



Thymic Program Directing the Functional Development of $\gamma\delta$ T17 Cells

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$\gamma\delta$ T cells comprise a unique T cell sublineage endowed with a wide functional repertoire, which allow them to play important—sometimes opposite—roles in many immune responses associated with infection, cancer, and inflammatory processes. This is largely dependent on the existence of pre-programmed discrete functional subsets that differentiate within the thymus at specific temporal windows of life. Since they represent a major early source of interleukin-17A in many models of immune responses, the $\gamma\delta$ T17 cell population has recently gained considerable interest. Thus, a better dissection of the developmental program of this effector $\gamma\delta$ T subset appears critical in understanding their associated immune functions. Several recent reports have provided new exciting insights into the developmental mechanisms that control $\gamma\delta$ T cell lineage commitment and differentiation. Here, we review the importance of thymic cues and intrinsic factors that shape the developmental program of $\gamma\delta$ T17 cells. We also discuss the potential future areas of research in $\gamma\delta$ T17 cell development especially in regards to the recently provided data from deep RNA sequencing technology. Pursuing our understanding into this complex mechanism will undoubtedly provide important clues into the biology of this particular T cell sublineage.

Keywords: $\gamma\delta$ T cells, innate immunity, interleukin-17A, thymus, transcription factor, development

Interleukin-17 (IL-17) is a highly conserved cytokine in vertebrates that plays a critical role in host homeostasis and immune response to pathogens especially at barrier sites (1, 2). Recent evidence indicates that IL-17 also emerges as a key contributor in immunity beyond the scope of infection, such as inflammation and cancer (3, 4). Given the pivotal role of lymphoid cell-derived IL-17 in orchestrating immune responses, its cellular sources have been extensively searched over the last decade. Initially believed to be mainly produced by conventional CD4⁺ T (Th17) cells (5, 6), the discovery of innate and innate-like lymphocytes endowed with potent capacities to produce IL-17 (7) suggests that this cytokine is well poised at the border between innate and adaptive immunity. These populations include $\gamma\delta$ T cells (8), natural killer T (NKT) cells (9), mucosal-associated invariant T (MAIT) cells (10), and group 3 innate lymphoid cells (ILC3) (11). Among those, $\gamma\delta$ T cells have been

Abbreviations: Ag, antigen; APC, Ag presenting cells; Blk, B lymphocyte kinase; DETC, dendritic epidermal $\gamma\delta$ T cell; IL, interleukin; IFN, interferon; ILC, innate lymphoid cells; Lef1, lymphoid enhancer-binding factor; MAIT, mucosal-associated invariant T; NKT, Natural Killer T; TCR, T cell receptor; TEC, thymic epithelial cells; TF, transcription factor.

demonstrated to be the main contributors in IL-17 production in many settings, such as infection, autoimmunity, and cancer. Here, we discuss the recent advances on our understanding of IL-17-producing $\gamma\delta$ T ($\gamma\delta$ T17) cell biology with a particular emphasis on the transcriptional road map that drives their innately “pre-programmed” effector fate.

CHARACTERISTICS OF $\gamma\delta$ T17 CELLS

Mouse $\gamma\delta$ T cells consist of a heterogeneous population of thymus-derived T lymphocytes characterized by distinct functional properties (e.g., cytokine profile and/or cytotoxic properties) and tissue distribution (12). Within this subset diversity, $\gamma\delta$ T17 cells can be defined based on their T cell receptor (TCR) repertoire usage and surface markers. Thus, $\gamma\delta$ T17 cells are almost exclusively restricted to $\gamma\delta$ T cells expressing either a V γ 6 or a V γ 4 TCR (N.B.: The Heilig and Tonegawa’s nomenclature (13) has been used in this review) (Table 1). In addition, many cell surface antigens (Ags) have been shown to distinguish $\gamma\delta$ T17 cells and can be defined as CD27⁻, NK1.1⁻, IL-7R α ^{high}, IL-18R^{high}, CD122⁻, and CCR6⁺ cells (14–16). $\gamma\delta$ T17 cells mainly establish residency at barrier sites including lung, skin, vagina, and oral cavity. However, they can recirculate in particular pathological situations including infections and cancer (17). The migratory capacity of $\gamma\delta$ T17 cells is regulated by the chemokine receptors CCR2 (during inflammation) and CCR6 (at homeostasis) (18). This preferential location at barrier sites might indicate a preferential interplay between $\gamma\delta$ T17 cells and the endogenous flora (19) as exemplified by the strong reduction in frequency of lung resident $\gamma\delta$ T17 cells in germ-free mice (20). Interestingly, while V γ 4⁺ $\gamma\delta$ T17 cells can be detected in the gastrointestinal tract (21), we and others failed to detect V γ 6⁺ $\gamma\delta$ T17 cells in this tissue in adult mice under steady-state condition (20, 22). This might suggest that the nature and/or diversity of commensals in the various mucosa could differentially influence the maintenance of $\gamma\delta$ T17 cell subsets.

$\gamma\delta$ T17 cells are characterized by their ability to promptly produce copious amounts of IL-17A/E, IL-22, IL-21, and GM-CSF (8, 23–25). This rapid capacity to produce these cytokines can be

mainly attributed to their innate-like feature. Despite expressing a fully functional rearranged TCR, $\gamma\delta$ T17 cells can respond to activating cytokines (IL-1 β , IL-23, and IL-18) even in absence of concomitant TCR engagement (8, 16). However, TCR ligation on naive $\gamma\delta$ T17 cells has been shown to license them by increasing activating cytokine receptor expression (e.g., IL-1R1 and IL-23R) and thus rendering them permissive to “innate” stimulation (26). The nature of these Ags is yet to be determined. Notably, the unprocessed form of the red algae protein phycoerythrin has been shown to interact with a small proportion of naive $\gamma\delta$ T cell TCRs irrespectively of their TCR repertoire (26). Even if the physiological relevance of phycoerythrin in the biology of mammalian cells is difficult to conceive, it is tempting to speculate that structurally related Ags could be relevant in the general selection and licensing of $\gamma\delta$ T cells. In addition, the fact that a single Ag can be recognized by various $\gamma\delta$ TCRs harboring distinct CDR3 regions is reminiscent with the NKT cell biology (27) and can suggest the existence of a restricted conformational “hot-spot” comprising few amino acid residues in CDR3 regions responsible for $\gamma\delta$ T cell antigenicity in mice. This structural basis for Ag recognition by innate-like T cells might have been conserved all through the evolution from jawless vertebrates (28).

Despite leaving the thymus with a pre-programmed effector fate, $\gamma\delta$ T17 cells have been shown to conserve a certain degree of plasticity in the periphery. This characteristic originates from an epigenetic regulation program for specific genes in $\gamma\delta$ T17 cells such as Dickkopf-related protein 3 (29). Thus, along with IL-17, $\gamma\delta$ T17 cells can also produce interferon (IFN)- γ under inflammatory conditions. The biological relevance of this plasticity has been revealed in various settings including *Listeria monocytogenes* infection (22). In this later model, long-lasting accumulation of V γ 6⁺ IFN- γ /IL-17 double producers was observed within intestinal lamina propria (22, 30). Since V γ 6⁺ $\gamma\delta$ T17 cells were reported to be absent from the gastrointestinal tract at steady-state (20), it is possible that the combination of the $\gamma\delta$ T17 cell epigenome and local environment modifications under this inflammatory condition favors their homing and survival in the gut tissue. On the other hand, it is interesting to mention that IFN- γ -producing pre-programmed $\gamma\delta$ T cells ($\gamma\delta$ T1) do not possess the capacity to

TABLE 1 | Origin, tissue distribution, and TCR repertoire of $\gamma\delta$ T17 cells.

