate character attributed to Vγ9Vδ2 T cells and intrathymic programming (see also section 5) (29,117,119). However, these cells still evolve in the periphery adapting on environmental signals they encounter (29,117). Such signals can already act in utero, as Vγ9Vδ2 T cells expand strongly upon congenital infection with the phosphoantigen-producing parasite Toxoplasma gondii (120) (Figure 2). This expansion is associated with differentiation towards potent cytotoxic effector cells as observed after birth upon environmental exposure (29,120) (Figure 2).

In contrast to our observations in congenital Toxoplasma infection, Cairo et al observed rather a depletion of phosphoantigen-reactive Vγ9Vδ2 T cells in placental malaria (121). A main difference between congenital Toxoplasma infection and placental malaria is that the malaria parasite very rarely crosses the placenta into the fetal circulation to establish an infection (122). Furthermore, the severity of placental malaria infection can have opposing effects on the immune system in early life, thus possibly contributing to the differential effect on the fetal Vγ9Vδ2 T cells (121,122). A well-defined characterization of placental malaria infection and a large sample size are probably needed to dissect the potentially diverse effects of maternal malaria on fetal Vγ9Vδ2 T cells.

Taken together, we propose that the first wave of T cells made by the human fetus, the Vγ9Vδ2 T cell subset, provides protection of the human fetus and newborn against infections in utero and early after birth. This does not exclude a possible immunopathological role of these cells like in conditions such as in sepsis of (premature) newborns and infants (123–125), which requires further investigation.

Follow-up of infants γδ TCR repertoire showed a remarkable stability of the repertoire (117). In infants vaccinated or not at birth with the phosphoantigen-containing BCG vaccine, the Vγ9Vδ2 T cell repertoire and function did not differ at 10 weeks of age (29). A transient effect may occur early after vaccination, but an immune equilibrium in the innate-like γδ T cells seems to settle quickly. This same state of effector function at 10 weeks in BCG- infants is probably attained by environmental encounters and microbiota establishment in the first weeks post-partum. This trend was also observed in the case of congenital T. gondii infection, where in the first days of post-natal life, the effect on the expansion and activation of (fetal) Vγ9Vδ2 T cells is greatly evident while later in life, from 2 months up to one year of age, the Vγ9Vδ2 T cells converge to a similar effector profile as the Toxo- infants (120). These observations are in line with the model of stereotypic immune system development proposed by Olin and colleagues based on a range of immune cells studied from birth until 3 years of age (note that γδ T cells were excluded in this study due to technical limitations) (126).

The dual fetal and post-natal wave of Vγ9Vδ2 T cell generation may provide different (specialized) immune players in the field. Extended analysis of the phenotype and function of early versus adult life Vγ9Vδ2 T cells showed that both populations show cytotoxic and immunomodulatory capacities, but they are not identical. For example, granzyme A is expressed at higher levels in early life in contrast to granulysin which is expressed mainly in adult Vγ9Vδ2 T cells (29). The differential response towards phosphoantigens in vitro may depict the need for tolerance especially during establishment of the microbiome. The adult Vγ9Vδ2 TCR repertoire is oligoclonal and private which is likely shaped by microbial exposure during lifespan (16,29,52,61). This adult Vγ9Vδ2 TCR repertoire may play an important role in immunosurveillance against cancer, although the most prevalent clonotypes do not necessarily represent the functional hierarchy of the clonotypes when measuring in vitro tumor-induced activation (46). Finally, Vγ9Vδ2 T cells might provide prolonged protection in

the elderly as they have been described to be resistant to senescence (127). While phosphoantigen-reactive γδ T cells do not exist in mice, the role of Vγ9Vδ2 T cells in rodents might be represented by other γδ T cells. For example, γδ T cells have been shown to play an important role against phosphoantigen-producing *Listeria monocytogenes* (Vγ6Vδ4) and Plasmodium (Vδ6.3; TRAV15-N1 according to IMGT) (38,85,128).

NonVγ9Vδ2 T cells are highly expanded upon congenital CMV infection, including a characteristic innate/public Vγ8Vδ1 TCR and the TRDV2-containing CDR3 motif that are highly prevalent in the fetal thymus (Table 2) (60,110). The TRGV8-containing CDR3 is the exact same sequence as the one that is highly enriched in the fetal thymus (CATWDTTGWFKIF) and the TRDV1-containing CDR3 (CALGELGDDKLIF) is very similar as the sequence that is highly enriched among the fetal thymic TRDV1-containing CDR3 sequences (CALGELGD*T*DKLIF) (60,110). In sharp contrast, anti-CMV reactivity in the adult is mediated by oligoclonal expansion of private clonotypes from nonVγ9Vδ2 T cells, including from Vδ1 and Vγ9-Vδ2+ T cells, and are thus rather part of the adaptive immune system (16,17,61,63). One such reactivity has been identified for a Vγ5Vδ4 TCR clonotype that was derived from one patient. Highlighting the striking private nature of this reactivity, efforts to identify the same Vγ5Vδ4 sequences and/or EPCR reactivities of other γδ TCR clonotypes failed (74).
There must be a propelling factor for a conserved extra layer of adaptive T lymphocytes on top of αβ T cells. A possibility is that the distinct biology of nonVγ9Vδ2 γδ T cells allowing clonal amplification towards diverse individual ligands (without MHC restriction) provides a much greater challenge for pathogens to evade (17,111).
In the (adult) mouse, γδ T cells have been shown to play a protective role against CMV infection in vivo (129,130). However, this role could only be revealed when other immune cells, such as αβ T cells, were absent. This indicates that CMV-reactive γδ T cells may be especially important in settings were the conventional adaptive immune system based on αβ T cells is not fully operational or biased towards tolerance such as in early life (60), or when it is compromised such as in transplant patients

(16,55). Interestingly, γδ T cells protect neonatal mice from influenza infection, which is due to the accumulation of IL17-producing γδ T cells in the neonatal lung (131). Thus, both in human and mouse, (semi-)invariant γδ T cells can play an important role against viral infections in early life.

