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Developmental origins of murine γδ T-cell subsets

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Summary

Murine γδ T cells display diverse responses to pathogens and tumours through early provision of pro-inflammatory cytokines such as interleukin-17A (IL-17) and interferon-γ (IFN-γ). Although it is now clear that acquisition of these cytokine-secreting effector fates is to a great extent developmentally pre-programmed in the thymus, the stages through which γδ progenitor cells transition, and the underlying mechanistic processes that govern these commitment events, are still largely unclear. Here, we review recent progress in the field, with particular consideration of how TCR-γδ signalling impacts on developmental programmes initiated before TCR-γδ expression.

Keywords: T-cell development; thymus; γδ T cells; IL-17; IFN-γ; TCR-γδ.

Thymocyte commitment to a γδ T-cell fate

Consensus holds that αβ and γδ T cells originate from a common thymic progenitor.[1] Expression of a pre-T-cell receptor (TCR-β paired with the invariant pre-TCR-α chain) directs $CD4^-$ $CD8^-$ double-negative (DN) cells to the αβ lineage and promotes their progression to a $CD4^+$ $CD8^+$ double-positive (DP) stage that serves as a precursor pool for conventional $CD4^+$ and $CD8^+$ T cells.[2] By contrast, DN cells that express TCR-γδ commit to the γδ lineage and become functionally mature without progression through the DP stage. Two basic models were proposed to explain αβ/γδ lineage commitment; a pre-commitment-selection model and a signal strength model.[3]

The pre-commitment-selection model suggested that lineage fate was determined before TCR-chain rearrangement. Subsequent TCR-γδ expression in γδ precursors or pre-TCR expression in αβ precursors then served to confirm lineage specification and promote further development of γδ lineage and αβ lineage cells, respectively. Cells with mismatched lineage specification/TCR were thought to die. In support of this, higher expression in early DN cells of both interleukin-7 receptor α (IL-7Rα),[4] and Sox13,[5] was shown to identify TCR^- thymocyte precursors with greater propensity to develop as γδ T cells. Despite this, the pre-commitment-selection model never really took hold. Indeed, recent reports suggest that only IL-17-secreting T γδ cells that use TCR-γ chain variable region-4 (Vγ4) (using Tonegawa nomenclature[6]) were absent in mice that lacked Sox13.[7,8]

By contrast, the signal strength model of αβ/γδ lineage choice has gained widespread support.[9,10] It proposes that TCR signal strength, rather than TCR identity, dictates αβ/γδ lineage fate. Hence, DN cells receiving a weak TCR signal commit to the αβ lineage, whereas DN cells perceiving a strong TCR signal develop as γδ T cells. This could also be considered an instructional model, as under normal conditions the pre-TCR provides a weak signal (presumably due to its very low surface expression), whereas TCR-γδ signals more strongly.

Although compelling evidence for the signal strength model now exists, it is still unclear how DN cells discriminate between different signal strengths. Activation of the extracellular signal-regulated kinase (ERK) pathway appears to be important, which can induce early growth response (Egr) family transcription factors and inhibitor of DNA binding 3 (Id3).[11] More recently, non-canonical targeting of docking site for ERK, FXF (DEF) domain containing ERK substrates through the ERK DEF-binding pocket has also been implicated in γδ T-cell fate commitment.[12] Hence, strong TCR signalling delivered mainly by TCR-γδ complexes drives DN cell commitment to the γδ T-cell lineage.

