T cell receptor signalling in cell development: strength isn't everything

Turchinovich, G; Pennington, DJ

TCRγδ signalling in γδ cell development; strength isn't everything

Gleb Turchinovich[1] and Daniel J. Pennington[2]

[1]London Research Institute, Cancer Research UK, and Peter Gorer Dept of Immunobiology, King's College School of medicine at Guy's Hospital, London SE1 9RT, UK.

[2]The Blizard Institute, Barts and The London School of Medicine, Queen Mary University of London, 4 Newark Street, London E1 2AT, UK.

Corresponding author: Pennington, D.J. (d.pennington@qmul.ac.uk)

Abstract

γδ cells are conserved across ~450 million years of evolution, from which they share the distinction, alongside αβ T cells and B cells, of forming antigen receptors by somatic gene recombination. However, much about these cells remains unclear. Indeed, although γδ cells display “innate-like” characteristics exemplified by rapid tissue-localised responses to stress-associated stimuli, their huge potential for T cell receptor (TCR)γδ diversity also suggests “adaptive-like” potential. Clarity requires a better understanding of TCRγδ itself, not only through identification of TCR-ligands, but also by correlating thymic TCRγδ signalling with commitment to γδ effector fates. Here, we propose that thymic TCRγδ-ligand engagement *versus* ligand-independent signalling differentially imprints innate-like *versus* adaptive-like characteristics on developing γδ cells, which fundamentally dictate their peripheral effector properties.

Unresolved role for TCRγδ in γδ cell development

γδ T cells are predominantly tissue-resident lymphocytes that display diverse responses against pathogens and tumours [1]. Indeed, novel immunotherapies that target γδ cells are now being explored to combat chronic viral infections, atopic and autoimmune pathologies, and various cancers [2,3]. Stimulation through TCRγδ is critical for γδ cell function [4]. However, by contrast to TCRαβ, signalling through which is absolutely required for thymic αβ T cell development, the role of TCRγδ in γδ cell development remains controversial. TCRγδ signalling is clearly necessary for commitment to the γδ lineage, but the initiation, regulation and molecular nature of this commitment signal are still uncertain. Moreover, ligand-mediated positive and negative selection through TCRγδ remain poorly understood, as too is the correlation between thymic TCRγδ signalling and subsequent γδ effector fates. Here, we build on recent studies that assess the initiation and consequences of TCRγδ signalling in immature thymocytes [5-7], to propose that thymic TCRγδ-ligand engagement *versus* ligand-independent TCRγδ signalling may differentially impose innate-like *versus* adaptive-like features on developing γδ cells.

Heterogeneity of peripheral γδ subsets

Discussion of the role of TCRγδ in γδ cell development first requires appreciation of the heterogeneous nature of peripheral and thymic γδ subsets. Functionally distinct γδ subsets have been extensively characterised by surface phenotype. For example, dendritic epidermal T cells (DETC), that reside in murine epidermis and predominantly express a TCRγδ that uses

TCRγ variable-region-5 (Vγ5) and TCRδ variable-region-1 (Vδ1) (nomenclature from [8]), are $CD44^+CD62L^-$, express CD103 (α_E integrin), and are $CD122^+$ consistent with their dependence on IL-15 (**Figure 1 and Table 1**) [9,10]. DETC also readily secrete IFN-γ when activated. This $CD44^+CD62L^-CD122^+$ IFN-γ-secreting phenotype is also shared by a minor population of lymphoid γδ cells (~0.5%) whose TCR binds to MHC class IB molecules $T10^b$ and $T22^b$, but only in mouse strains expressing $T10^b$ and $T22^b$ [7]. Moreover, it also characterises a $CD90^{dull}CD27^+$ "NKT-like" γδ subset that uses a restricted $V\gamma1^+V\delta6.3^+$ (or $V\delta6.4^+$) TCR and is known to secrete both IFNγ and IL-4 [11].

By contrast to DETC, Vγ4-biased γδ cells (i.e. a γδ subset with over-representation of Vγ4-containing TCRγ chains) of the murine dermis secrete IL-17A, are $CD44^+CD122^-$, and express CCR6 and the scavenger receptor SCART2 [9,12,13] (**Figure 1 and Table 1**). These cells are likely $CD27^-$, as they closely resemble IL-17A-secreting Vγ6-biased γδ cells from the peritoneal cavity and female reproductive tract that are $CD27^-CD44^+CD122^-$ and $CD25^+$ [14,15]. A $CD27^-CD44^+CD62L^-CD122^-$ phenotype accompanied by CCR6 and SCART2 expression is also shared by a minor population of IL-17A-producing γδ cells from the secondary lymphoid organs [13,16,17]. Nonetheless, the majority of lymphoid γδ cells from naive mice secrete large amounts of IFN-γ when activated, but no IL-17A [16]. These cells are $CD27^+$, with a contrasting (for example, to DETC) $CD44^-CD62L^+CD122^-$ phenotype (**Figure 1 and Table 1**). Finally, Vγ7-biased γδ intraepithelial lymphocytes (IELs) of the gut are $CD27^+$ and express IFN-γ on activation [18]. These cells are often described as "partially activated", and may be under constant stimulation from gut-associated antigens [19,20].

Thymic γδ subsets

TCRγδ$^+$ thymocytes are first evident from embryonic day-14 (E14), being initially dominated by a population of Vγ5$^+$Vδ1$^+$ DETC progenitors [21]. Shortly after a population of Vγ6$^+$Vδ1$^+$ progenitors emerge that are destined for the female reproductive tract, peritoneal cavity and tongue. Thymic terminal transferase (TdT) is not expressed during these prenatal stages, resulting in simple V-D-J joins that characterize the canonical TCRs of fetal-derived γδ cells [21]. By contrast, postnatal thymic precursors of gut and lymphoid γδ cells possess diverse V-D-J joins in their Vγ1, 2, 4 and 7-containing TCRs. This sequential progression of γδ cell output is partly due to ordered Vγ-region transcription and rearrangement. Nonetheless, other intrinsic differences between fetal/adult thymic progenitors [22,23], and requirement for age-specific thymic stromal factors [24], are also thought to influence subsequent γδ cell fate.