Besides the role against infections of human innate Vγ9Vδ2 and nonVγ9Vδ2 T cells in early life, we propose that these innate cells can also perform non-immune functions. For example, the high granzyme A expression in fetal Vγ9Vδ2 T cells, without co-expression of other mediators usually associated with cytotoxic granules such as perforin or granzyme B, could serve a role adapted for fetal tissue and infant development (29,132,133). Observations related to alternative roles of γδ T cells are increasing in mouse models such as in body temperature regulation by adipose-tissue-associated γδ T cells (134,135) and brain/short-term memory development by meningeal-resident γδ T cells (136). In both mouse models, these non-immune functions appear to be dependent on IL17 produced by fetal-derived innate Vγ6+ γδ T cells. In human, programmed γδ T cells producing IL17 have not been detected. However, a range of genes described to have a role in IL17 production in γδ T cells were enriched in fetal compared to post-natal nonVγ9Vδ2 γδ thymocytes: MAF, RORC (RORG), TGFBR3 and SMAD, IL23R and IL1R, BLK, and CCR6 (110). Since the subcutaneous adipose tissue of human skin appears to be highly enriched for Vγ9-Vδ2+ cells (75), it is possible that innate IL17 producing γδ T cells could also play an important role in the thermogenesis in human, for example at birth. Such double 'immune' and 'non-immune' function attributed to the same innate immune cells may be a way for the fetus to use its energy sources in an efficient way at a time of rapid growth and tissue remodeling.

Overall, the human fetus possesses a set of innate γδ T cell subsets that show a division of labor in order to react against infections in early life: Vγ9Vδ2 T cells against (phosphoantigen-producing) parasites (and possibly bacteria) and nonVγ9Vδ2 T cells against viral (CMV) infections. In parallel, these cells can provide non-immunological functions, as recently demonstrated for innate γδ T cell subsets in mouse models. In later life, human Vγ9Vδ2 T cells remain the main γδ subset with innate

characteristics despite their post-natal development, while the nonVγ9Vδ2 T cells become now players of the adaptive immunity.

5. How: Mechanisms in the generation of innate versus adaptive γδ T cells

Despite the discrepancies in γδ TCR repertoire and γδ T cells subsets between human and mouse, our recent work has demonstrated that there are several similarities in the mechanisms that drive the generation and programming of innate γδ T cells. This includes the importance of short homology repeats in the fusion of gene segments during V(D)J recombination and the intrinsic role of hematopoietic stem and precursor cells (HSPC) caused by the high expression of the RNA binding protein Lin28b (52,110). We discuss here these findings in the context of fetal versus adult hematopoiesis, highlight the similarities between human and mouse γδ T cell generation and compare this with the generation of B1 B cells, a well-defined innate B cell subset in the mouse that is generated early in life (111,137–139).

The presence of a couple of identical nucleotides at the edge of two coding ends is proved to drive the VDJ recombination at the specific site by keeping the alluded nucleotides once in the final exon (140–142). They are called short homology repeats and the proximity of such short homology repeats in two combining coding ends is favored by the absence of N additions (142) (Figure 3). In human, the expression of TdT, the enzyme adding N additions during V(D)J recombination, is very low in fetal life, starting at around 12wk of gestation in the liver and 20wk in thymus (143). This absence or low expression is due to an active suppressive mechanism as we have recently shown that the downregulation of TdT is due to the high expression of the RNA-binding protein Lin28b in fetal HSPC (110). We propose that this active inhibition of TdT expression in developing γδ thymocytes results in no (or very low level of) N nucleotide insertions, allowing the recombination of the short homology repeats to occur (Figure 3). We have recently identified short homology repeats in several human TRGV/TRDV, TRDD and TRGJ/TRDJ gene segments that are likely contributing to the generation of invariant γδ TCR sequences in the human fetus, similar to mouse invariant γδ TCR sequences (52,110,142). The short homology repeats are highlighted in the invariant human and mouse γδ TCR

sequences in Table 2. In some cases, the short homology repeats are formed after involvement of P additions (palindromic template nucleotides) at the coding ends (52,115,140,144) (Table 2). Although not proposed in the original references, we found that the mouse Vδ5-containing CDR3 common sequence (previously called BID) (95,145), is likely driven by short homology repeats recombination both in the V-D and D-J junction following P additions (Table 2). Of note, short homology repeats have also been described to be involved in the generation of the BCR of mouse B1 cells (146). Besides its effect on the generation of the γδ TCR repertoire, we found that the source of HSPC and associated Lin28b expression plays also an important role in the acquisition effector functions in fetal γδ thymocytes (110), which we discuss further below.

Lin28b and its paralog, Lin28a, are RNA-binding proteins that are involved in many physiological and cellular processes such as metabolism (147), growth (148,149), aging (150), tissue development (151) or cancer (149,152). Although some of these actions are elicited by regulating the stability or the of different mRNA transcripts (153–155), the vast majority of them involve the control of microRNA Let-7 biogenesis (156). Regarding fetal to adult HSPC transition, it has been shown that Lin28b determines the high self-renewal potential of mouse fetal hematopoietic stem cells (157). Further downstream, Lin28b appears also to play an important role in driving the generation of fetal characteristics of cells within the erythroid (human fetal hemoglobin expression) (155,158,159), myeloid (mouse mastocytes) (160) and lymphoid lineages (human and mouse γδ T cells (110,161), mouse CD8 T cells (162), mouse B1 cells (163,164) and human regulatory T cells (165)). Furthermore, Lin28b is involved in the fetal erythroid-dominant hematopoiesis (166). While the generation of macrophages in the mouse fetus is akin to γδ T cell developmental waves (167,168), it remains to be determined whether this is also driven by Lin28b.