Waves of thymic γδ T-cell development

It is long established that γδ T cells develop in waves that are associated with the usage of certain Vγ-regions and eventual tissue location.[13] Murine γδ T cells begin to appear during the mid-to-late fetal period (~E13.5). $V\gamma5^+$ precursors of skin-resident dendritic epidermal T cells

develop first, closely followed by Vγ6$^+$ cells that locate to various epithelial sites such as the reproductive tract. Vγ7$^+$ precursors of ileal intraepithelial lymphocytes appear next, around birth, followed by Vγ1$^+$, Vγ2$^+$ and Vγ4$^+$ cells that are the dominant subsets in tissues such as the dermis, lung, colon and liver, and in peripheral lymphoid organs. Interestingly, the sequential appearance of Vγ5$^+$, Vγ6$^+$, Vγ7$^+$ and then Vγ4$^+$ cells reflects the order of these Vγ regions at the Cγ1-TCR-γ locus.[14]

Thymic $\gamma\delta$ T-cell development can also be correlated to acquisition of distinct effector potentials.[15] The initial skin-homing Vγ5$^+$ cells are reported to secrete interferon-γ (IFN-γ).[16] There then follows a perinatal period dominated by generation of IL-17-secreting $\gamma\delta$ T cells that include initially all Vγ6$^+$ cells and then later a majority of Vγ4$^+$ cells. Nonetheless, Vγ1$^+$ and Vγ2$^+$ cells also develop during this period (alongside Vγ4$^+$ cells) with IL-17-secreting potential.[17] The perinatal period also sees the development of Vγ7$^+$ precursors of ileal intraepithelial lymphocytes, and a natural killer T (NKT) -like $\gamma\delta$ T-cell subset that uses a Vγ1Vδ6.3/6.4$^+$ invariant TCR $\gamma\delta$, can secrete IFN-γ, IL-4 and IL-15, and that homes preferentially to the liver.[18] Interferon-γ-secreting $\gamma\delta$ T ($\gamma\delta$IFN) cells without IL-4 secretion also begin to appear in the neonatal period and continue to be produced well into adulthood.[19] Finally, a subset of seemingly uncommitted $\gamma\delta$ T cells is also generated from the neonatal period onwards. These appear to possess 'naive'-like qualities and may retain the potential to adopt multiple effector fates in the periphery.[20–22]

Development of IFN-γ-secreting $\gamma\delta$ T-cell subsets

TCR-$\gamma\delta$ signalling above a certain threshold is clearly required to commit DN cells to the $\gamma\delta$ lineage. However, beyond this commitment step, TCR-$\gamma\delta$ signalling also plays a key role in thymic acquisition of $\gamma\delta$ T-cell effector fate.[16,17,23–25] Thymic TCR-agonist selection events are associated with development of Vγ5Vδ1$^+$ dendritic epidermal T cells,[16,26] Vγ1Vδ6.3/6.4$^+$ NKT-like $\gamma\delta$ T cells[27,28] and Vγ4Vδ5$^+$ T10/T22-specific $\gamma\delta$ T cells[23,25] that all acquire the potential to secrete IFN-γ. Conversely, attenuation of TCR-$\gamma\delta$ signal strength adversely affects the generation of $\gamma\delta$IFN cells.[16,17,23,24]

Up-regulation of several surface proteins appears to mark TCR-$\gamma\delta$-mediated progression of CD24$^+$ $\gamma\delta$ precursors toward a general IFN-γ-secreting phenotype (Fig. 1).[16,17,19,23,29,30] However, an understanding of how these markers relate to ligand-independent (akin to tonic signalling) versus ligand-induced TCR-$\gamma\delta$ signalling (which are both presumably of sufficient strength for commitment to the $\gamma\delta$ lineage) is challenging. Recent evidence from adult mice suggests that ligand-independent TCR-$\gamma\delta$ signalling may be sufficient to generate naive-like $\gamma\delta$ T cells that progress from a CD25$^+$ CD371$^+$ CD24$^+$ stage by down-regulating first CD25 and then CD371 to generate CD25$^-$ CD371$^-$ CD24$^+$ cells that can exit the thymus to seed peripheral lymphoid organs.[30] By contrast, increased TCR signal strength (as induced for example by anti-TCR-δ antibody engagement) up-regulates CD200, closely followed by CD73 and CD45RB.[16,17,30] However, it is not yet clear whether these events are a consequence of only TCR $\gamma\delta$/ligand interactions, or whether stronger ligand-independent signalling is also sufficient.[31] Nonetheless, it is more certain that *bona fide* cell-bound agonist/TCR engagement (e.g. for selection of Vγ1Vδ6.3/6.4$^+$ NKT-like $\gamma\delta$ T cells[28]) drives up-regulation of additional markers such as CD44, NK1.1 and CD122 that identify various terminally differentiated $\gamma\delta$IFN cell subsets.[27] Many of these also become CD24$^-$, especially when developing during the neonatal period.[30]