Subset	Windows of development	Steady-state tissue distribution	Origin	V(D)J diversity
V γ 1 ⁺	Mainly perinatal (day 3–8)	Barrier sites and lymphoid tissues	Natural	Intermediate to high
V γ 2/3 ⁺	Late embryonic and perinatal (from E17 to day8)	Barrier sites and lymphoid tissues	Natural	–
V γ 4 ⁺	Late embryonic and postnatal (E18 onward)	Barrier sites and lymphoid tissues	Natural: for V γ 4 ⁺ T cells of fetal origin including at least the V γ 4V δ 5 subset	Invariant for natural $\gamma\delta$ T17: V γ 4J γ 1/V δ 5D δ 2J δ 1
			Inducible: after Ag encounter in the periphery without extensive clonal expansion	Intermediate to high for inducible $\gamma\delta$ T17
V γ 5 ⁺	Embryonic only (from E13 to E16)	Epidermis	Reprogrammed: unknown mechanism (TCR?)	Invariant: V γ 5J γ 1C γ 1/V δ 1D δ 2J δ 2
V γ 6 ⁺	Embryonic only (from E14 to birth)	Barrier sites at the exception of the gut	Natural	Invariant: V γ 6J γ 1C γ 1/V δ 1D δ 2J δ 2

E, embryonic day; V, variable gene segment; D, diversity gene segment; J, junction gene segment; Ag, antigen; TCR, T cell receptor.

produce IL-17 (31). However, a small proportion of epidermal $\gamma\delta$ T1 (e.g., V γ 5⁺) cells has been demonstrated to produce IL-17 *in vivo* upon skin wounding (32). The molecular determinants involved in giving rise to this cytokine production capacity are currently unknown but seem to rely on TCR signaling (33).

The existence of $\gamma\delta$ T17 cells in humans is still a matter of debate (34). Actually, the thymic program of $\gamma\delta$ T cells in humans seems to differ from the one described in mice (35). Most of the data available suggest that human $\gamma\delta$ T cells might not be “innately” programmed to produce IL-17 during their thymic development but rather acquire this capacity under inflammatory conditions once in the periphery akin to CD4⁺ Th17 cells. Thus, circulating V γ 9V δ 2⁺ [using Lefranc’s nomenclature (36)] T cells from adult healthy donors produce no or little IL-17 (37) except under complex stimulatory protocol including both activating cytokines and TCR engagement (38). However, it is important to mention that purified V γ 9⁺ $\gamma\delta$ T cells from cord blood seem more prone to produce IL-17 (37, 39, 40) that could suggest an embryonic origin for human $\gamma\delta$ T17 cells similar to the murine situation. It is, therefore, possible that these putative $\gamma\delta$ T17 cells occupy particular niches of the body that render them difficult to assess under homeostatic conditions. Murine pre-committed $\gamma\delta$ T17 cells are often characterized by the expression of an almost clonal TCR (41). Thanks to next-generation sequencing, the existence of clonal TCR-expressing $\gamma\delta$ T cell subsets in humans has recently emerged. Upon cytomegalovirus reactivation, a recent study demonstrated the massive proliferation of diverse $\gamma\delta$ T cell clones in patients after allogeneic-hematopoietic-stem-cell transplantation (42). However, the TRG and TRD sequences of these clones were not shared among individuals at the nucleotide level (42). In addition, analysis of the human V δ 1⁺ T cells in healthy adults indicates that this repertoire is dominated by few private clonotypes (43). Determining the cytokine profile of these clones will be helpful to better appreciate the existence and origins of $\gamma\delta$ T17 cells in humans. Whatever the mechanisms that drive their emergence in humans, the capacity of human $\gamma\delta$ T cells to produce IL-17 has been demonstrated in various immune responses including infection, cancer, and autoimmunity (38, 44–46).

DEVELOPMENT OF $\gamma\delta$ T17 CELLS

Dealing With the Concept of “Innate/Natural” vs “Adaptive/Inducible” Origins of $\gamma\delta$ T17 Cells

Mouse $\gamma\delta$ T cells develop in a standardized manner by sequential waves that can be conveniently followed based on their V γ chain usage (47). This process starts during embryonic life from day 13 (E13) onward. The first wave is exclusively constituted of the IFN- γ -producing V γ 5⁺ cells and lasts for about 4 days. This is shortly followed by a developmental wave of “natural” IL-17-producers comprising both canonical V γ 6⁺ (from E14 to birth) and restricted subsets of V γ 4⁺ (E18 onward) (48, 49). Around birth, IFN- γ -producing V γ 1⁺ and V γ 4⁺ subsets start to develop along with the IL-4/IFN- γ -double producers V γ 1⁺V δ 6⁺ subset. After birth, developing $\gamma\delta$ T cells mainly exhibit a naive uncommitted profile (47).

According to this scheme, natural $\gamma\delta$ T17 cell development is believed to be restricted to the gestational period. To support this notion, Haas and colleagues demonstrated that transplantation of bone marrow from IL-17-competent mice into lethally irradiated *Il17af*-deficient adult recipients failed to induce $\gamma\delta$ T17 cell development (49). Partial $\gamma\delta$ T17 cell development in the thymus could be achieved in reconstituted *Il17af*-deficient neonate recipients but failed to give rise to $\gamma\delta$ T17 cells in the periphery. In addition, inducible expression of *Rag1* in T cell precursors in adult mice did not restore *de novo* generation of $\gamma\delta$ T17 cells (49). Likewise, CCR6⁺ IL-17-producing dermal $\gamma\delta$ T cells failed to reconstitute 8 weeks after bone marrow transplantation unless the mice received an additional transfer of neonatal thymocytes (33). Surprisingly, the same lab reported the presence of V γ 4⁺ CCR6⁺ (but not V γ 4⁻ CCR6⁺) $\gamma\delta$ T17 cells in lymph nodes of recipient TCR δ ^{-/-} mice following bone marrow transplantation in absence of neonatal thymocytes at 12 weeks post-grafting (50). The basis for this difference remains unclear but one can argue that, in the 12 weeks model, authors have reconstituted “inducible” $\gamma\delta$ T17 cells only. Several studies have also reported that the peripheral pool of $\gamma\delta$ T17 cells decreased with age (51, 52), which further support the embryonic origin of natural $\gamma\delta$ T17 cells.

Despite suggesting a strict favorable temporal window for natural $\gamma\delta$ T17 cell development during fetal life, these studies also raised some additional interrogations. Is this developmental model imposed by intrinsic (nature of $\gamma\delta$ T precursors) or extrinsic (embryonic vs adult thymic environment) factors? Zúñiga-Pflücker’s team recently started to provide an answer to this question. Culture of $\gamma\delta$ TCR-transduced fetal or adult hematopoietic precursors with OP9-Delta-like protein 4 (Dll4) cells led to the development of $\gamma\delta$ T cells with IL-17 production capacity in a similar manner (53). Thus, these data suggest that adult progenitors conserve their intrinsic capacity to develop as $\gamma\delta$ T17 cells once in an appropriate environment. However, since the authors used a clonal TCR (V γ 4V δ 5) (54) for transfection, it cannot be excluded that in addition to the favorable environment, the nature of the TCR expressed plays a role in the IL-17 effector fate, in particular regarding the recent discovery of clonal V γ 4J γ 1/V δ 5D δ 2J δ 1 T cells with a strongly biased IL-17-producing profile (55). In addition, recent studies have shown that adult peripheral $\gamma\delta$ T cells (from adult bone marrow-derived precursors) can convert into “induced” $\gamma\delta$ T17 cells upon inflammatory conditions (56, 57). Both studies highlighted a critical role for the cytokines IL-23 and IL-1 β , and to a lesser extent TCR signaling, in this process (56, 57). Importantly, the potential to give rise to inducible $\gamma\delta$ T17 cells seems to be restricted to the IL-2R β ⁻ $\gamma\delta$ T cell subset (57). To further illustrate this peripheral polarization capacity, Buus and colleagues recently provided a RNA-Seq analysis of adult $\gamma\delta$ thymocytes indicating IL-17 potential in certain subsets including notably IL-2R α ⁺ Clec12A⁺ V γ 1⁺ and V γ 4⁺ cells (58).