How Lin28b drives the generation of the functional effector program in human fetal γδ thymocytes is unclear and is probably indirect (Figure 4)(110). The transcriptomic profile of human fetal thymocytes

is particularly enriched for the transcription factor promyelocytic leukemia zinc finger (PLZF, also known as ZBTB16) (110), an important regulator of different innate lymphocytes (90). Interestingly, since PLZF has been described as a target of Let-7 in the mouse model (169), it is a main candidate to regulate functional invariant effector programming downstream of Lin28b (Figure 4). However, it remains unclear how PLZF actually promotes the generation of innate lymphocytes (90,169). Another candidate transcription factor downstream of Lin28b is Eomesodermin (EOMES) (Figure 4), that shows enriched expression in fetal γδ thymocytes (110) and is described as a Let-7 target in mouse CD8+ αβ T cells (170). Insulin Like Growth Factor 2 mRNA-Binding Protein 1 (IGF2BP1), another RNA-binding protein with similar functions as Lin28b (171–173), is also highly enriched in human fetal γδ thymocytes (110). This protein is a target of Let-7 (174,175) and, in cancer cells (176), a stabilizer of the Lin28b transcript due to its interaction with the lncRNA Lin28-AS1 (Figure 4). Furthermore, Insulin Like Growth Factor 2 mRNA-Binding Protein 3 (IGF2BP3), another member of IGF2BP RNA binding proteins and also a target of Let-7 (177), has been shown to enhance Lin28b-induced hematopoietic reprogramming in the development of mouse B1 cells (163,178) by stabilizing mRNA transcripts in a Let-7 independent way (154) (Figure 4). Finally, recent data indicate that Lin28b can even bind gene promoters (179). Thus while Lin28b overexpression results in downregulation of Let-7 during the generation of human innate γδ T cells (110), it does not exclude Let-7-independent mechanisms (Figure 4). What drives or controls the timing of Lin28b itself in the fetal immune system is not well established. A candidate is the polycomb-group gene Ezh2 that is a negative regulator of Lin28b expression in adult mouse HSPC (180) and a repressor of mouse fetal B-1–like cell phenotype acquisition (181) (Figure 4).

Another striking conservation between human and mouse is the relation between the TRDV and TRGV gene segment usage during lifespan on the one hand and their position in the TRD and TRG loci on the other hand. TRDV2, the first TRDV gene segment used in the human fetus, is most proximal to the TRDD gene segments (at the 5’ side), like the mouse TRDV4, the first gene segment used in

mouse. Likewise, TRGV9, the first gene segment used in the human fetus, is the most proximal functional TRGV gene segment to the TRGJ gene segments, just like the mouse TRG5 gene segment. Furthermore, human TRGV8 and mouse TRGV6 are located 5' of TRGV9 and TRGV5 respectively, and thus in 'second' position in order to generate the second wave of human and mouse γδ T cells. Importantly, it has been shown that the position of the TRGV5 and TRGV6 gene segments proximal to the TRGJ gene segment plays a key role for their usage in the early fetal rearrangement (182). It is likely that the same applies for the other 'early' gene segments present in the mouse TRD locus and human TRG/TRD loci, that are also located at a proximal position 5' of the TRD or TRJ gene segments. In addition, other factors may also contribute to these selected TRD/TRG gene segment usage, including localized changes in chromatin structure (183). Of note, besides its inhibitory effect on TdT enzyme expression and thus inhibition of N additions, Lin28b can also influence the TRDV usage (110). How Lin28b influences the human TRDV2 (fetal) versus TRDV1 (post-natal) usage (110) remains to be determined.

Our data regarding the nonfunctional TRGV10-TRJP1 CDR3 recombination and CDR3 data derived from T cell progenitors strongly indicate that the high prevalence of invariant γδ TCRs among human fetal γδ thymocytes does not depend on a γδ TCR-dependent signal (110). Similar conclusions are made for mouse invariant γδ T cells, based on CDR3 data obtained from transgenic mice with a nonfunctional TRGV5 gene segment (141) and in TRD gene mutant mice (140). In contrast, TCR signaling strength is known to play an important role in the functional programming in mouse γδ T cell subsets; for example a strong TCR signal leads to an IFNγ program in mouse thymocytes (90,91). Similarly, mouse B1 cell development has been described to be dependent on a strong BCR signal (184). The role of TCR signaling in the acquisition of a functional effector program in human fetal γδ thymocytes remains to be investigated. Furthermore, the interaction of TCR signal with Lin28b function in the thymic programming of mouse and human γδ thymocytes should be analyzed. In the mouse model, it has been described that besides intrinsic properties of HSPC, also the fetal

environment plays an important role in the development of innate mouse Vγ5Vδ4 T cells (104,185). In line with this, thymic epithelial expression of *Skint1*, a BTNL family member, was shown to be critical for the selective expansion and maturation of the Vγ5Vδ4 T thymocytes (186,187). It is still unclear whether Skint1 directly interacts with the invariant Vγ5Vδ4 TCR or whether it interacts with a hitherto uncharacterized receptor expressed exclusively by Vγ5Vδ4 thymocytes (186). This dependency on the fetal environment could explain why Yuan et al did not find these cells in a mouse model with ectopic overexpression of Lin28b in adult HSPC (163). We have used the in vitro OP9DL1 system to compare the generation of human γδ T cells from various HSPC sources and found that in the same 'thymic environment' only fetal HSPC sources were able to generate invariant γδ T cells. However, this does not exclude that signals from other HSPC-derived cells are important in this model of human γδ T cell generation. Indeed, in the mouse it has been shown that 'transconditioning' mediated by double-positive thymocytes, in a mechanism dependent on lymphotoxin B, CD27 and CD70 (15,188), are important to establish an IFNγ programming in mouse fetal γδ thymocytes (189). Whether a similar process is involved in the functional programing of human fetal γδ thymocytes remains to be determined. While it is clear that other members of the BTN(L) family (BTN2A1 and BTN3A1) are crucial for phosphoantigen-reactivity of human Vγ9Vδ2 T cells, it will be important to investigate whether these transmembrane proteins are involved in the (fetal) thymic development of this human innate γδ T cell subset like Skint1 for the mouse innate Vγ5Vδ4 cells.