IL-17-secreting $\gamma\delta$ T-cell development

There is presently no convincing model that adequately explains the distinctive characteristics of thymic $\gamma\delta$17 cell development. Such a model would need to describe the restricted perinatal generation of $\gamma\delta$17 cells, which is generally attributed to largely undefined features of the perinatal thymic environment that are different to the adult thymus.[15] Nonetheless, adoptive transfer of adult bone marrow into IL-17-deficient recipients fails to reconstitute the $\gamma\delta$17 cell compartment.[32] In addition, adult thymocyte precursors also fail to generate $\gamma\delta$17 cells in a grafted embryonic thymus, suggesting that capacity to initiate an IL-17-specific $\gamma\delta$ T-cell programme is intrinsic to embryonic progenitors.[32] However, substantial evidence to support this idea is still largely lacking.

Particularly perplexing for the development of $\gamma\delta$17 cells is the role of TCR-$\gamma\delta$ signal strength.[17] A recent report that observed CD73 expression (purported to indicate TCR $\gamma\delta$ engagement) on adult thymic $\gamma\delta$17 cells suggested a requirement for strong TCR-$\gamma\delta$ signals.[33] As too did absence of $\gamma\delta$17 cells in mice with defective Zap-70 function.[34] However, this Zap-70 dependence was not obviously correlated with strong TCR-$\gamma\delta$ signalling, and the role of Zap-70 has since been disputed by claims that $\gamma\delta$17 cells instead require Syk.[35] Furthermore, CD73 and CD45RB (that also marks TCR-$\gamma\delta$ engagement[16,33]) are both absent on thymic $\gamma\delta$17 cells that develop in the perinatal window.[17]

By contrast, a body of evidence instead suggests that $\gamma\delta$17 cell development requires weak (or even absent) TCR $\gamma\delta$ signalling.[16,17,19,23,25] For example, generation of $\gamma\delta$17 cells is favoured by the reduction of ERK activity.[12,17] In addition, Vγ5Vδ1$^+$ $\gamma\delta$ T cells developing in the absence of Skint1 adopt IL-17-secreting characteristics.[16] Also, engagement of TCR-$\gamma\delta$ with even very low concentrations of activating anti-TCR-δ antibody reduces the generation of all IL-17-committed $\gamma\delta$ T cells dramatically.[17]