This situation illustrates the recent concept of “natural” vs “inducible” $\gamma\delta$ T17 cells (48). While “natural” (V γ 6⁺ and V γ 4⁺ subsets) $\gamma\delta$ T17 cells are committed to this effector fate during their embryonic/perinatal thymic developmental program, “inducible” $\gamma\delta$ T17 cells stem from naive (V γ 1⁺ and V γ 4⁺ subsets) $\gamma\delta$ T cells within the periphery upon inflammatory conditions through cytokine, and/or Ag recognition akin to conventional CD4⁺

Th17 cells (26). Thus, it is tempting to view “natural” $\gamma\delta$ T17 cells as innate-like T cells whereas “inducible” $\gamma\delta$ T17 cells can rather be considered adaptive. This is reminiscent with the situation in the $\alpha\beta$ lineage comprising innate-like T17 (NKT17 and MAIT17) cells and adaptive conventional Th17 cells.

Thymic Molecular Determinants of $\gamma\delta$ T17 Cell Effector Fate

In this section, we will review the thymic determinants that drive $\gamma\delta$ T cell differentiation into an IL-17 effector fate by deciphering how “natural” IL-17-committed $\gamma\delta$ T cells emerge from the rest of the $\gamma\delta$ T cell compartment. Before commitment into $\gamma\delta$ T17 cell sublineage, thymic precursors have to first undergo a bifurcation into $\alpha\beta$ or $\gamma\delta$ lineages. Mechanisms driving this initial dichotomy are beyond the scope of this review, but it is worth mentioning that the strength of TCR engagement in thymocytes emerges as a driving force in this process [see Ref. (53, 59) for reviews]. However, requirement for TCR ligation in IL-17-committed $\gamma\delta$ T cell differentiation is still an intense matter of debates that will be discussed later. Commitment toward the $\gamma\delta$ T lineage happens at double negative (DN)2 and DN3 stages (60, 61). Interestingly, the effector fate of “natural” $\gamma\delta$ T cell subsets (IFN- γ - vs IL-17A-producing) appears to be already predetermined at this stage. Thus, commitment to “natural” $\gamma\delta$ T17 cells exclusively arises from the late DN2 stage in a B cell leukemia/lymphoma 11b-dependent manner (46).

Further differentiation into the IL-17 effector fate is a complex and highly dynamic process involving multiple molecular and cellular interactions. For the sake of clarity, we distinguish here the (1) extrinsic factors (e.g., thymic environmental cues) and (2) intrinsic factors [e.g., intracellular signaling pathways and transcription factors (TFs)] that tune $\gamma\delta$ T17 precursors into mature $\gamma\delta$ T17 cells.

Extrinsic Factors

Molecular and cellular players within the thymic microenvironment are crucial for acquisition of $\gamma\delta$ T cell effector fate (Figure 1). Anatomically, precursors migrate from the cortex to the medulla during this process, where they receive multiple signals that dictate their differentiation. In this three-dimensional environment, it is important to keep in mind that time should be considered as a fourth dimension when deciphering the “natural” $\gamma\delta$ T17 cells ontogeny.

Cytokines

Many cytokines have been reported to directly or indirectly regulate thymic $\gamma\delta$ T17 cell differentiation/development.

IL-7 is a critical and non-redundant cytokine in lymphopoiesis (62). IL-7R α -deficient mice completely lack $\gamma\delta$ T cells (63, 64) partly due to the role of IL-7 in V-J recombination of TCR γ genes (63, 65). Its specific role in survival and proliferation of $\gamma\delta$ T17 cells

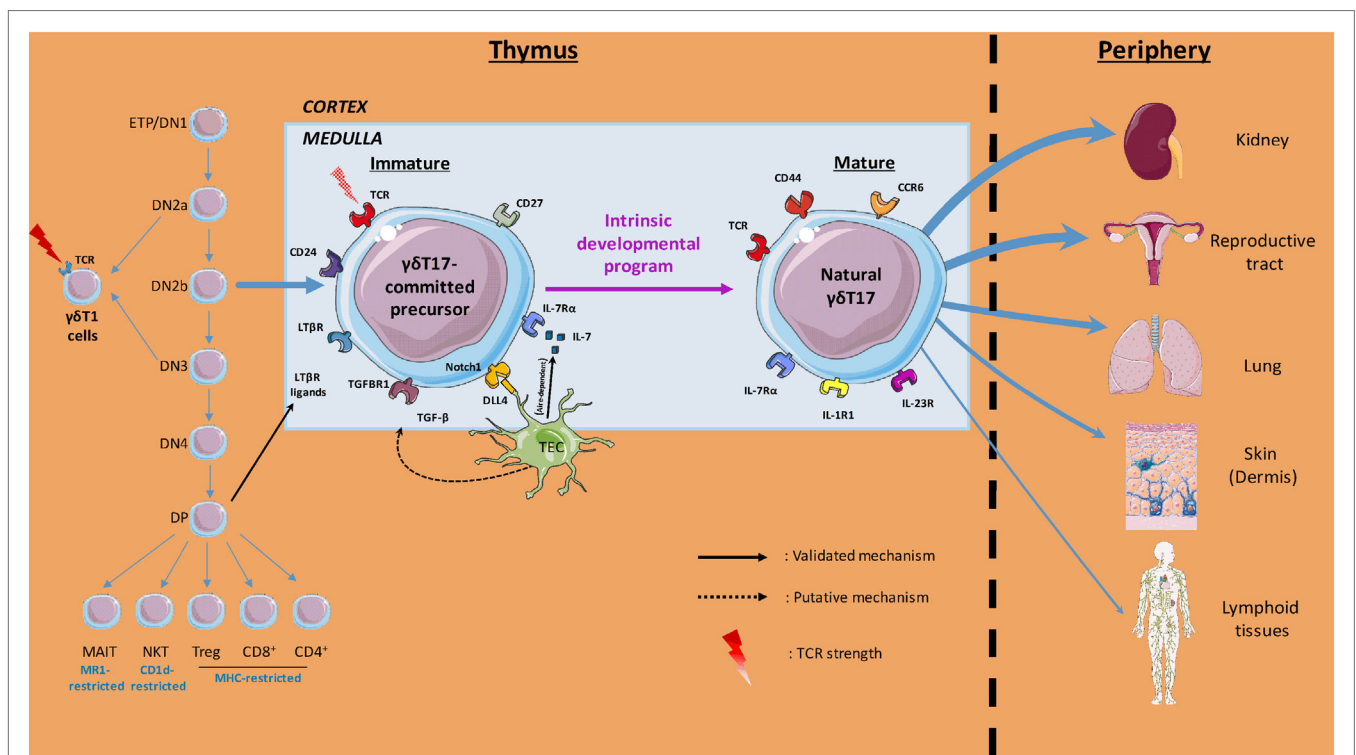


FIGURE 1 | Overview of thymic $\gamma\delta$ T17 cell ontogeny. Initial intrathymic pathways leading to pre-committed $\gamma\delta$ T17 cells divergence from other T cell lineages are illustrated. The thymic (cortical and medullary) environmental cues involved in $\gamma\delta$ T17 cell effector fate and the preferential peripheral niches of mature $\gamma\delta$ T17 cells are also depicted. Labels indicate the cells, soluble factors, proteins, surface markers, and T cell receptor (TCR) signal strength involved in the $\gamma\delta$ T17 cell program of differentiation.

in peripheral tissues under homeostatic and pathological situations has been demonstrated (37, 66, 67). Even if this question has never been directly addressed, many studies imply a requirement for IL-7 in the proper natural $\gamma\delta$ T17 cell development. First, in a model of conditional abrogation (RBP-J κ) that precludes IL-7R α (CD127) expression, but in which the initial generation of $\gamma\delta$ precursors (DN2 and DN3 stages) is maintained, the pool of both thymic and peripheral $\gamma\delta$ T17 cells is markedly reduced (68). Second, addition of recombinant IL-7 in fetal thymic organ culture from E16 thymus promotes $\gamma\delta$ T17 cell expansion over IFN- γ -producing $\gamma\delta$ T cells (37). Understanding whether IL-7 directly participates in the differentiation program or solely in the post-differentiation expansion of $\gamma\delta$ T17 cells will require further investigations. In line with the favorable temporal window for $\gamma\delta$ T17 cell development around birth, it is important to mention that thymus is particularly enriched for IL-7 [especially in thymic epithelial cells (TECs)] in neonates and its presence declines with age (69, 70).