6. Concluding remarks and perspectives

Unlike other unconventional T cells such as invariant NKT cells and MAIT cells, there is no obvious conservation of γδ T cell subsets between mice and human. However, recent research revealed conservation of key elements between these two species, thus highlighting more similarities between human and mouse γδ T cells than previously appreciated. This includes the mechanism of invariant γδ TCR generation in early life (short homology repeat-mediated recombination; use of proximal TR gene segments; role of the RNA binding protein Lin28b) (29,52,110) and the importance of BTN(L) molecules in the selection and/or TCR reactivity of γδ T cells (186). Both in human and mice an innate γδ TCR repertoire is generated in early life, which is paralleled by thymic functional programming, playing an important role against infections during this period. Upon aging, γδ T cells become more adaptive-like. Whether the functional programming observed in the human fetal thymus (e.g. enriched expression of IFNγ and granzyme A) is associated with particular functional and/or CDR3-biased γδ T cell subsets or clusters like in the mouse remains to be investigated, e.g. via single-cell RNA sequencing analysis. Such analysis may also clarify whether during early human gestation, a γδ thymocyte cluster is present that is specialized in IL17 production (110).

Despite similarities in the generation of human and mouse innate γδ T cells, there are also differences. The first T cells made in the mouse, Vγ5Vδ4 T cells, are maintained in the skin epidermis as DETC until adulthood by clonal self-renewal (107), while adult blood human Vγ9Vδ2 T cells are generated anew later in life(52). This may allow to develop strategies to enhance the de novo generation of Vγ9Vδ2 T cells in cancer patients. The species-specific aspects such as the presence of DETC in the mouse skin epidermis and phosphoantigen-reactive Vγ9Vδ2 T cells in human blood may be related to species-specific needs in immunity. It can also be related to particular biological needs not directly related to immunity since innate γδ T cells appear to intertwine immunological and non-immunological physiological roles. Such physiological roles have also been described for human fetal

intestinal CD4+ αβ T cells, which are absent in the mouse (190,191). Interestingly, the associated αβ TCR repertoire also contains public TRB CDR3 sequences, although the level of publicity was far inferior to that observed in innate γδ T cells (190). What remains to be elucidated is the anticipated developmental waves in the thymus, which could be related to a bias in the αβ TCR repertoire generation (192–194).

While γδ TCR repertoire analysis by HTS advanced significantly the insight into the innate versus adaptive role of human γδ T cells and their development, the possible homing of specific fetal innate γδ T cell subsets to particular solid tissues in human is unclear. Adult liver and intestine do not appear to contain such tissue-associated (semi-)invariant γδ TCRs (32,33). But since the persistence of human fetal-derived γδ T cells can be different than in mouse (52), it is possible that the adaptive-like repertoire observed in these adult human solid tissues is preceded by fetal-derived innate γδ T cells. That thesis can be investigated by γδ TCR repertoire analysis by HTS of fetal solid tissues.

Finally, because of the development and importance of γδ T cells in early life, as discussed in this review, the role of the microbiome during this early 'window of opportunity' in the imprinting of these cells deserves a more in depth investigation (29,117,195–197).

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Conflict of interest

None

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Tables

Table 1. Prevalence of γδ T cells in human and mouse adult tissues (ex vivo).

	Human adult		
Tissue	**% γδ of CD3***	**enriched for**	**Reference**
Blood	1-6% (mean 3.7%)	Vγ9Vδ2	(29,30)
Lymph node	2.2%	Vδ2	(198)
Lung	1.4% (BAL)	Vδ1 (in TB lesions)	(199)
	2-4% lung lesion		(78)
Skin epidermis	8.7% (repres. plot)	Vδ1	(75)
Skin dermis	3.8% (repres. plot)	Vδ1	(75)
Subcutaneous adipose tissue	3.7% (repres. plot)	Vδ2	(75)
Intestine (epithelia)	7-8%	Vδ3 (Vδ1>Vδ2)	(68)
	20-21%	Vδ1	(32,69)
	37%		(70)
Lamina propria	5%	Vδ3 (Vδ2)	(68,69)
	2%		(32)
Liver	around 12%	Vδ1	(33)
Decidua	10-16%	Vδ1	(81)
Breast	mean around 3% (until 30%)**	Vδ1	(80)
Spleen (red pulp)	12%	Vδ1	(198)
Thymus	1.40%	Vδ1	(198)
	Mouse adult		
Tissue	**% γδ of CD3**	**enriched for**	**Reference**
Blood	3%	Vγ1 & Vγ4	(200)
	2%		(134)
Lymph node	1%	Vγ1	(201)
	2%		(200,202)
Lung	3%	Vγ4	(134)
	5-10%		(203)
Skin epidermis	around 99%	Vγ5	(204)
Skin dermis	15%	Vγ4 & Vγ6	(205)
Intestine (epithelia)	42.1%	Vγ7	(73)
Liver	1.8%	Vγ1	(202)
	4.5%		(134)
Decidua	around 17%	Vγ6	(200)
Spleen	8%	Vγ1	(206)
	2.50%		(134)

*based on flow cytometry

**"grid" explant culture system

IMGT nomenclature used. (repres.: representative).

Table 2. List of main invariant CDR3 sequences of human and mouse γδ T cells.