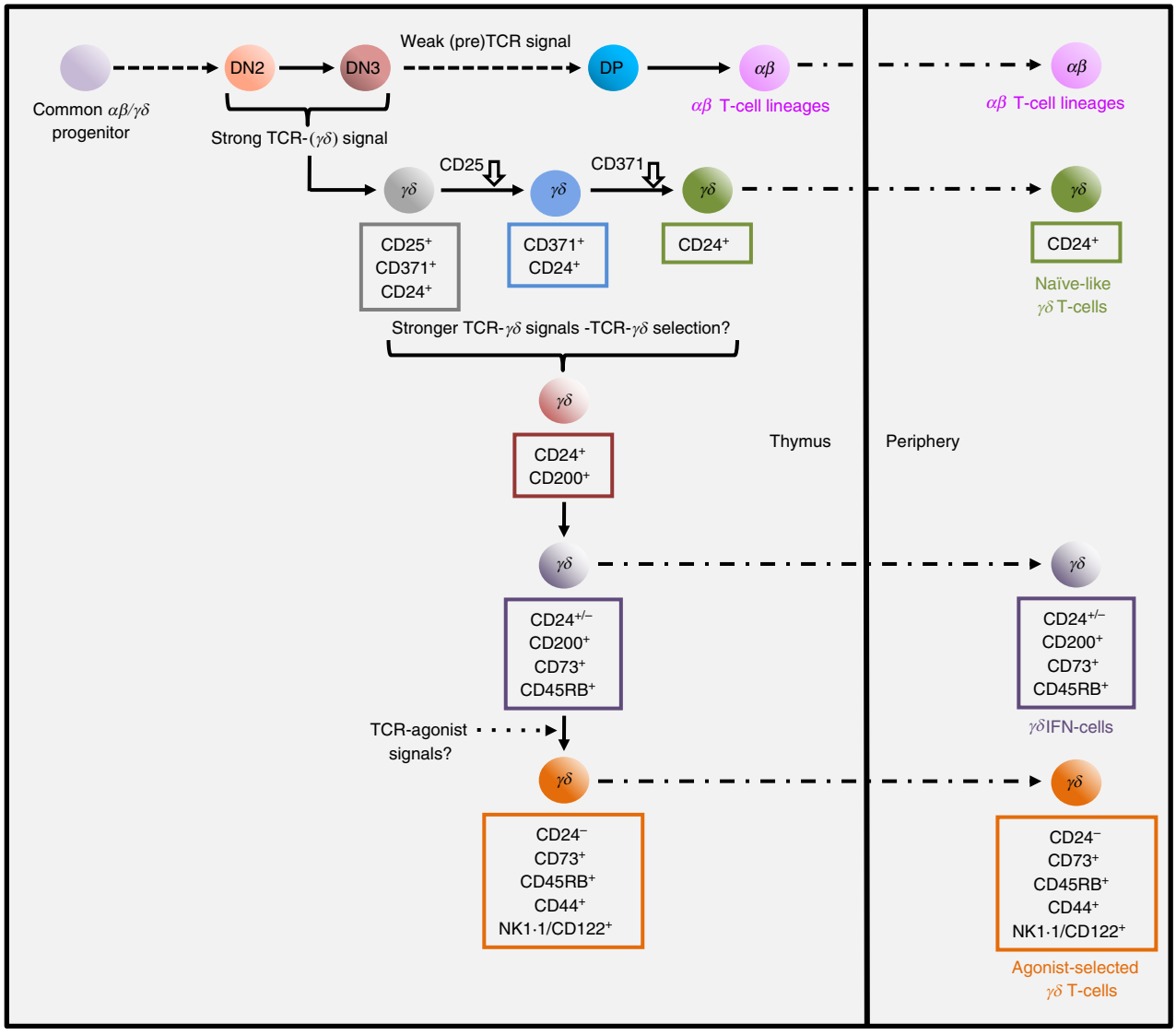

Figure 1. Murine thymic γδ T-cell development from a common αβ/γδ progenitor. αβ/γδ progenitors are diverted into the γδ lineage as a result of strong T-cell receptor (TCR) signals. Stronger TCR γδ signal strength (ligand-independent or cell-bound agonist-driven) appears to drive γδ thymocytes to interferon-γ (IFN-γ) -committed (γδIFN) or agonist-selected γδ T-cell fates (the latter possibly aided by co-stimulatory signals). Phenotypes of thymic γδ subsets and their peripheral counterparts are indicated. Downward arrow indicates marker down-regulation. '?' denotes ongoing uncertainty.

Whether weak TCR-γδ signalling equates to ligand-independent tonic signalling (as has been suggested[23]) remains unclear. Indeed, it is even possible that weak TCR-γδ signalling actually translates as 'alternative' TCR-γδ signalling in which the TCR acts simply as a scaffold to enable certain downstream pathways to operate. Such 'adapter-like' signalling has been described for the B-cell receptor that is suggested (at certain stages of B-cell development) to act as an adaptor protein that brings Syk to the BAFF receptor.[36] Nonetheless, a conclusion that only weak or alternative/absent TCR γδ signalling is compatible with the generation of γδ17 cells is difficult to reconcile with the requirement for strong TCR-γδ signalling to commit DN progenitors to the γδ lineage. Unfortunately, this consistently ignored paradox has yet to be adequately resolved.