Akin to conventional $\alpha\beta$ Th17 cells, TGF- β 1 has also been proposed to participate in $\gamma\delta$ T17 cell development (71). In addition, this report indicates that some other Th17-driving cytokines, such as IL-23 and IL-21, appear to be dispensable. However, since the authors have performed their investigation in 11-day-old mice, a time at which natural $\gamma\delta$ T17 cells already egressed the thymus, it is difficult to precisely evaluate the contribution of TGF- β 1 in natural $\gamma\delta$ T17 cell differentiation.

The role of IL-6 in $\gamma\delta$ T17 cell development is somehow controversial. While several studies indicated that IL-6 deficiency did not influence $\gamma\delta$ T17 cell homeostasis and cytokine production capacity (71, 72), others reported a defect in $\gamma\delta$ T17 cells in both thymus (73) and peripheral organs (74). Thus, the contribution of IL-6 in natural $\gamma\delta$ T17 cell differentiation remains unclear. In addition, mRNAs for IL-6R α (CD126) are barely expressed on both immature and mature thymic natural $\gamma\delta$ T17 cells (from the ImmGen database). However, it cannot be firmly excluded that IL-6 influences the thymic microenvironment to support $\gamma\delta$ T17 cell differentiation as previously hypothesized (73).

Thus, this indicates that the cytokine network within the thymic microenvironment has to be tightly regulated in a time-dependent manner to allow natural $\gamma\delta$ T17 cell differentiation.

Thymic Epithelial Cells

Akin to other thymocytes, interaction with TEC is likely critical in $\gamma\delta$ T17 cell differentiation, even though few experimental data are currently available. Thus, medullary (m)TEC has been implicated in regulating V γ 6⁺ $\gamma\delta$ T17 cell development through the TF autoimmune regulator (Aire) (69). In *Aire*^{-/-} mice, IL-7 production is up-regulated in mTEC, and this is accompanied by an overproduction of V γ 6⁺ $\gamma\delta$ T17 thymocytes. Interestingly, other subsets of natural $\gamma\delta$ T17 cells, especially V γ 4⁺ subsets were not affected by specific Aire deletion in mTEC (69). This feature also indicates that the various natural $\gamma\delta$ T17 cell subsets probably require different signals to develop.

Cortical (c)TEC has also been recently shown to control $\gamma\delta$ T17 cell development. Using a mouse model with specific cTEC ablation, Nitta and colleagues observed a strong dysregulation in the proportion of natural $\gamma\delta$ T17 cell subsets (75). Specifically, absence

of cTEC skewed the $\gamma\delta$ T17 TCR repertoire toward V γ 6 expression at the expense of the V γ 4⁺ T cell subset during the postnatal period while the proportions of V γ 6⁺ and V γ 4⁺ $\gamma\delta$ T17 cells remained normal during embryonic life. Authors hypothesize that in their mouse model, the postnatal thymic microenvironment resembles to the fetal microenvironment, which in turn favors the V γ 6⁺ subset. In addition, since they have been proposed to be a prime source of TGF β (76), cTEC could also participate in thymic development of $\gamma\delta$ T17 cells through this mechanism (71).

The importance of Dll4, a Notch ligand expressed by TEC (77) has also been proposed in $\gamma\delta$ T17 cell differentiation (78). In a co-culture model of E15 thymocytes with stromal cells, absence of Dll4 expression by stromal cells led to an abrogation in $\gamma\delta$ T17 cell development (78). This phenotype indicates that Dll4 is likely to be a common factor for the differentiation of all natural $\gamma\delta$ T17 cell subsets.

Finally, a recent study proposed that signaling through NF- κ B-inducing kinase (NIK) in TEC is essential for the generation of a fully functional pool of $\gamma\delta$ T17 cells (79). However, the molecular factors regulated by NIK in TEC are yet to be determined. Understanding the NIK-dependent pathways in TEC will certainly provide important clues in the TEC- $\gamma\delta$ T17 precursor interaction mechanisms that drive $\gamma\delta$ T17 cell effector fate.

Altogether, the fetal and perinatal thymic environment offers a temporal window of opportunity for $\gamma\delta$ T17 cell differentiation. However, the available literature indicates the requirement for differential factors according to the subset of naturally occurring $\gamma\delta$ T17 cells. This might somewhat rely on the intrinsic nature of the $\gamma\delta$ T17 precursors. It can also be hypothesized that these precursors (V γ 6⁺ and V γ 4⁺) require timely expressed TCR ligands in the thymic environment. However, no host-derived Ags have been proposed to date to participate in $\gamma\delta$ T17 cell differentiation.

Intrinsic Factors

A Requirement for TCR Ligation: Still an Open Question?

Beyond its importance into $\gamma\delta$ lineage commitment, TCR signal strength is also involved in the functional maturation of $\gamma\delta$ -committed thymocytes. Specifically, TCR signal strength drives the IL-17- vs IFN- γ -producing $\gamma\delta$ T cell dichotomy. However, it appears difficult to clearly attribute a specific strength to a specific effector fate. While the literature tends to demonstrate consensually that a strong TCR signaling in $\gamma\delta$ thymocytes drives their commitment toward a Th1-like effector fate (31, 80–82), the situation in $\gamma\delta$ T17 cell differentiation remains highly debated.

Chronologically, a first set of data suggested that $\gamma\delta$ T17 cell differentiation occurred in the absence of TCR cognate ligands (80). However, (1) this study used adult thymocytes and focused on peripheral organs that are weakly if not populated with natural $\gamma\delta$ T17 cells and (2) it cannot be excluded that ligand-independent TCR signaling plays a part in this model. Therefore, these results are likely to provide specific information about the requirement of TCR signals for inducible $\gamma\delta$ T17 cells. In this sense, these data perfectly fit with the concept that inducible $\gamma\delta$ T17 cells egress the thymus with a naive uncommitted profile and need further encounter with peripheral Ags to gain their capacity to produce IL-17. Few years later, the lab of Adrian Hayday highlighted the butyrophilin-like molecule Skint-1 as a molecular determinant in

Th1-like effector fate of $V\gamma 5^+$ T cells (31). Interestingly, in absence of Skint-1, the differentiation of $V\gamma 5^+$ T cells resulted in the generation of cells displaying a phenotype of natural $\gamma\delta$ T17 cells (31). Indeed, Skint-1 engagement in $V\gamma 5^+$ thymocytes induces the upregulation of TCR-dependent genes that subsequently repress the transcriptional differentiation program of natural $\gamma\delta$ T17 cells. In line, Pennington and colleagues recently demonstrated that differentiation of E15 $\gamma\delta$ thymocytes in presence of an anti-TCR δ mAb (GL3) blunted their commitment toward a $\gamma\delta$ T17 cell profile (82). Altogether, weak or no TCR signals seem required to allow proper $\gamma\delta$ T17 cell development. Thus, the developmental program of natural $\gamma\delta$ T17 cells appears to be a TCR Ag-free process acquired by “neglect.”

On the other hand, mice presenting a reduced function in the TCR proximal signaling kinase ZAP-70 displayed a reduced pool of both IL-17A-producing $V\gamma 6^+$ and to a lesser extent $V\gamma 4^+$ T cells in neonate thymocytes (83). In the same line, Silva-Santos and colleagues observed a reduction in the frequency of IL-17A-producing $V\gamma 6^+$ subset using double-heterozygous mice for the CD3 subunits γ and δ in which TCR signaling is attenuated (81).

These apparently contradictory results may have multiple explanations. Notably, it is assumable that the different subsets of $\gamma\delta$ T17 precursors may require a specific and fine-tuned TCR signals to engage in their differentiation program. Specifically, $V\gamma 6^+$ may require an “intermediate” TCR signals while $V\gamma 4^+$ subsets may need weak or no signals. Moreover, intensity of TCR signaling can be hardly compared from one experimental setting to another, making any generalization risky. In this context, the importance of TCR signaling in programming $\gamma\delta$ T17 cell differentiation is still an open question. This also raises the putative existence of TCR self-ligands for natural $\gamma\delta$ T17 cells. The recent discovery of butyrophilin-like molecules as Ags for mouse $\gamma\delta$ T cells (84, 85) opens a new exciting avenue of research in the field. Identification of the enlarged butyrophilin family in both mouse and human $\gamma\delta$ T cell biology might offer an interesting anchoring point for future translational studies.