Human						
Nucleotype	**Clonotype**	**V usage**	**D usage**	**J usage**	**Enriched in**	**Reference**
TGTGCCACCTGGG**ATA**CCACTGGTTGGTTCAAGATATTT	CATWDTTGWFKIF	TRGV8		TRGJP1	fetal thymus, blood congenital CMV	(60,110)
TGTGCCTTGTGGGAGGT**G**CAAGAGTTGGGCAAAAAAATCAAGGTATTT	CALWEVQELGKKIKVF	TRGV9		TRGJP	fetal thymus, fetal/infant blood	(52,101)
TGTGCTCTTGGGGA**ACT**GGGGGACGATAAACTCATCTTT	CALGELGDDKLIF	TRDV1	TRDD3	TRDJ1	blood congenital CMV infection	(60)
TGTGCCTGTGAC**AC**TGGGGGATACTGGGACACCCGACAGATGTTTTTC	CACDTGGYWDTRQMFF	TRDV2	TRDD3	TRDJ3	fetal thymus, blood congenital CMV	(60,110)
TGTGCCTGTGACATACTGGGGGAT**AC**CGATAAACTCATCTTT	CACDILGDTDKLIF	TRDV2	TRDD3	TRDJ1	fetal liver, infant blood	(29,99)
TGTGCCTGTGAC**GT**ACTGGGGGAT**AC**CGATAAACTCATCTTT	CACDVLGDTDKLIF	TRDV2	TRDD3	TRDJ1	fetal liver, infant blood	(29,99)
TGTGCCTGTGACACCGTACTGGGGGATAC(C/G)TGGGACACCCGACAGATGTTTTTC	CACDTVLGDTWDTRQMFF	TRDV2	TRDD3	TRDJ3	cord blood, infant blood	(29,61)
Mouse						
TGTGCAGTCTGGAGATCAGGCACATCATGGGTCAAGATATTT	CAVWRSGTSWVKIF	TRGV1		TRGJ4	liver, spleen, lung, LN, neonatal thymus	(113,130)
TGTTCCTACGGCTTATATAGCTCAGGTTTTCACAAGGTATTT	CSYGLYSSGFHKVF	TRGV4		TRGJ1	LN, spleen	(95)
TGTGCCTGCTGGG**AT**AGCTCAGGTTTTCACAAGGTATTT	CACWDSSGFHKVF	TRGV5		TRGJ1	fetal thymus, skin epidermis	(94,105)
TGTGCATGCTGGG**AT**AGCTCAGGTTTTCACAAGGTATTT	CACWDSSGFHKVF	TRGV6		TRGJ1	fetal thymus, lung, uterus, tongue, skin dermis	(94,112)
TGTGGGTCAGAT**ATC**GGAGGGAGCTCCTGGGACACCCGACAGATGTTTTTT	CGSDIGGSSWDTRQMFF	TRDV4	TRDD2	TRDJ2	fetal thymus, skin, lung, uterus, tongue	(94,105,112)
TGTGCCTCGGGGTAT**AT**CGGAGGGATACGAG**CT**ACCGACAAACTCGTCTTT	CASGYIGGIRATDKLVF	TRDV5	TRDD2	TRDJ1	LN, spleen	(95,145)
TGTGCTCTCTGGGAGCTGGTCGGAGGGATACGAG**CT**ACCGACAAACTCGTCTTT	CALWELVGGIRATDKLVF	TRAV15-1-DV6-1	TRDD2	TRDJ1	liver, spleen	(113–115)

Nucleotides involved in short homology repeat recombination (SHR) are in bold and underlined. In blue, P additions involved in SHR. IMGT nomenclature used. (LN: lymph node).

Figure legends

Figure 1

γδ TCR repertoire nomenclature. Characterizations based on

A. diversity level
B. sharing between individuals
C. previous antigen encounters

These terms can be overlapping, for example invariant sequences are usually innate.

Plots: in A, tree maps depicting CDR3 clonotype usage, each rectangle represents one CDR3 clonotype and its size corresponds to its relative frequency in the repertoire; in B & C-left, pair comparisons of repertoire; C-right, tracking of repertoire during 5 timepoints.

Figure 2

Development and function of innate Vγ9Vδ2 T cells.

The fetal thymus generates generally germline repertoire which is similar to what is observed in fetal blood. Congenital infection or post-natal environmental exposure leads to expansion of the fetal public repertoire and gain of effector functions. Adult blood Vγ9Vδ2 T cells originate mainly from the diverse repertoire generated in the post-natal thymus, containing high N additions, and display additional effector functions. Granules containing granzymes, perforin and granulysin are depicted in colored dots to show highest prevalence.

Medical art modified from (207).

Figure 3

The generation of invariant γδ TCRs depends on Lin28b and short homology repeats.

Lin28b blocks the insertion of random nucleotides (N additions) by the TdT enzyme which allows germline identical nucleotides from the Vend and Jstart to be found at proximal sites and give rise to invariant/public TCRs through short homology repeats recombination.

Example of human TRGV8-TRGJP1 recombination leading to invariant TCR (via short homology repeats) (left) and to a variable TCR containing N additions (in red; inserted by TdT) (right).

Protein forms modified from (207).

Figure 4

Potential Lin28b interaction partners driving the development of fetal innate γδ T cells.

Lin28b may regulate the expression of key drivers of fetal innate γδ T cell developmental programming either by inhibiting Let-7 biogenesis or by Let-7 independent actions. In the latter, IGF2BP family members could play a major role by stabilizing Lin28b (IGF2BP1) or by promoting the

stability of transcripts involved in acquisition of fetal innate γδ features (IGF2BP3). IGF2BP1, Insulin Like Growth Factor 2 mRNA Binding Protein 1; IGF2BP3, Insulin Like Growth Factor 2 mRNA Binding Protein 3; EZH2, Enhancer Of Zeste 2 Polycomb Repressive Complex 2 Subunit; PLZF, Promyelocytic Leukemia Zinc Finger; EOMES, eomesodermin.

Protein forms modified from (207).