A further defining feature of γδ17 cell development is involvement of distinct signalling modalities that are not required for the generation of other γδ T-cell subsets. For example, thymic γδ17 cells are absent or significantly reduced in mice deficient for the following: Hes-1, which is downstream of Notch-1 signalling;[37] HEB, an E protein that functions alongside the E2A proteins (E12 and E47);[38] Syk, which is possibly linked in γδ17 cells to phosphoinositide 3-kinase;[35] Blk, a B-cell kinase;[39] and transforming growth factor-β and Smad3.[40] At present,

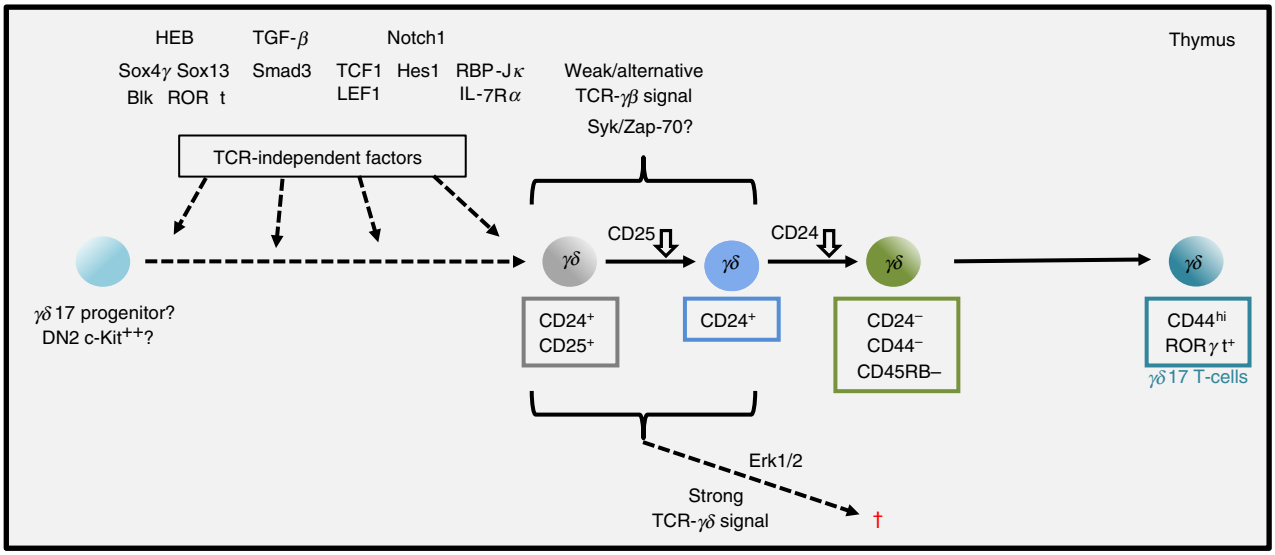

Figure 2. Model for the development of murine interleukin-17 (IL-17) -secreting ($\gamma\delta$17) $\gamma\delta$ T cells. IL-17-secreting $\gamma\delta$ T cells may develop from a separate pool of progenitors with a pre-determined 'IL-17 programme' that is established by expression of transcription factors (depicted) before expression of the T-cell receptor (TCR). Subsequently, the programme is reinforced by weak or alternative signalling mediated by the expression of specific TCR-$\gamma\delta$. In the event of increased signal strength, TCR-$\gamma\delta$-expressing thymocytes may undergo programmed cell death (†). Downward arrow indicates marker down-regulation. '?' denotes ongoing uncertainty.

the interconnection of these pathways is unclear. As too is their relationship to the weak/absent TCR-$\gamma\delta$ signalling that is permissive for $\gamma\delta$17 cell development.