In the adult murine thymus, CD27, CD25, CD24 and CD44 identify five distinct γδ cell populations [16,25] (**Figure 1 and Table 1**). The most immature TCRγδ$^+$ cells are CD27$^+$CD25$^+$CD24$^+$CD44$^-$, express low TCR levels but are highly proliferative [16,25]. These progenitors down-regulate CD25 to become CD27$^+$CD25$^-$CD24$^+$CD44$^-$ cells, that can possibly already colonise the periphery [26]. They also likely represent precursors for three “mature” γδ thymocyte populations that lack surface expression of CD24. This includes a CD27$^-$ subset that is CD44$^+$CD62L$^-$, largely CCR6$^+$ [17], and is already committed to IL-17A secretion [16]. By contrast, mature CD27$^+$CD24$^-$ γδ thymocytes, that have potential to secrete IFN-γ, can be further sub-divided into CD44$^+$CD62L$^-$CD122$^+$ and CD44$^-$CD62L$^+$CD122$^-$ subsets. The former lack CCR9 and are largely NK1.1$^+$ [17,20], being enriched for precursors of NKT-like γδ cells [11]. Conversely, CD27$^+$CD24$^-$CD44$^-$CD62L$^+$CD122$^-$ γδ thymocytes are likely progenitors to

those of similar phenotype in peripheral lymphoid organs (see previous section). Thus, the thymus generates distinct γδ populations with clear phenotypic links to peripheral γδ subsets.

Thymic commitment to a γδ cell fate

γδ and αβ T cells share a common $CD4^-CD8^-$ double negative (DN) thymic progenitor in which TCRγ, TCRδ, and TCRβ rearrangements initiate [21]. DN cells that express a preTCR (TCRβ paired with invariant preTCRα chain), traverse a "β-selection" checkpoint to a $CD4^+CD8^+$ double positive (DP) stage that marks commitment to the αβ lineage [27]. By contrast, TCRγδ expression appears to commit DN cells to a γδ fate. These observations initially suggested a qualitatively instructional role for preTCR and TCRγδ in αβ versus γδ fate determination [28]. However, this model failed to explain development of TCRγδ-dependent DP cells in preTCR-deficient mice [29,30], or that precocious expression of transgenic-TCRαβ induced appearance of "γδ-like" cells [31].

A competing "pre-commitment" model for αβ versus γδ lineage choice alternatively proposed that fate determination occurred prior to TCR expression. This initially correlated to heterogeneity in CD127 expression in $CD44^+CD25^+$ DN (DN2) cells; $CD127^{hi}$ cells being biased toward the γδ lineage [32]. More recently, expression status of Sox-13 has been similarly implicated [33], while commitment potential to the γδ lineage clearly varies with both ontogeny [22], and with the developmental stage at which TCRγδ is initially expressed [34]. Nonetheless, the extent to which subsequent TCRγδ signalling can override these pre-committed states remains unclear [35].

"Strong" TCR signalling promotes a γδ cell fate

Available data now best fit a model in which quantitative differences in TCR signal strength, irrespective of TCR identity, dictate αβ versus γδ fate determination; "strong" signalling promotes a γδ fate, and "weaker" signalling generates αβ-committed DP cells [36,37]. Operationally, this equates to an instructional model, as TCRγδ largely provides strong signals while preTCR signalling is weaker. Although stronger signalling from TCRγδ appeared to correlate with increased ERK1/2 phosphorylation, induction of Egr family transcription factors, and up-regulation of Id3 [36], the molecular pathways that define γδ commitment are only now being defined (as discussed later). Recent investigations using Delta-like-1-expressing OP9 (OP9-DL1) stromal cell co-culture of TCRγδ$^{(+)}$ DN3 thymocytes supported a "signal-strength" model [35]. Thus, strong TCRγδ signalling combined with age and/or stage-specific pre-commitment factors promote a γδ fate (**Figure 2**).

Generating strong TCR signals; engaging thymic TCRγδ ligands

The paucity of known murine TCRγδ ligands has made investigation of ligand engagement during thymic γδ cell development problematic. Nonetheless, at least three γδ subsets are implicated in thymic ligand binding; thymus leukemia (TL)-specific γδ cells, NKT-like γδ cells, and DETC.

Interaction of TL-specific γδ cells (in either KN6 or G8 TCRγδ-transgenic mice) with cognate T10^{b} or T22^{b} ligand (from the MHC TL region) during thymic development was variously reported to cause tolerance, deletion, or trafficking of cells to the gut epithelium [38-40].

However, recent experiments with a T22-tetrameric FACS-staining reagent have instead suggested that thymic ligand-engaging TL-specific γδ cells develop to secrete IFN-γ, whereas thymic ligand-naive TL-specific γδ cells secrete IL-17A [7]. This study further reported that T22-tetramer-negative γδ cells, which constitute ~99% of those observed in wild-type mice, share phenotypic features of ligand-naive (i.e. IL-17A-secreting) TL-specific γδ cells, somewhat contradicting the perceived view of γδ cells as predominantly IFN-γ-secreting. Importantly, the development of ligand-naive γδ cells was suggested to result from TCR-oligomerization-mediated ligand-independent TCRγδ signalling (see below) [7]. A subsequent report additionally suggested that TL-specific TCRγδ$^{(+)}$ IELs also lack evidence of thymic ligand engagement [20].

TCR-ligand-mediated selection is also assumed for thymic development of NKT-like γδ cells, as their characteristic Vγ1$^+$Vδ6.3$^+$ (or Vδ6.4$^+$) TCR displays restricted Vδ-CDR3 length and amino acid composition [11,41]. Like TCRαβ$^+$ NKT cells, NKT-like γδ cells are dependent on SAP signalling [42] and the transcription factors ThPOK [43] and PLZF [44], the latter being necessary for IL-4 and IFN-γ secretion [44]. However, although PLZF is induced by TCRγδ cross-linking [44], disruption of the LAT–Itk–Id3 signalling pathway, that functions downstream of TCRγδ, paradoxically promotes expansion of Vγ1$^+$Vδ6.3$^+$ cells [42,45-47]. Consistent with this, attenuation of TCR signalling appeared to expand the Vγ1$^+$Vδ6.3$^+$ subset, and elevated PLZF levels in those expanded cells [42]. Thus, very strong ligand-dependent TCRγδ signalling may not favour development of NKT-like γδ cells.