Costimulatory Molecules

On top of the TCR, its accessory receptors have been proposed to participate in $\gamma\delta$ T cell differentiation. In addition to be a convenient marker to distinguish $\gamma\delta$ T functional subsets, the costimulatory receptor CD27 has been shown to participate in $\gamma\delta$ T cell development (14). Indeed, $\gamma\delta$ thymocytes from *Cd27^{-/-}* mice presented altered expression of *ifng*. Although CD27 deficiency did not influence the pool of $\gamma\delta$ T17 cells, CD27 gain of function in thymic cultures resulted in lower IL-17 transcripts by CD27⁻ $\gamma\delta$ thymocytes (14). Thus, CD27 appears as a thymic regulator in $\gamma\delta$ T cell effector fate.

Inducible T cell co-stimulator (ICOS) signaling pathway has also recently emerged as a possible determinant in $\gamma\delta$ T17 cell (at least for the $V\gamma 4^+$ subset) development (86). Agonistic activity of anti-ICOS mAb in fetal thymic organ culture significantly impaired $V\gamma 4^+$ $\gamma\delta$ T17 development. In line, genetic ablation of ICOS tends to increase the pool of thymic $V\gamma 4^+$ $\gamma\delta$ T17 cells (86). Thus, this study indicates that ICOS-dependent intracellular pathways in thymocytes controls $\gamma\delta$ T17 cell effector fate.

Besides, one must keep in mind, that, alongside with TCR and costimulation receptor signaling, multiple other signals have to be integrated by embryonic thymocytes to, *in fine*, engage toward the $\gamma\delta$ T17 effector fate.

Soluble Mediator Receptor Signaling Pathways

$\gamma\delta$ T17 precursors express specific receptors for various soluble factors produced in the thymic environment by hematopoietic and non-hematopoietic cells. Signals provided by these mediators have to be integrated by $\gamma\delta$ T cells to fully develop.

For instance, TGF β receptor (TGF β R) signaling pathway in developing $\gamma\delta$ T17 cells could be important in their effector fate. Mice deficient for Smad3, a critical component of the TGF β R signaling pathway presented a striking defect in frequency of thymic $\gamma\delta$ T17 cells compared with littermate controls (71). However, since the authors did not provide direct evidence (bone marrow chimera and OP-9 models) for an intrinsic role of the TGF β R signaling pathway, it cannot be excluded that this pathway is indirectly linked to $\gamma\delta$ T17 cell development.

In addition, the targeting of lymphotoxin- β receptor through double positive thymocytes-derived ligands (87) also controls the generation of $\gamma\delta$ T17 cells by regulating the expression of TFs from the NF- κ B family namely RelA and RelB (88).

As mentioned above, the IL-7/IL-7R α axis is important to generate a normal pool of $\gamma\delta$ T17 cells. However, a better understanding of the downstream molecular cascade involved will be helpful to understand whether this axis controls the differentiation program or the homeostasis of $\gamma\delta$ T17 cells.

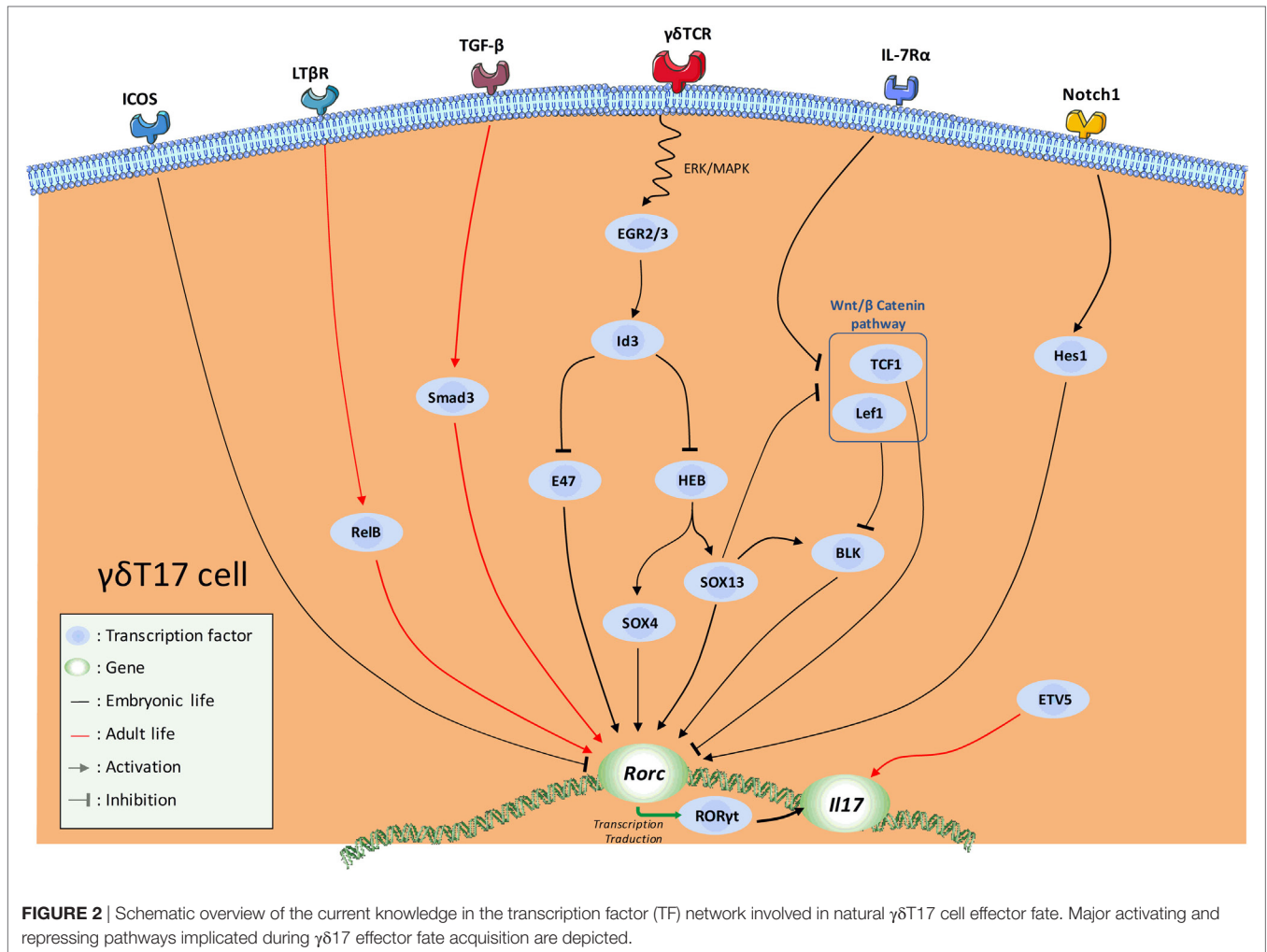
On the other hand, IL-15R α signaling disruption favors the development of $\gamma\delta$ T17 cells in thymus of neonates (65). The molecular mechanisms responsible for this are currently unknown but might rely on the activation of repressing factors in the IL-15R α signaling pathway or could be indirect by reducing the competition with $\gamma\delta$ T17-driving γ -chain-dependent cytokines such as IL-7.

The expression of the prostacyclin (PGI₂) receptor (IP) on thymocytes has also been demonstrated to control $\gamma\delta$ T17 cell development. This was evidenced by a failure to generate $\gamma\delta$ T17 cells in the thymus of IP^{-/-} mice (89). However, the molecular determinants involved in this process are yet to be defined.