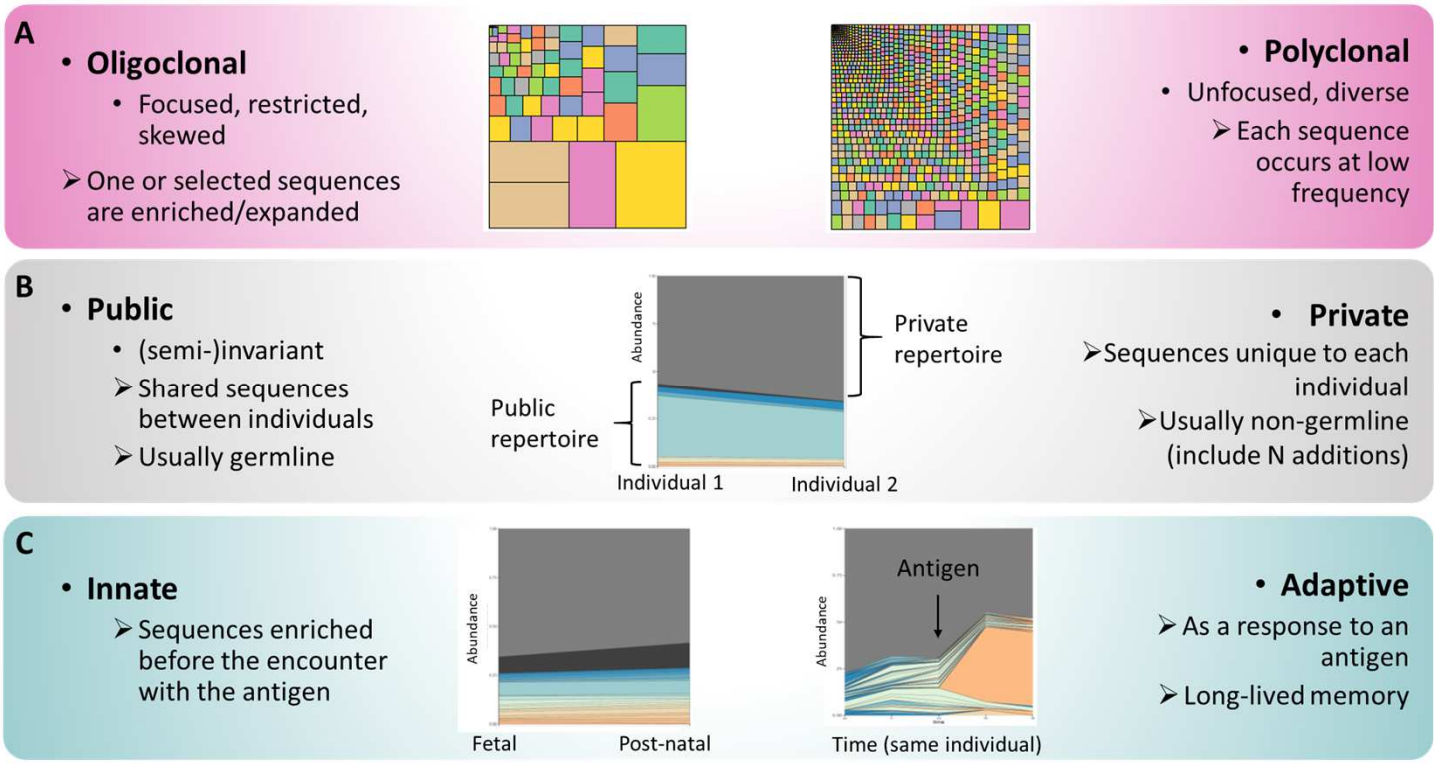

Figure 1. γδ TCR repertoire nomenclature. Characterisations based on: A. diversity level, B. sharing between individuals, C. previous antigen encounters. These terms can be overlapping, for example invariant sequences are usually innate. Plots: in A, tree maps depicting CDR3 clonotype usage, each rectangle represents one CDR3 clonotype and its size corresponds to its relative frequency in the repertoire; in B & C-left, pair comparisons of repertoire; C-right, tracking of repertoire during 5 timepoints.