Finally, selection through TCRγδ is also implicated in DETC development, which correlates with thymic stromal expression of immunoglobulin superfamily gene *Skint1* [48,49]. Although

not necessarily a direct ligand for the TCR, Vγ5$^+$Vδ1$^+$ fetal thymic progenitors that engage Skint1$^+$ stromal cells upregulate *Egr3* that, together with NFAT and NFκB signalling, promote the DETC phenotype that involves up-regulation of *Tbx21* and IFN-γ-secreting potential [5]. By contrast, Vγ5$^+$Vδ1$^+$ progenitors that develop in the absence of Skint1 fail to induce *Egr3* and *Tbx21*, but express both *Sox13* and *Rorc* that jointly promote what can be called a "non-selected" phenotype that includes IL-17A-producing potential. Importantly, the reciprocal regulation of *Egr3 versus Sox13*/*Rorc* could be demonstrated in adult γδ thymocytes by cross-linking with agonist anti-TCRδ antibody. Thus, this study begins to provide critical insight into the molecular mechanisms that relate γδ cell functional specification to thymic ligand engagement.

Ligand-independent TCRγδ signalling

Despite the acknowledged presence of certain thymic TCRγδ-ligands, and that TCRγδ signalling is considered ligand-driven in peripheral immune responses, ligand engagement may not mediate all instances of TCRγδ signal initiation in DN thymocytes. Ligand-independent signal initiation has long been demonstrated for preTCR [50], being variously ascribed to pTα-mediated lipid-raft association [51], preTCR oligomerization mediated by the extracellular Ig-loop of pTα [52,53], or to an intrinsically low signalling threshold in DN thymocytes [54]. Thymic ligand-independent signalling was similarly proposed for TCRγδ, possibly mediated by oligomerization of the variable region of TCRδ [7]. In addition, a recent study suggested that "TCRγδ" complexes that lack variable domains, or that lack both variable and constant Ig-like domains, can still initiate signals that drive RAG-2-deficient thymocytes toward a "γδ-like" fate

[6]. This implies that appropriate surface pairings of TCRγ and TCRδ chains that possibly bring CD3ε-containing signalling modules into close proximity of available Lck is sufficient for TCRγδ signal initiation in DN thymocytes. Thus, strong TCRγδ signalling may not only be a consequence of ligand engagement; additionally, efficiently paired TCRγ/TCRδ chains that are expressed at the cell surface above a certain critical threshold will also commit DN progenitors to a γδ cell fate [6].

Mapping thymic TCRγδ signalling to peripheral γδ effector fate

Studies on DETC, NKT-like, and antigen-experienced TL-specific γδ cells clearly associate thymic ligand binding with a $CD44^+CD62L^-CD122^+$ phenotype and IFN-γ-secreting potential [5,7,44]. Nonetheless, the majority of $CD27^+$ lymphoid γδ cells also produce abundant IFN-γ when activated [16], despite displaying a contrasting $CD44^-CD62L^+CD122^-$ phenotype that implies an absence of thymic TCR-ligand engagement. Thus, we suggest that thymic ligand-independent TCRγδ signalling may be sufficient to promote γδ cell commitment to subsequent IFN-γ production (and not to IL-17A production as suggested in previous reports [7]). Moreover, we also propose that a significant component of thymic TCR-ligand engagement may actually be interaction with ligand-presenting cells that provide critical additional signals for subsequent γδ cell effector function; this may include provision of SAP-dependent signalling for NKT-like γδ cells, or access to Skint1 for developing DETC.

By contrast to IFN-γ secretion, IL-17A production by γδ cells has been proposed as a default pathway in which $TCR\gamma\delta^+$ thymocytes do not encounter agonist TCR-ligand [7]. However, IL-17A-secreting $CD27^-$ γδ thymocytes display a uniformly “activated” $CD44^+CD62L^-$ thymic

phenotype similar to ligand-experienced γδ cells (although without CD122 or NK1.1 expression) [17,25]. Indeed, early studies suggested that $V\gamma6^+V\delta1^+$ thymic progenitors (that mature to secrete IL-17A) undergo ligand-driven TCR selection for canonical CDR3 sequences to a similar degree as $V\gamma5^+V\delta1^+$ DETC progenitors [55]. This notwithstanding, it is unlikely that any such IL-17A-inducing thymic TCR-ligand would behave as a full TCRγδ agonist [5,7].

Whatever the nature of the inductive event for IL-17A-secreting potential, the thymic progenitors of IL-17A-secreting γδ cells appear to enter a complex program of development [5] that results from some significant degree of fetal thymus-associated pre-commitment [14]. It also appears to require signalling pathways that involve B lymphoid kinase [56], TGFβ1 [57], and Hes-1 [15]. Thus, it presently remains unclear whether an IL-17A-secreting γδ fate truly represents a ligand-independent γδ cell developmental pathway, or whether fetal/neonatal γδ progenitors of IL-17A-secreting γδ cells must also interact with thymic ligands that results in distinct but overlapping phenotypic changes to those observed for DETC and NKT-like γδ cells.