The $\gamma\delta$ T17 Cell Transcriptional Program

The dynamic integration of these multiple signals leads to the implementation of a complex transcriptional program that dictates $\gamma\delta$ T17 cell effector fate (Figure 2). The aim of this program is ultimately to maintain and/or to favor the expression of Rorc (encoding ROR γ t), the cardinal TF for IL-17-secreting cells (90) including $\gamma\delta$ T cells (72). The recent advances in RNA deep sequencing analysis allowed a better understanding of the transcriptional regulation involved in $\gamma\delta$ T17 development.

Thus, SRY-related HMG-box (SOX) 4 and SOX13, two members of the high mobility group box TF family constitute a central node of regulation in this program (50, 91, 92). SOX4/13 are paramount in the acquisition of the $\gamma\delta$ T17 effector fate by (1) directly controlling *Rorc* transcription, (2) possibly enhancing important $\gamma\delta$ T17 cell-driving pathways such as IL-7R α signaling, and (3) possibly inhibiting *Rorc*-repressing TFs (31, 92).



In this later mechanism, SOX13 was suggested to inhibit the *Rorc*-repressing activity of two downstream mediators of the Wnt/ β -catenin signaling pathway namely lymphoid enhancer-binding factor 1 (Lef1) and transcription factor 1 (TCF1) (92). In this context, *Tcf7* (encoding for TCF1) deficiency leads to an aberrant high proportion of $\gamma\delta$ T17 cells (92). At this stage, it is also important to mention that this pathway also regulates the development of the IFN- γ -producing $V\gamma 1V\delta 6.3^+$ and $V\gamma 5^+$ cells (92). The mechanisms by which the TCF1–Lef1 axis counteracts the $\gamma\delta$ T17 transcriptional program are not fully understood. First, it is possible that TCF1 and Lef1 control *Rorc* expression through epigenetic (histone deacetylase) activity as suggested in conventional T cells (93). Alternatively, this axis could also indirectly repress *Rorc* expression by inhibiting the transcription of B lymphocyte kinase (Blk) (92), an important signal transducer in $\gamma\delta$ T17 cell development (94). However, how Blk controls ROR γ t expression is currently unknown. It is noteworthy that, using *blk*^{-/-} mice, $V\gamma 6^+$ T cells were shown to be more Blk-dependent than $V\gamma 4^+$ (94). As SOX13 was shown to regulate Blk expression and to control $V\gamma 4^+$, but minimally $V\gamma 6^+$, subset development (92), these results appear somewhat contradictory. However, $V\gamma 4$ and $V\gamma 6$ subsets develop at different temporal windows; therefore,

they are likely to integrate different thymic signals. As a result, this might modulate the relative importance of a same regulatory axis, and eventually leading to different effects on their respective transcriptional program. Thus, regulatory network required for $V\gamma 4^+$ ontogeny may be more SOX13-dependent than $V\gamma 6^+$ subset. Regarding the differential contribution of the TCR signaling in the $\gamma\delta$ T17 effector fate of these populations, Blk may play a role at this stage. According to its regulatory activity on TCR signaling, Blk could act as a “rheostat” to fine-tune signals delivered by the thymic $\gamma\delta$ T cell ligands. Thus, Blk deficiency might affect more $V\gamma 6^+$ ontogeny as TCR signaling has been proposed to control the development of these latter but not $V\gamma 4^+$. Of note, Blk overexpression has been shown to enhance IL-7 responsiveness in B cells (95).

As stated earlier, the TCR signaling pathway strongly influences the acquisition of the $\gamma\delta$ T17 effector fate. Mechanistically, TCR engagement induces upregulation of proteins of the early growth response (Egr) family namely Egr2 and Egr3 (31, 81). These two TFs positively regulate the DNA-binding protein inhibitor Id3. Thus, Id3 impairs $\gamma\delta$ T17 cell differentiation through (1) inhibition of HeLa E-box binding protein (HEB)-dependent *Sox4* and *Sox13* expressions (96) and (2) inhibition of the *Rorc* promoter

E47 (91). This scheme is also in line with the differential requirement for TCR strength in $\gamma\delta$ T17 cell effector fate of $V\gamma 6^+$ and $V\gamma 4^+$ further emphasizing the differences in the developmental programs of $V\gamma 6^+$ vs $V\gamma 4^+$ $\gamma\delta$ T17 cells.

In addition, the promyelocytic leukemia zinc finger (PLZF) protein is a key TF in the development of some innate and innate-like lymphocytes that dictates their acquisition of a Th-like effector program (97–99). PLZF was shown to control $V\gamma 6^+$ differentiation into $\gamma\delta$ T17 cells (100). The molecular mechanisms that govern PLZF activity in $V\gamma 6^+$ development are currently unknown and require further investigations. PLZF contribution in $V\gamma 4^+$ $\gamma\delta$ T17 cell development has not been assessed yet. However, it is noteworthy that PLZF does not appear to be expressed in neonates $V\gamma 4^+$ precluding a role of this TF for this particular subset (100).

Among the intracellular pathways that dictate the $\gamma\delta$ T17 effector fate, Notch signaling contributes to the generation of $\gamma\delta$ T17 cells through the helix-loop-helix protein Hes1 (78). Since it mainly exerts transcriptional repressing activities, it is possible that Hes1 acts in one of the pathways discussed above. Unrevealing the Hes1 interactome in developing $\gamma\delta$ T17 cells will be informative to get insight into the molecular factors that regulate this mechanism. An additional pathway by which the Notch signaling pathway could influence $\gamma\delta$ T17 cell development is by promoting IL-7R α expression through the RBP-Jk pathway (68). However, this pathway was only described in peripheral $\gamma\delta$ T17 cells of adult mice to control their homeostasis and self-renewal.

In addition to its role in proliferation/survival of $\gamma\delta$ T17 cells during development, the IL-7R α signaling pathway is also likely to contribute to their transcriptional program. Indeed, IL-7/IL-7R α signaling in fetal thymocytes was demonstrated to blunt both *Lef1* and *Tcf7* expression (101).

In silico analyses have also been fruitful to understand the transcriptional program of $\gamma\delta$ T17 cells. Using an algorithm that predicts important regulators across various lineages, the TF ETV5, along with SOX13 was proposed as a master regulator in $V\gamma 4^+$ $\gamma\delta$ T17 cell differentiation (102). Conditional ablation of ETV5 in T cells confirmed the role of this TF in $V\gamma 4^+$ $\gamma\delta$ T17 effector fate (102). Absence of ETV5 in developing $V\gamma 4^+$ slightly reduced ROR γ t expression but severely impaired IL-17 secretion. This is reminiscent with the situation for Th17 cell differentiation in which ETV5 directly promotes *il17a* and *il17f* expressions but has no influence on *Rorc* (103). The role of ETV5 in $V\gamma 6^+$ development remains to be determined; however, ETV5 is highly expressed on immature fetal $V\gamma 6^+$ and strongly repressed upon maturation (<http://www.immgen.org/databrowser/index.html>).

Originally thought to be acquired by “neglect,” this literature underlines that the $\gamma\delta$ T17 effector fate is under the control of a very active process in which the SOX4/13 axis acts as a guardian for proper ROR γ t expression. In addition, the discrepancies in the phenotypes observed for $V\gamma 4^+$ and $V\gamma 6^+$ $\gamma\delta$ T17 cells imply different programs for natural $\gamma\delta$ T17 cell development. Thus, further transcriptomic analyses at single cell resolution are clearly required to better decipher the overlapping and/or specific developmental “trajectories” that drive the effector fate of these subsets.

What Can We Learn From Transcriptomic Analysis of Developing Natural $\gamma\delta$ T17 Cells?

As stated above, the recent advances in the quality of whole genome analyses allowed to validate and/or to predict the involvement of numerous genes in the transcriptional signature of many cell populations including $\gamma\delta$ T cells. In addition, normalized and comparative analysis of various gene sets among lymphocyte lineages led to the identification of conserved and/or distinct signature pathways in their effector program including Th17(-like) effector fate (104).