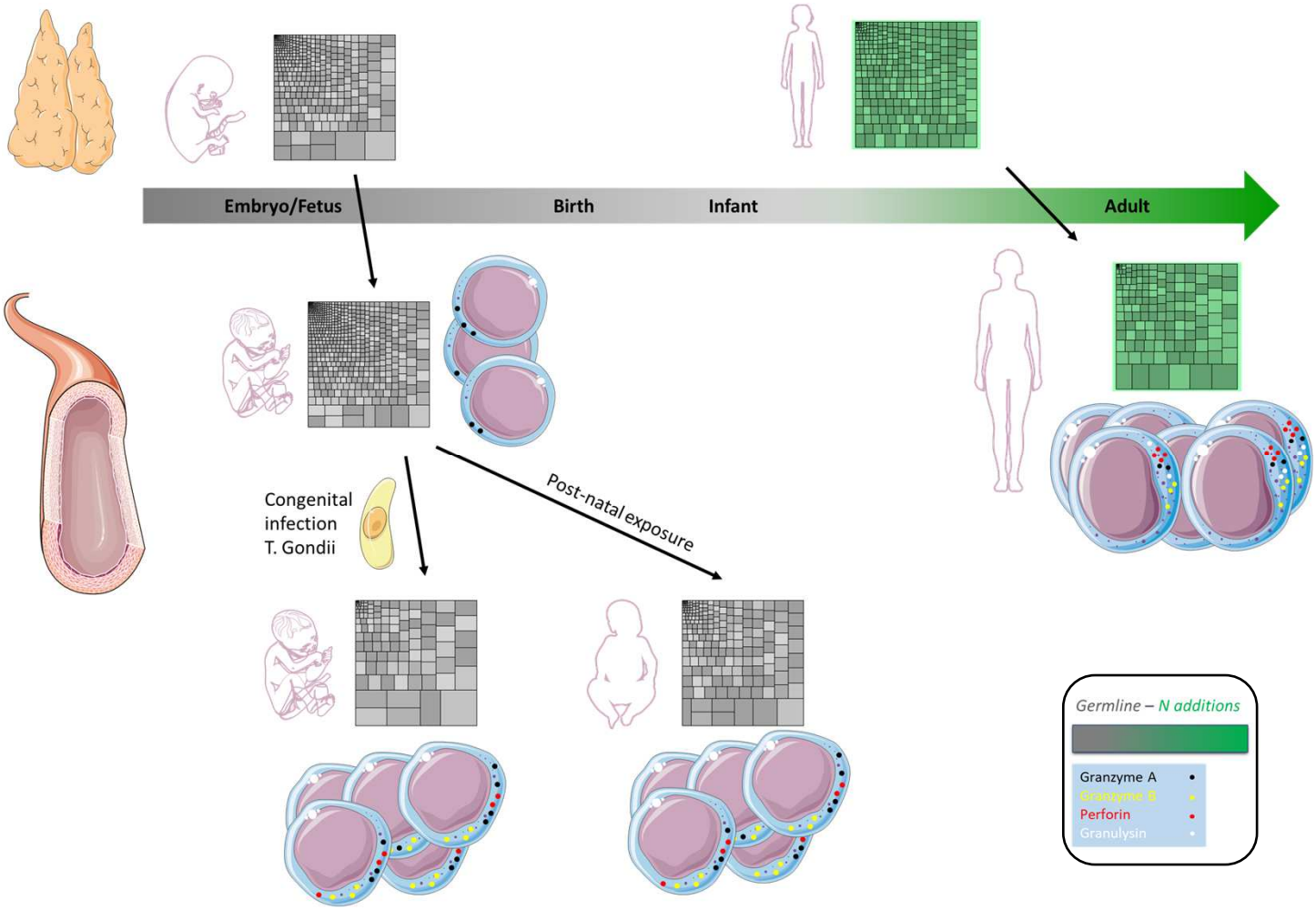

Figure 2. Development and function of innate Vγ9Vδ2 T cells. The fetal thymus generates generally germline repertoire which is similar to what is observed in fetal blood. Congenital infection or post-natal environmental exposure leads to expansion of the fetal public repertoire and gain of effector functions. Adult blood Vγ9Vδ2 T cells originate mainly from the diverse repertoire generated in the post-natal thymus, containing high N additions, and display additional effector functions. Granules containing granzymes, perforin and granulysin are depicted in coloured dots to show highest prevalence. Medical art modified from (207).