Implications for γδ cell function

The common description of γδ cells as “innate-like” perhaps more accurately reflects tissue-associated γδ subsets with highly focused TCR specificities that represent prototypic stress-surveillance lymphocytes with rapid responses to autologous stress-antigens [1]. As discussed above, precursors of these populations are likely selected on thymic ligands that endow them with specific effector functions, or reinforce homing to certain body locations [11,23]. These cells respond *en masse* to local tissue insults and make critical contributions to both immune protection and tissue integrity [1]. IFN-γ-secreting DETC represent a well-studied example of

these stress-surveillance lymphocytes. However, it is tempting to also speculate whether IL-17A-producing γδ subsets, such as Vγ6$^+$Vδ1$^+$ cells from the female reproductive tract and peritoneal cavity, or Vγ4-biased dermal γδ cells, might also contribute to stress-surveillance. These subsets are predominantly tissue-located, and share the "activated" CD44$^+$CD62L$^-$ phenotype of DETC and NKT-like γδ cells that could suggest some degree of thymic TCRγδ ligand engagement [16,25]. Certainly their rapid responsiveness to cytokines such as IL-1 and IL-23 would be consistent with an "innate-like" existence [58,59]. Investigations that determine whether IL-17A-producing γδ progenitors require thymic TCR-ligand interaction for development should clarify this issue. Moreover, they should test the hypothesis that "innate-like" γδ subsets require TCRγδ ligand engagement during their thymic development.

Adaptive T cell responses are generally defined as clonal expansions of relatively few antigen-specific lymphocytes. Interestingly, this feature may well be shared by CD27$^+$CD44$^-$ CD62L$^+$CD122$^-$ γδ cells that comprise the majority of γδ cells in secondary lymphoid organs [16]. This subset rapidly and extensively expands to secrete abundant IFN-γ on activation through TCRγδ (and CD27), and was shown to include Murid herpesvirus-4 responsive cells [59]. Thus, γδ cells which lack evidence of thymic TCR-ligand engagement (i.e. with a CD27$^+$CD44$^-$CD62L$^+$CD122$^-$ phenotype), that likely develop as a consequence of ligand-independent TCRγδ signalling, appear to demonstrate peripheral "adaptive-like" responses, that includes abundant secretion of IFN-γ, on recognising non-thymic (possibly pathogen-associated) antigens. Nonetheless, there is little evidence of γδ memory cell generation or "fixing" of TCR specificities in the TCRγδ repertoire as a consequence of these expansions

[7,26]; instead expanded γδ cell clones appear relatively short-lived, being replaced by “fresh” naive γδ cells that presumably maintain a diverse TCRγδ repertoire.

Similarities with non-T lymphocyte development

Despite persistent temptation to align γδ cells with their αβ T cell cousins, comparison with non-T lymphocytes may instead reveal much about γδ cell biology. For example, developing B cells whose B cell receptors (BCRs) engage self-antigen often develop as B-1 B cells, a subset with "innate-like" features that includes rapid functional responses and a restricted BCR repertoire [60]. Conversely, B cells expressing BCRs with no apparent self-reactivity primarily differentiate into "conventional" follicular B cells with classic “adaptive-like” qualities. Interestingly, BCR signalling of “intermediate” strength in response to limiting self-antigen drives marginal zone B cell generation in a BAFF-dependent manner [61]. Here, BCR signalling induces expression of non-canonical NFκB pathway substrate p100 which suppresses survival and differentiation unless converted to active p52 by BAFF signalling [62]. This demonstrates that different B cell fates are generated by different “qualities” of BCR signalling that may or may not require ligand engagement and/or input from additional signalling pathways [60]. Thus, an alternative developmental perspective from a distantly-related lymphocyte relative may provide fresh insight on the generation of different γδ effector fates that possibly result from similar differences in TCRγδ signalling and co-stimulation.

Concluding remarks

Recent studies have reinforced the importance of γδ cell responses in infections, cancer and autoimmunity [1]. Indeed, the administration of autologous activated human $V\gamma9^{+}V\delta2^{+}$ γδ cells now represents a promising approach for immunotherapy in diverse disease scenarios [2,3]. Clearly, an improved knowledge of γδ cell biology is essential, and great strides have been taken to characterise γδ subset phenotypes and functions throughout the body. A thorough understanding of thymic γδ cell development is equally important, at the forefront of which is elucidation of TCRγδ-mediated selection events. Here, we propose that thymic engagement of TCRγδ ligands generates “innate-like” γδ subsets with rapid cytokine responses to stress-associated stimuli. By contrast, γδ cells that do not recognise thymic ligands develop as “adaptive-like” γδ cells that expand clonally to secrete IFN-γ in response to non-thymic and potentially foreign TCRγδ-ligands. Thus, we contend that thymic TCR selection plays a critical role in ascribing appropriate functional responses to self/non-self TCRγδ specificities.

Box 1. Outstanding Questions

- What are the cognate ligands for murine TCRγδ? Do these include self-ligands? Have canonical γδTCRs been preserved through evolution for self-ligand recognition?
- To what extent are thymic progenitors pre-committed to "innate-like" γδ effector fates? Do "innate-like" γδ and αβ T cells share a common thymic progenitor?
- To what extent is thymic TCRγδ-agonist engagement required for γδ cell development? What contribution does thymic ligand-independent TCRγδ signalling make to the peripheral γδ pool? How does ligand-independent TCRγδ signalling initiate?
- How is the IL-17A-secreting effector program initiated during thymic γδ cell development? And how do additional inputs, such as TGFβ or Notch signalling, affect this process?
- What are the signalling pathways, molecular mechanisms, and transcription factors that regulate thymic commitment to diverse γδ cell effector fates?