Transcription factor networks

Much recent effort has been directed at understanding the transcriptional networks that underpin the thymic development of $\gamma\delta$ T cells. Strong TCR-$\gamma\delta$ signalling, for example in Vγ5Vδ1$^+$ progenitors that engage Skint1$^+$ cells,[16] up-regulates Egr3 and Id3 in an ERK-dependent manner.[11] Egr3 induces T-bet (and CD45RB), while suppressing Sox13 and RORγt, that appear to specify a $\gamma\delta$17 fate (Sox13 also down-regulates the $\gamma\delta$IFN markers NK1.1 and CD27).[16] Moreover, Id3 antagonizes the function of the E protein transcription factor HEB, which directs $\gamma\delta$17 development by up-regulation of Sox4 and Sox13.[38] Sox4$^{-/-}$ and Sox13$^{-/-}$ mice specifically lack Vγ4$^+$ $\gamma\delta$17 cells (although the Vγ6$^+$ $\gamma\delta$17 pool is still largely intact).[7] Indeed, Sox4 and Sox13 deficiency dramatically decreased RORγt and Blk (in Vγ4$^+$ $\gamma\delta$17 cells). This study also demonstrated a dramatic increase in the ratio of $\gamma\delta$17 : $\gamma\delta$IFN cells in both the Vγ1$^+$ and Vγ4$^+$ compartments of TCF1$^{-/-}$ mice, and suggested that antagonism between TCF1 (which is induced by Notch-1 signalling) and Sox13 may control downstream regulators such as TCF1-induced LEF1 to influence $\gamma\delta$17 versus $\gamma\delta$IFN fates.[7] Notch-1 signalling via RBP-Jκ can also increase IL-7Rα expression.[41] IL-7 was previously shown to expand $\gamma\delta$17 cells during fetal thymic development, largely through activation of signal transducer and activator of transcription 3.[42] Moreover, IL-7 in combination with IL-1β, IL-21 and IL-23 was suggested to 'dampen' strong TCR-$\gamma\delta$ signals by reducing Id3 expression to allow $\gamma\delta$ precursors to adopt a $\gamma\delta$17 cell fate.[43] Finally, evidence also suggests possible roles for nuclear factor of activated T cells and nuclear factor-κB in $\gamma\delta$IFN development,[16] whereas generation of Vγ1Vδ6.3/6.4$^+$ NKT-like $\gamma\delta$ T cells that can secrete both IFN-γ and IL-4 also involves the TCR-signalling-induced transcription factors promyelocytic leukaemia zinc finger (PLZF) and T-helper inducing POZ-kruppel like factor (ThPOK).[27,44,45]

Re-thinking $\gamma\delta$ T-cell development

Weight of evidence now suggests that an alternative view of $\gamma\delta$ T-cell development should be considered. Consensus has long held that all $\gamma\delta$ T-cell subsets (i.e. $\gamma\delta$IFN and $\gamma\delta$17) stem from a common progenitor that can also generate conventional $\alpha\beta$ T cells.[1] Certainly, development of naive-like $\gamma\delta$ T cells, $\gamma\delta$IFN cells, and the known agonist-selected $\gamma\delta$ subsets fits with this idea, with strong (compared with the pre-TCR) TCR-$\gamma\delta$ signalling first committing a common $\alpha\beta$/$\gamma\delta$ progenitor to the $\gamma\delta$ lineage, before then determining effector fate (i.e. naive-like versus $\gamma\delta$IFN) based on levels of TCR signal strength with perhaps additional factors directing specific fates (e.g. SLAM signalling for NKT-like $\gamma\delta$ T cells). Importantly, such a model that excludes the development of $\gamma\delta$17 cells does not generate the paradox of requiring weak or absent TCR-$\gamma\delta$ signalling for acquisition of (IL-17-secreting) $\gamma\delta$ T-cell effector function, while at the same time requiring

those cells to have experienced strong TCR-$\gamma\delta$ signalling for entry into the $\gamma\delta$ lineage.