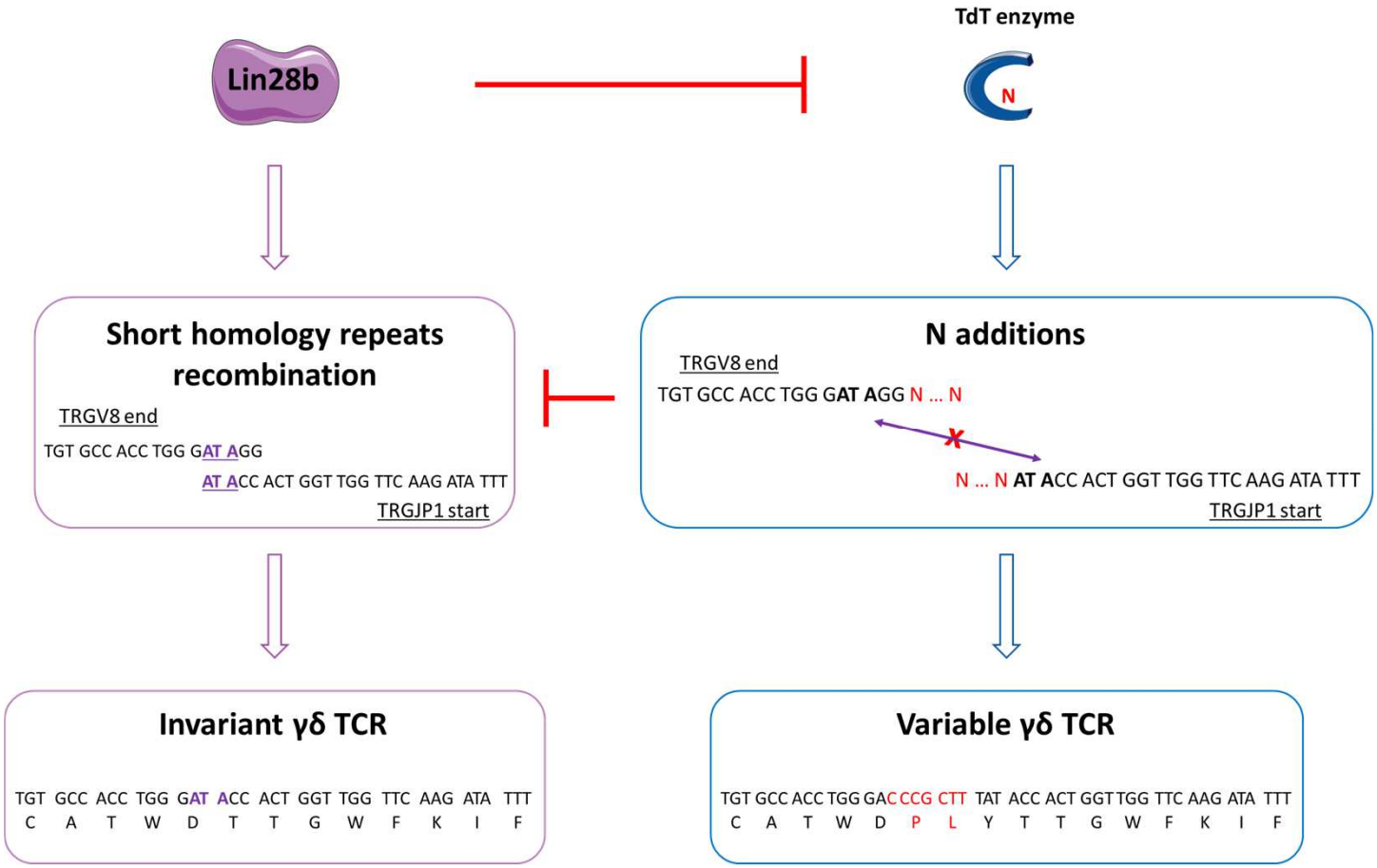

Figure 3. The generation of invariant γδ TCRs depends on Lin28b and short homology repeats. Lin28b blocks the insertion of random nucleotides (N additions) by the TdT enzyme which allows germline identical nucleotides from the Vend and Jstart to be found at proximal sites and give rise to invariant TCRs through short homology repeats recombination. Example of human TRGV8-TRGJP1 recombination leading to invariant TCR (via short homology repeats) (left) and to a variable TCR containing N additions (in red; inserted by TdT) (right). Protein forms modified from (207).

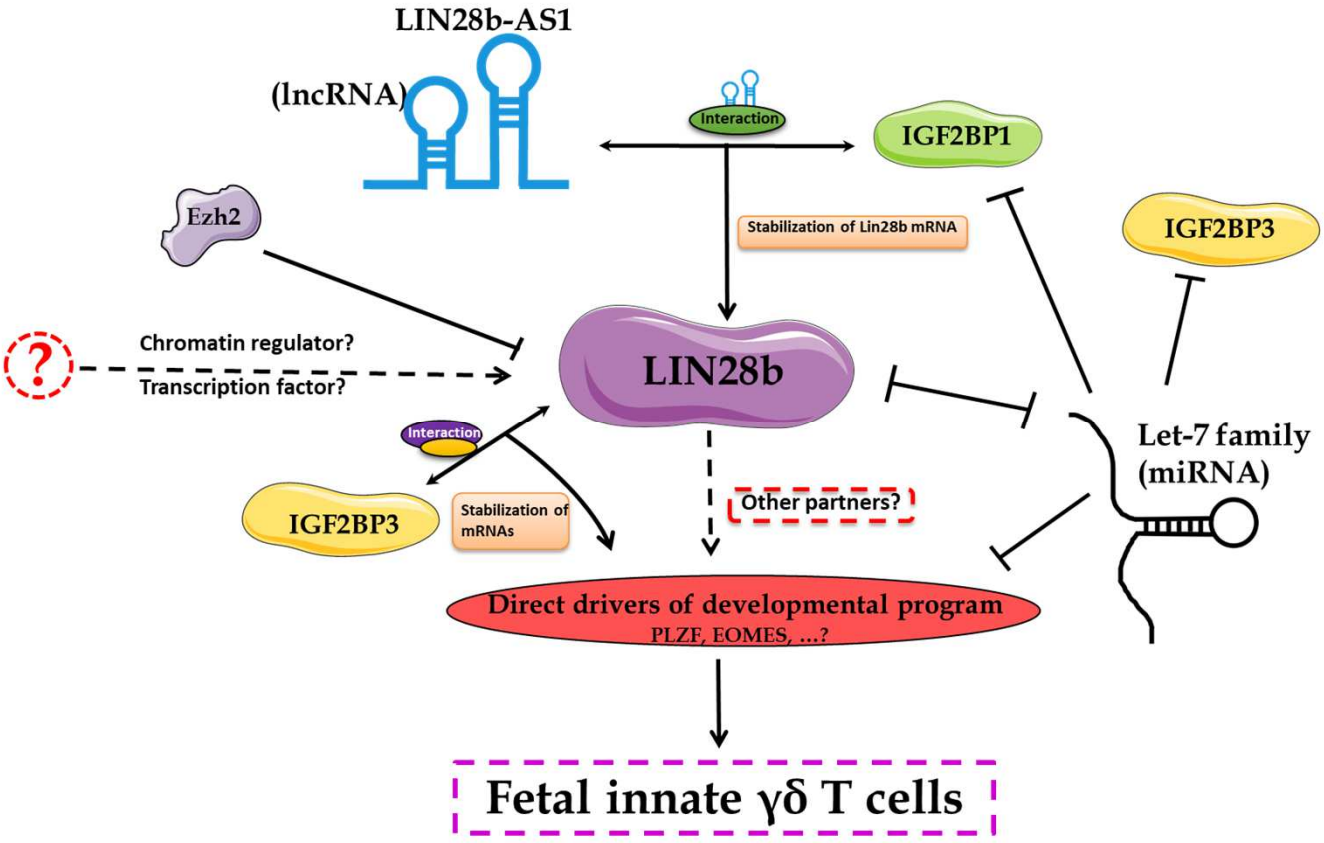

Figure 4. Potential Lin28b interaction partners driving the development of fetal innate γδ T cells. Lin28b may regulate the expression of key drivers of fetal innate γδ T cell developmental programming either by inhibiting Let-7 biogenesis or by Let-7 independent actions. In the latter, Insulin Like Growth Factor mRNA-Binding Protein family (IGFBP) members could play a major role by stabilizing Lin28b (IGF2BP1) or by promoting the stability of transcripts involved in acquisition of fetal innate γδ features (IGF2BP3). IGF2BP1, Insulin Like Growth Factor 2 mRNA Binding Protein 1; IGF2BP3, Insulin Like Growth Factor 2 mRNA Binding Protein 3; EZH2, Enhancer Of Zeste 2 Polycomb Repressive Complex 2 Subunit; PLZF, Promyelocytic Leukemia Zinc Finger; EOMES, eomesodermin. Protein forms modified from (207).