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Figure Legends

Figure 1. Thymic and peripheral γδ subsets. Phenotypic characterisation of thymic and peripheral γδ subsets based on surface expression of CD25 (purple segments; "25"), CD24 (green segments; "24"), CD27 (yellow segments; "27"), CD44 (blue segments; "44") and CD122 (red segments; "122"). The predominant cytokines (IFN-γ, IL17A and IL-4) produced by a subset on activation *ex vivo* are illustrated. The term "Vγ4-biased" indicates a γδ subset that uses the Vγ4 region of TCRγ more frequently than expected. TCRγδ expression is first seen in the thymus on $CD27^{+}CD24^{+}CD25^{+}$ progenitors (**a)** that do not express CD44 or CD122. This population rapidly down-regulates CD25 and increases TCRγδ surface expression to generate immature $CD27^{+}CD24^{+}CD25^{-}CD44^{-}CD122^{-}$ cells (**b**) that are likely precursors of at least three mature $CD24^{-}$ thymic subsets. TCR-agonist engagement preserves CD27 expression and up-regulates CD44, CD122 (**c**). This population likely includes precursors of peripheral NKT-like γδ cells (**d**) that predominantly express a $V\gamma1^{+}V\delta6.3^{+}$ (or $V\delta6.4^{+}$) TCR; and possibly TL-specific γδ cells that develop in a $T10^{b}$ or $T22^{b}$-expressing background (**e**). Interestingly, this thymic phenotype (c) is shared by thymic DETC progenitors (**f**) during early ontogeny that are also thought to be TCR-agonist selected. Mature DETC (**g**), NKT-like, and TL-specific γδ cells all secrete abundant IFN-γ. Progenitor (b) also likely generates $CD27^{+}CD44^{-}CD122^{-}$ cells (**h**) through prolonged ligand-independent signalling, that seed the peripheral lymphoid organs (**i**) (and possibly the gut; not shown), where they generate abundant IFN-γ on activation. Progenitor (b) also appears to generate thymic IL-17A-secreting cells that are $CD27^{-}$ $CD44^{+}CD122^{-}$ (**j**). It is presently unclear how this transition proceeds; we suggest that TCRγδ engagement may be required, although both ligand-independent TCRγδ signalling and some element of pre-commitment have also been proposed. These cells (j) are likely precursors of

IL-17A-producing γδ cells from the peritoneal cavity and female reproductive tract (**k**), dermis (**l**) and lymph nodes (**m**). Data is from references [7,9-17,20,25,47,48,63].

Figure 2. TCR signal strength and pre-commitment factors dictate γδ versus αβ lineage fate. Strength of TCR signalling rather than type of TCR complex (i.e. preTCR *vs.* TCRγδ) appears to dictate γδ versus αβ lineage fate; stronger TCR signals commit early thymocyte progenitors to a γδ fate, whereas weaker signals promote an αβ T cell fate. Operationally, this represents an instructional model of commitment, as TCRγδ is known to signal more strongly than preTCR (predicted ranges of signal strength for preTCR and TCRγδ are indicated). Nonetheless, factors unrelated to strength of TCR signalling, such as age (fetal *vs.* adult), developmental stage at which TCR signalling initiates (i.e. DN2 *vs.* DN3 *vs.* DN4), and expression of certain "pre-commitment" factors (e.g. CD127 or Sox-13), will impact on the likelihood that a particular signal strength will result in commitment to either lineage.

References

1 Hayday, A.C. (2009) Gammadelta T cells and the lymphoid stress-surveillance response. *Immunity* 31 (2), 184-196

2 Bonneville, M. and Scotet, E. (2006) Human Vgamma9Vdelta2 T cells: promising new leads for immunotherapy of infections and tumors. *Curr Opin Immunol* 18 (5), 539-546

3 Beetz, S. *et al.* (2007) Human gamma delta T cells: candidates for the development of immunotherapeutic strategies. *Immunol Res* 37 (2), 97-111

4 Bonneville, M. *et al.* (2010) Gammadelta T cell effector functions: a blend of innate programming and acquired plasticity. *Nat Rev Immunol* 10 (7), 467-478

5 Turchinovich, G. and Hayday, A.C. (2011) Skint-1 Identifies a Common Molecular Mechanism for the Development of Interferon-gamma-Secreting versus Interleukin-17-Secreting gammadelta T Cells. *Immunity*

6 Mahtani-Patching, J. *et al.* (2011) PreTCR and TCR{gamma}{delta} Signal Initiation in Thymocyte Progenitors Does Not Require Domains Implicated in Receptor Oligomerization. *Sci Signal* 4 (182), ra47

7 Jensen, K.D. *et al.* (2008) Thymic selection determines gammadelta T cell effector fate: antigen-naive cells make interleukin-17 and antigen-experienced cells make interferon gamma. *Immunity* 29 (1), 90-100

8 Heilig, J.S. and Tonegawa, S. (1986) Diversity of murine gamma genes and expression in fetal and adult T lymphocytes. *Nature* 322 (6082), 836-840

9 Sumaria, N. *et al.* (2011) Cutaneous immunosurveillance by self-renewing dermal {gamma}{delta} T cells. *J Exp Med* 208 (3), 505-518

10 Van Beneden, K. *et al.* (2002) Expression of inhibitory receptors Ly49E and CD94/NKG2 on fetal thymic and adult epidermal TCR V gamma 3 lymphocytes. *J Immunol* 168 (7), 3295-3302

11 Azuara, V. *et al.* (1997) A novel subset of adult gamma delta thymocytes that secretes a distinct pattern of cytokines and expresses a very restricted T cell receptor repertoire. *Eur J Immunol* 27 (2), 544-553

12 Gray, E.E. *et al.* (2011) Cutting Edge: Identification of a Motile IL-17-Producing {gamma}{delta} T Cell Population in the Dermis. *J Immunol*

13 Kisielow, J. *et al.* (2008) SCART scavenger receptors identify a novel subset of adult gammadelta T cells. *J Immunol* 181 (3), 1710-1716

14 Shibata, K. *et al.* (2008) Identification of CD25+ gamma delta T cells as fetal thymus-derived naturally occurring IL-17 producers. *J Immunol* 181 (9), 5940-5947

15 Shibata, K. *et al.* (2011) Notch-Hes1 pathway is required for the development of IL-17-producing {gamma}{delta} T cells. *Blood*

16 Ribot, J.C. *et al.* (2009) CD27 is a thymic determinant of the balance between interferon-gamma- and interleukin 17-producing gammadelta T cell subsets. *Nat Immunol* 10 (4), 427-436

17 Haas, J.D. *et al.* (2009) CCR6 and NK1.1 distinguish between IL-17A and IFN-gamma-producing gammadelta effector T cells. *Eur J Immunol* 39 (12), 3488-3497