However, *in silico* analysis of the transcriptional program of $\gamma\delta$ T17 cells has been mainly discussed regarding the maturation of the $V\gamma 4^+$ cell subset in adult mice (91, 92, 104). Thus, this population comprises both “inducible” $\gamma\delta$ T17 cells as well as non-IL-17-producing subsets. To focus on “natural” $\gamma\delta$ T17 cells that develop during embryonic life, we reanalyzed the datasets of developing $V\gamma 6^+$ T cells (immature/CD24^{hi} vs mature/CD24^{low}) (GSE37448). Bioinformatic analysis generated a gene set of the top 1,000 transcripts significantly regulated during the effector fate acquisition of this subset (Table S1 in Supplementary Material). Interestingly, while similar analysis on developing fetal $V\gamma 5^+$ T cells (GSE15907) indicated that 87.8% of the 1,000 top regulated genes were up-regulated, only 48.7% did so in the $V\gamma 6^+$ dataset (Figure 3). This emphasizes the fact that, unlike $\gamma\delta$ T1, $\gamma\delta$ T17 cell effector fate is rather acquired using a repressing model.

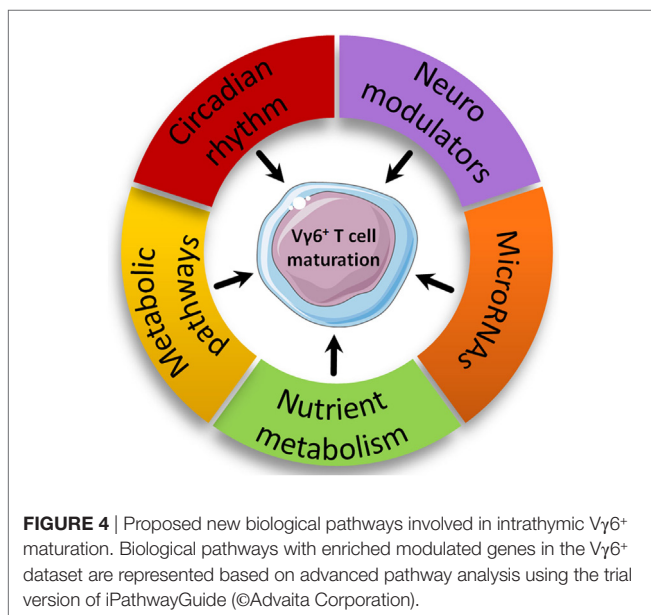
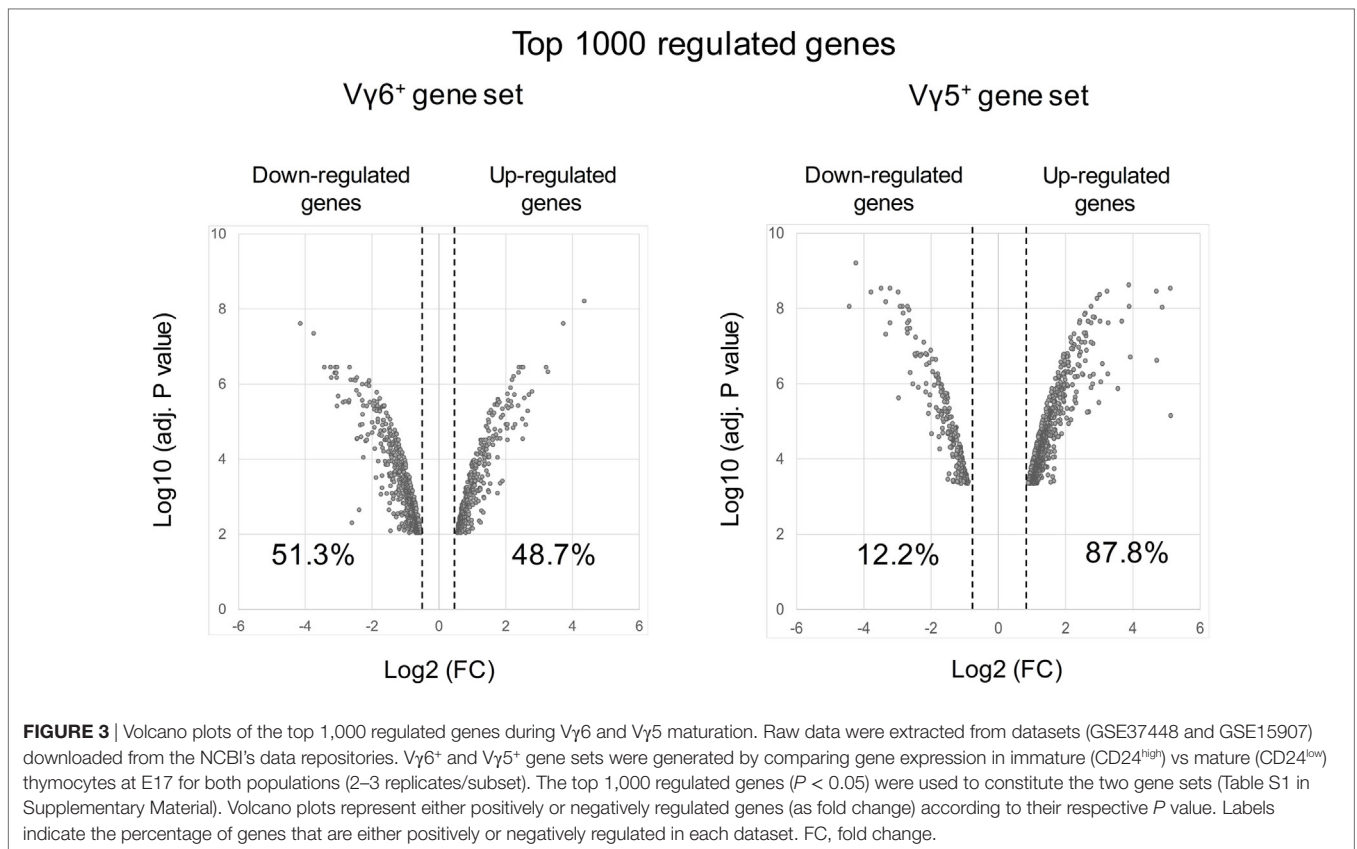
As expected, among the list of gene generated, we found many up-regulated genes shared with other innate(-like) or adaptive IL-17-producing lymphocytes including *Rorc*, *Il17a*, and *Il17f* and the cytokine/chemokine receptors *Il1r1*, *Il2rb*, *Il7r*, *Il17rc*, *Il17re*, *Il23r*, *Il18r1*, *Ccr6*, and *Cxcr6*. This is paralleled by a silencing of genes involved in Th1 and Th2 differentiation, such as *Il2ra*, *Il12rb2*, *Lck*, *Gata3*, and *Maml2*.

In addition, we noted numerous genes involved in TCR signaling including *Lck*, *Nck2*, *Pak1*, *Plcg*, *Prkcq*, *Ptpn22*, and *Nfkbie*. Notably, all these transcripts were down-modulated during $V\gamma 6^+$ maturation. Moreover, genes involved in costimulation, such as *Cd27*, *Cd28*, *Icos*, *Themis*, *Slamf1*, *Slamf6*, and *Pik3r2*, were also repressed upon differentiation. In line, it is noteworthy that the transcript encoding for the nuclear receptor Nur77 (*Nr4a1*), a faithful marker of TCR strength (105) is strongly repressed during $V\gamma 6^+$ maturation. Given the controversy discussed previously, these observations clearly suggest that the TCR signaling pathway has to be maintained under tight regulation to allow $V\gamma 6^+$ T cell differentiation.

More importantly, using advanced pathway analysis, we pinpointed, in the $V\gamma 6^+$ T cell gene set, multiple family of genes involved in biological processes and molecular pathways that might be involved in their developmental program (Figure 4).

Nutrient Metabolism

Along with *Nr4a1*, other genes encoding for nuclear receptors such as receptors of vitamin A (*Rarg*) and D (*Vdr*), two vitamins reported to participate in ILC, $\gamma\delta$ T and NKT (25, 106) homeostasis and development are up-regulated during $V\gamma 6^+$ maturation.