But what about $\gamma\delta$17 cells; how then do they develop? The answer may lie with a re-imagining of the pre-commitment-selection model (Fig. 2). Such a model would propose that a $\gamma\delta$17 programme be established in a distinct thymic $\gamma\delta$17-progenitor before the expression of TCR-$\gamma\delta$. Subsequent to this specification, appropriate alternative/weak signalling from certain TCR-$\gamma\delta$ would allow full manifestation of the pre-committed $\gamma\delta$17 transcriptional programme. By contrast, stronger (e.g. ligand-dependent) TCR-$\gamma\delta$ signalling may be incompatible for further development or survival.[17] Importantly, this $\gamma\delta$17-progenitor may have different developmental potentials when compared with the conventional common $\alpha\beta$/$\gamma\delta$ progenitor (which gives rise to other $\gamma\delta$ subsets). Hence, a 'committing' TCR-$\gamma\delta$ signal may not be required to divert the $\gamma\delta$17-progenitor from an alternative (e.g. $\alpha\beta$ T-cell) fate. Some evidence for such a model already exists; DN cells (i.e. TCR$^-$) with IL-17-secreting potential and with an open TCR-δ locus have been observed,[32] and fetal or adult c-kit$^+$ CD44$^+$ CD25$^-$ 'early thymic progenitors', but not DN3 cells, were shown to favour development of Vγ4$^+$ CCR6$^+$ CD27$^-$ $\gamma\delta$ T cells on OP9-DL1 cultures.[7] These observations were further extended to show that fetal c-kit^{++} DN2 cells but not c-kit$^-$ DN3 cells could generate $\gamma\delta$17 cells, possibly in a Bcl11b-dependent manner.[46] Moreover, expression in all DN cells of a Vγ4$^+$ transgenic TCR-$\gamma\delta$ did not markedly increase generation of $\gamma\delta$17 cells, implying that a restricted IL-17-progenitor pool exists.[7] Certainly, more evidence is required to confirm and extend these initial observations, and to assess whether all $\gamma\delta$17 cells could develop from such an alternative source.

Concluding remarks

An alternative developmental origin for IL-17-secreting immune cells may hark back to very early evolutionary times as IL-17-like genes are found in a variety of invertebrates that possess only innate-like immune systems.[47] Moreover, $\gamma\delta$17-like cells may be a modern-day example of one of the first types of lymphocyte. Support for this has come from recent work in jawless vertebrates (e.g. lampreys), whose lymphocytes express antigen receptors (variable lymphocyte receptors) that contain activation-induced cytidine deaminase-dependent somatically rearranged leucine-rich repeat modules.[48] Three divisions of lymphocytes can be identified in these animals, two of which resemble conventional T cells, and B cells. Interestingly, the third lymphocyte lineage largely occupies epithelial sites such as the skin and gut, can respond rapidly to challenge, and expresses both Sox13 and IL-17.[49] These $\gamma\delta$17-like cells that pre-date RAG and vertebrate T-cell receptors appear to have an evolutionarily ancient history.[50] It may therefore not be a surprise that their developmental origins are segregated from the developmental pathways of more recently evolved lymphocyte subsets (e.g. $\gamma\delta$IFN cells). Obviously, such innate-like origins invoke a relationship with the recently described family of innate lymphoid cells.[51] The transcriptional programmes of $\gamma\delta$17 cells and certain subsets of innate lymphoid cell type 3 show clear similarities (e.g. expression of RORγt, CD127, CD25, CCR6).[52] Further investigation of the links between these two cell types may therefore provide further insight into the developmental peculiarities of these highly enigmatic $\gamma\delta$ T cells.

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Disclosure

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