18 Kohyama, M. *et al.* (1999) Cytolytic and IFN-gamma-producing activities of gamma delta T cells in the mouse intestinal epithelium are T cell receptor-beta-chain dependent. *Proc Natl Acad Sci U S A* 96 (13), 7451-7455

19 Shires, J. *et al.* (2001) Biological insights into TCRgammadelta+ and TCRalphabeta+ intraepithelial lymphocytes provided by serial analysis of gene expression (SAGE). *Immunity* 15 (3), 419-434

20 Jensen, K.D. *et al.* (2009) Cutting edge: Gammadelta intraepithelial lymphocytes of the small intestine are not biased toward thymic antigens. *J Immunol* 182 (12), 7348-7351

21 Hayday, A.C. (2000) [gamma][delta] cells: a right time and a right place for a conserved third way of protection. *Annu Rev Immunol* 18, 975-1026

22 Heyborne, K. *et al.* (1993) Evidence that murine V gamma 5 and V gamma 6 gamma delta-TCR+ lymphocytes are derived from a common distinct lineage. *J Immunol* 151 (9), 4523-4527

23 Jin, Y. *et al.* (2010) Cutting edge: Intrinsic programming of thymic gammadeltaT cells for specific peripheral tissue localization. *J Immunol* 185 (12), 7156-7160

24 Ikuta, K. *et al.* (1990) A developmental switch in thymic lymphocyte maturation potential occurs at the level of hematopoietic stem cells. *Cell* 62 (5), 863-874

25 Prinz, I. *et al.* (2006) Visualization of the earliest steps of gammadelta T cell development in the adult thymus. *Nat Immunol* 7 (9), 995-1003

26 Tough, D.F. and Sprent, J. (1998) Lifespan of gamma/delta T cells. *J Exp Med* 187 (3), 357-365

27 Fehling, H.J. *et al.* (1995) Crucial role of the pre-T-cell receptor alpha gene in development of alpha beta but not gamma delta T cells. *Nature* 375 (6534), 795-798

28 Saint-Ruf, C. *et al.* (2000) Different initiation of pre-TCR and gammadeltaTCR signalling. *Nature* 406 (6795), 524-527

29 Kersh, G.J. *et al.* (1995) Efficient maturation of alpha beta lineage thymocytes to the CD4+CD8+ stage in the absence of TCR-beta rearrangement. *J Immunol* 154 (11), 5706-5714

30 Buer, J. *et al.* (1997) Role of different T cell receptors in the development of pre-T cells. *J Exp Med* 185 (9), 1541-1547

31 Bruno, L. *et al.* (1996) The alpha beta T cell receptor can replace the gamma delta receptor in the development of gamma delta lineage cells. *Immunity* 5 (4), 343-352

32 Kang, J. *et al.* (2001) Evidence that gammadelta versus alphabeta T cell fate determination is initiated independently of T cell receptor signaling. *J Exp Med* 193 (6), 689-698

33 Melichar, H.J. *et al.* (2007) Regulation of gammadelta versus alphabeta T lymphocyte differentiation by the transcription factor SOX13. *Science* 315 (5809), 230-233

34 Ciofani, M. *et al.* (2006) Stage-specific and differential notch dependency at the alphabeta and gammadelta T lineage bifurcation. *Immunity* 25 (1), 105-116

35 Kreslavsky, T. *et al.* (2008) T cell receptor-instructed alphabeta versus gammadelta lineage commitment revealed by single-cell analysis. *J Exp Med* 205 (5), 1173-1186

36 Haks, M.C. *et al.* (2005) Attenuation of gammadeltaTCR signaling efficiently diverts thymocytes to the alphabeta lineage. *Immunity* 22 (5), 595-606

37 Hayes, S.M. *et al.* (2005) TCR signal strength influences alphabeta/gammadelta lineage fate. *Immunity* 22 (5), 583-593

38 Dent, A.L. *et al.* (1990) Self-reactive gamma delta T cells are eliminated in the thymus. *Nature* 343 (6260), 714-719

39 Bonneville, M. *et al.* (1990) Self-tolerance to transgenic gamma delta T cells by intrathymic inactivation. *Nature* 344 (6262), 163-165

40 Lin, T. *et al.* (1999) Autospecific gammadelta thymocytes that escape negative selection find sanctuary in the intestine. *J Clin Invest* 104 (9), 1297-1305

41 Azuara, V. *et al.* (1998) The homogeneity of the TCRdelta repertoire expressed by the Thy-1dull gammadelta T cell population is due to cellular selection. *Eur J Immunol* 28 (11), 3456-3467

42 Alonzo, E.S. *et al.* (2010) Development of promyelocytic zinc finger and ThPOK-expressing innate gamma delta T cells is controlled by strength of TCR signaling and Id3. *J Immunol* 184 (3), 1268-1279

43 Park, K. *et al.* (2010) TCR-mediated ThPOK induction promotes development of mature (CD24-) gammadelta thymocytes. *EMBO J* 29 (14), 2329-2341

44 Kreslavsky, T. *et al.* (2009) TCR-inducible PLZF transcription factor required for innate phenotype of a subset of gammadelta T cells with restricted TCR diversity. *Proc Natl Acad Sci U S A* 106 (30), 12453-12458

45 Qi, Q. *et al.* (2009) Enhanced development of CD4+ gammadelta T cells in the absence of Itk results in elevated IgE production. *Blood* 114 (3), 564-571

46 Ueda-Hayakawa, I. *et al.* (2009) Id3 restricts the developmental potential of gamma delta lineage during thymopoiesis. *J Immunol* 182 (9), 5306-5316

47 Verykokakis, M. *et al.* (2010) Inhibitor of DNA binding 3 limits development of murine slam-associated adaptor protein-dependent "innate" gammadelta T cells. *PLoS One* 5 (2), e9303

48 Lewis, J.M. *et al.* (2006) Selection of the cutaneous intraepithelial gammadelta+ T cell repertoire by a thymic stromal determinant. *Nat Immunol* 7 (8), 843-850