In addition, genes encoding for vitamin transporters (*Slc23a2* and *Slc2a3*) were also modulated in the V γ 6⁺ gene set. This could imply an important role for vitamins in $\gamma\delta$ T17 cell development. Somewhat related, expression of *gpr183*, a sensor of oxysterols was strongly up-regulated (3.6 fold) in developing V γ 6⁺ T cells.

Interestingly, GPR183 has recently emerged as a critical player in the control of ILC3 homeostasis (107). Moreover, oxysterols are ligands for ROR γ t and drive Th17 cell differentiation (108). The influence of nutrient-derived metabolites on lymphocyte immunity including early development has recently gained considerable attention (2). The recent discovery of vitamin B2 metabolites as Ags for MAIT cells (109) will certainly reinforce the interest of immunologists for nutrient metabolism. The availability of vitamins *in utero* has also been shown to control the quality of the immune system in later life (110). According to these arguments and the temporal window of development for $\gamma\delta$ T17 cells, investigating the nutrient metabolism in $\gamma\delta$ T17 cell biology will be likely to provide new interesting data on the influence of maternal diet in shaping immunity.

Neuroimmunology

In line with the emerging concept of neuroimmunology, numerous members of the “neuroactive ligand-receptor interaction” pathway were present in the V γ 6⁺ gene set including genes encoding for neuroactive substance receptors of neuropeptide (*Gpr83*), hormones (*Sstr2*, *Rxfp1*, and *Calcr1*), prostanoids (*Ptger4*, *Ptgfrn*, and *Ptgir*), leukotrienes (*Cysltr2* and *Ltb4r1*), nucleotides (*P2rx7*, *Adora2a*, and *Lpar4*), and amino acids (*Gabbr1*, *Gabbr2*, and *Gria3*). Interestingly, the above-mentioned genes encoding for prostanoids, hormones, and nucleotide receptors are down-regulated during V γ 6⁺ T cell maturation, while those encoding for leukotrienes are up-regulated.

The relationship between neuromodulators and immune cell development is largely unexplored. However, there are evidence for an expression of neuromodulators and their associated-receptors in TEC and on thymocytes, respectively (111). Furthermore, *ex vivo* somatostatin (ligand for *Sstr2*) addition in FTOC increased thymocyte numbers and maturation. By contrast, both neuropeptide Y (ligand for *Gpr33*) and calcitonin (ligand for *Calcr1*) reduced thymocyte numbers (112). Last, *Ptgir* (encoding for the prostaglandin I₂ receptor) has already been biologically validated to participate in natural $\gamma\delta$ T17 cell development (89). Regarding this, a broad analysis of the eicosanoid family in $\gamma\delta$ T17 cell development should be encouraged.

Circadian Rhythm

Among highly regulated genes, our analysis also retrieved genes related to circadian rhythm. Thus, we found that *Nr1d1*, *Nr1d2*, and *Bhlhe40*, which encodes for REV-ERB α , REV-ERB β , and Dec1 proteins, respectively, were all up-regulated in mature V γ 6⁺ T cells. These proteins are critical repressors of *Arntl* (encoding for Bmal1), *Npas2*, and *Clock*, three master clock genes (113, 114). Of note, *Arntl*, *Npas2*, and *Clock* were also significantly regulated in developing V γ 6⁺ T cells. Recent literature has emphasized the importance of circadian rhythms in fine-tuning immune responses (113, 115). Interestingly, ROR γ t expression has been shown to be under circadian regulation through a REV-ERB α -dependent mechanism (116). Thus, these data may suggest a role for the circadian rhythm in V γ 6⁺ T cell biology. Of note, NFIL3 (E4BP4), a repressor of the circadian clock, is implicated in the differentiation/development of ILCs and NK cells (117–119). In addition, we can speculate that the regulation of these clock genes helps mature V γ 6⁺ T cells to integrate and to regulate circadian cues once in the periphery, as demonstrated for many other cellular actors of innate immunity (113).

Immunometabolism

Regarding the growing interest for understanding immunometabolic pathways implicated in leukocyte biology, we searched for genes involved in metabolic pathways. Our analysis revealed that many genes involved in the six major metabolic pathways specifically glycolysis (*Aldh2*, *Ldhd*, *Acss1*, and *Acss2*), tricarboxylic acid cycle (*Idh1*, *Idh2*, and *Aco1*), pentose phosphate pathway (*Fbp1*), fatty acid oxidation (*Cpt1a*, *Nr4a3*, *Peci*, *Auh*, *Pex5*, and *Ivd*), fatty acid synthesis (*Fads2* and *Slc45a3*), and amino acid metabolism (*Aco1* and *Bcat1*) are down-regulated in mature V γ 6⁺ T cells.

Interestingly, fatty acid oxidation is associated with regulatory T cell differentiation while glycolysis is a major metabolic pathway in effector T cell differentiation (120, 121). Of note, expression of *Cpt1a* was reduced in Th17 cells compared with regulatory T cells (120). Enhanced fatty acid synthesis and glycolysis in immune cells, especially T cells, have been regarded as markers of inflammatory cells required for acquisition of effector functions upon inflammatory conditions (122). This adds a metabolic argument into the fact that V γ 6⁺ T cells are “preset” cells with metabolic programming occurring during development, to be immediately and fully functional once in peripheral tissues.

MicroRNAs

To date, there is limited literature on the role of microRNAs in leukocyte development. In our gene set, we detected the presence of five microRNAs (*mir15b*, *mir181a-1*, *mir181a-2*, *mir181b-1*, and *mir181b-2*), all of them being down-regulated in mature V γ 6⁺ T cells. Of note, miR-181 was reported to be essential in NKT cell development (123, 124). Interestingly, the group of Immo Prinz studied the impact of miR-181a/b-1 deficiency on $\gamma\delta$ T cell development. While thymic V γ 1⁺ and V γ 4⁺ T cell subsets were unaltered in the absence of miR-181a/b-1, authors reported a higher frequency of thymic, but not peripheral V γ 6⁺ cells in miR-181a/b-1-deficient mice (125). The reason that underlines this feature is currently unknown. However, since miR-181 is a well-known positive regulator of the TCR signal strength (126), it is tempting to hypothesize that a reduced TCR signaling confers an advantage for V γ 6⁺ T cell differentiation. Thus, defining the miRome of developing $\gamma\delta$ T17 cells will potentially bring a novel layer of complexity in their developmental program.

Even if these *in silico* analyses suggest a role for scantily explored biological pathways in $\gamma\delta$ T17 cell development and/or maintenance, supportive experimental data are clearly required to explore these predictive hypotheses. It is noteworthy that these proposed biological pathways are not meant to be V γ 6⁺ cell-specific and, therefore, it does not preclude that other cell types including other $\gamma\delta$ T cell subsets may rely on similar biological pathways to develop. In addition, instruction that drives $\gamma\delta$ T17 effector fate is likely to start early in thymocyte development [even before TCR rearrangement (49)]; therefore, comparing immature vs mature populations probably induced an important bias in our analysis.

CONCLUDING REMARKS

Despite the considerable body of work performed in the field of $\gamma\delta$ T17 cell ontogeny, many questions remain unsolved and sometimes appear more complex than initially thought. Even if revealing bulk transcriptomes have been informative to predict the developmental program of $\gamma\delta$ T17 cells, they present a major drawback since this kind of analysis does not reflect the dynamic aspect of the development. In this context, the recent and rapid evolution in single cell deep RNA sequencing and bioinformatics technologies will undoubtedly help to reveal the developmental “trajectories” that dictate $\gamma\delta$ T17 cell effector fate. In addition, other layers of regulation such as post-transcriptional (especially epigenetic) regulation and implication of microRNAs deserve further investigations and will have to be integrated in order to better decipher the general mechanism(s) driving $\gamma\delta$ T17 cell development. Given the critical role of $\gamma\delta$ T17 cells in major health concerns such as infections and cancer, advances in these fundamental biological processes are clearly mandatory.

AUTHOR CONTRIBUTIONS

YJ, EP, MH, MS-T, TB, and CP prepared and wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at <https://www.frontiersin.org/articles/10.3389/fimmu.2018.00981/full#supplementary-material>.

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