49 Barbee, S.D. *et al.* (2011) Skint-1 is a highly specific, unique selecting component for epidermal T cells. *Proc Natl Acad Sci U S A* 108 (8), 3330-3335

50 Irving, B.A. *et al.* (1998) Thymocyte development in the absence of pre-T cell receptor extracellular immunoglobulin domains. *Science* 280 (5365), 905-908

51 Aifantis, I. *et al.* (2002) A critical role for the cytoplasmic tail of pTalpha in T lymphocyte development. *Nat Immunol* 3 (5), 483-488

52 Yamasaki, S. *et al.* (2006) Mechanistic basis of pre-T cell receptor-mediated autonomous signaling critical for thymocyte development. *Nat Immunol* 7 (1), 67-75

53 Pang, S.S. *et al.* (2010) The structural basis for autonomous dimerization of the pre-T-cell antigen receptor. *Nature* 467 (7317), 844-848

54 Haks, M.C. *et al.* (2003) Low activation threshold as a mechanism for ligand-independent signaling in pre-T cells. *J Immunol* 170 (6), 2853-2861

55 Itohara, S. and Tonegawa, S. (1990) Selection of gamma delta T cells with canonical T-cell antigen receptors in fetal thymus. *Proc Natl Acad Sci U S A* 87 (20), 7935-7938

56 Laird, R.M. *et al.* (2010) Unexpected role for the B cell-specific Src family kinase B lymphoid kinase in the development of IL-17-producing gammadelta T cells. *J Immunol* 185 (11), 6518-6527

57 Do, J.S. *et al.* (2010) Cutting edge: spontaneous development of IL-17-producing gamma delta T cells in the thymus occurs via a TGF-beta 1-dependent mechanism. *J Immunol* 184 (4), 1675-1679

58 Sutton, C.E. *et al.* (2009) Interleukin-1 and IL-23 induce innate IL-17 production from gammadelta T cells, amplifying Th17 responses and autoimmunity. *Immunity* 31 (2), 331-341

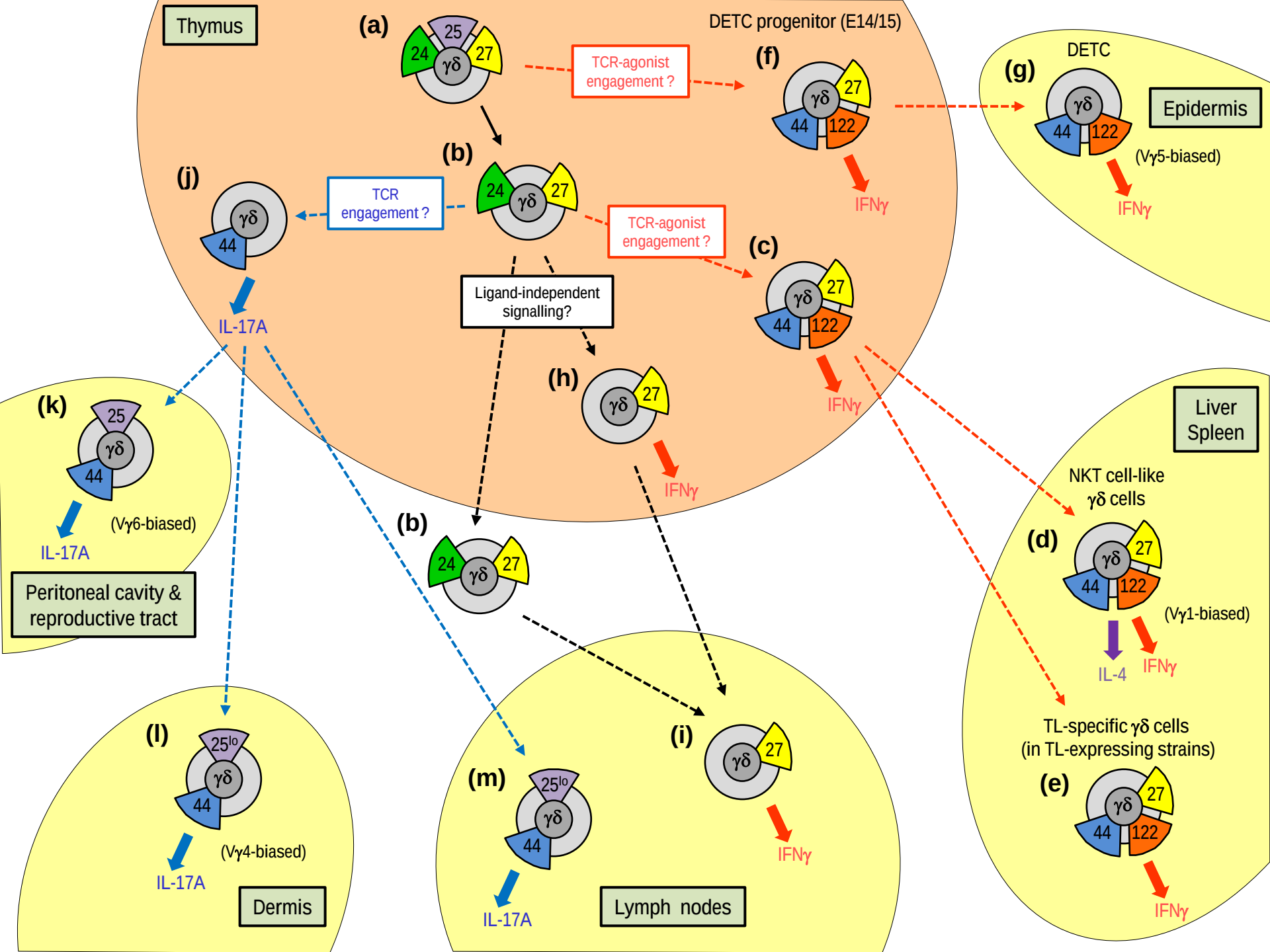
59 Ribot, J.C. *et al.* (2010) Cutting edge: adaptive versus innate receptor signals selectively control the pool sizes of murine IFN-gamma- or IL-17-producing gammadelta T cells upon infection. *J Immunol* 185 (11), 6421-6425

60 Hardy, R.R. *et al.* (2007) The protean nature of cells in the B lymphocyte lineage. *Immunity* 26 (6), 703-714

61 Wen, L. *et al.* (2005) Evidence of marginal-zone B cell-positive selection in spleen. *Immunity* 23 (3), 297-308

62 Stadanlick, J.E. *et al.* (2008) Tonic B cell antigen receptor signals supply an NF-kappaB substrate for prosurvival BLyS signaling. *Nat Immunol* 9 (12), 1379-1387

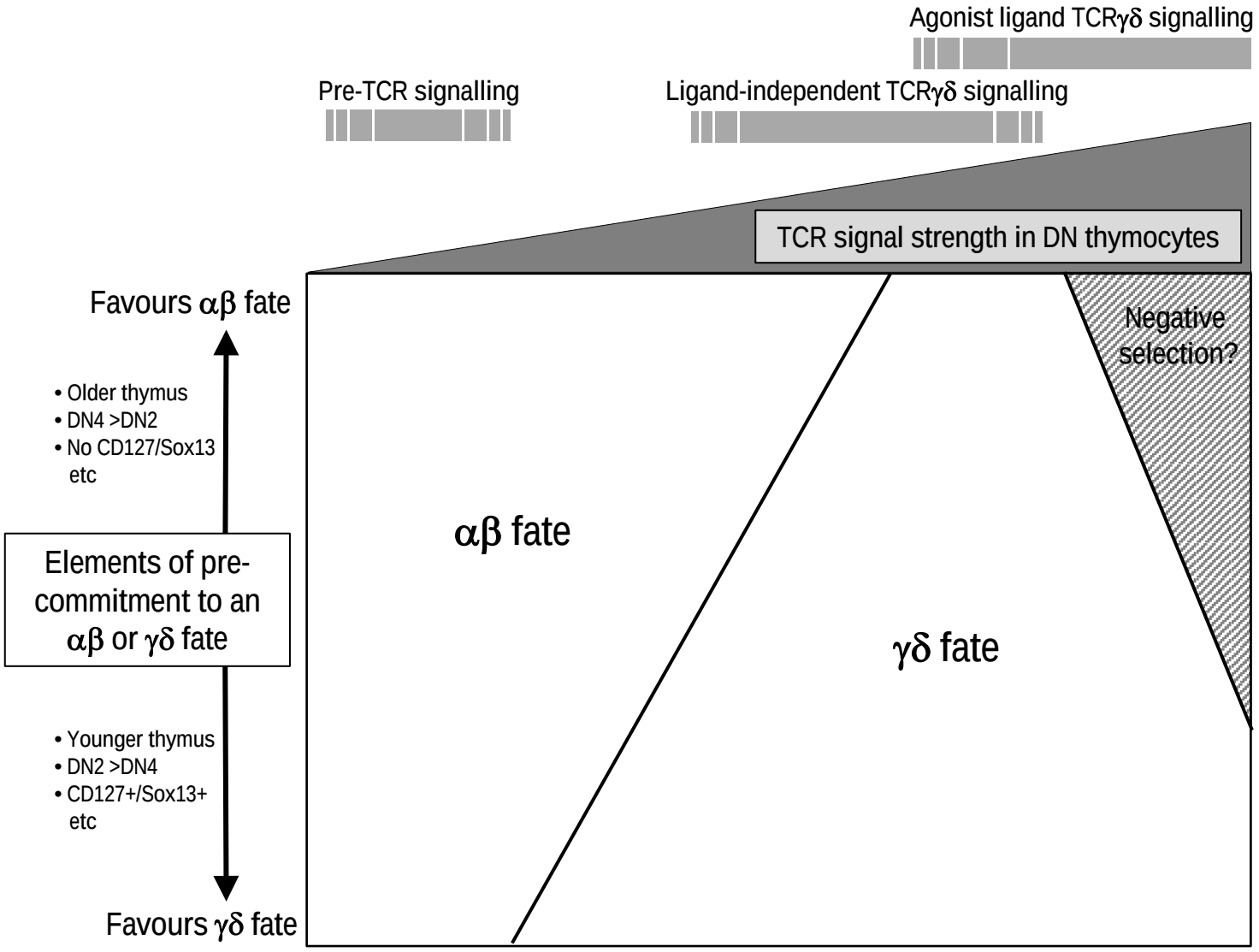
63 Malinarich, F.H. *et al.* (2010) Constant TCR triggering suggests that the TCR expressed on intestinal intraepithelial gammadelta T cells is functional in vivo. *Eur J Immunol* 40 (12), 3378-3388


Thymus
DETC progenitor (E14/15)
(a)
25
24
γδ
27
TCR-agonist engagement ?
(f)
27
γδ
44
122
IFNγ
(g)
DETC
Epidermis
(Vγ5-biased)
IFNγ
(b)
24
γδ
27
(j)
γδ
44
TCR engagement ?
TCR-agonist engagement ?
(c)
27
γδ
44
122
IFNγ
Ligand-independent signalling?
IL-17A
(h)
γδ
27
IFNγ
(k)
25
γδ
44
(Vγ6-biased)
IL-17A
Peritoneal cavity & reproductive tract
(b)
24
γδ
27
Liver
Spleen
NKT cell-like γδ cells
(d)
27
γδ
44
122
(Vγ1-biased)
IL-4
IFNγ
TL-specific γδ cells (in TL-expressing strains)
(e)
27
γδ
44
122
IFNγ
(l)
25lo
γδ
44
(Vγ4-biased)
IL-17A
Dermis
(m)
25lo
γδ
44
IL-17A
(i)
27
γδ
IFNγ
Lymph nodes

	IFN-γ	IL-17A	CD24	CD25	CD27	CD44	CD62L	CD122	NK1.1	CCR6	CCR9	SCART2
DETC (Vγ5-biased)	+ [5] (Thy)	- [5,9] (Thy Sk)	- [48] (Thy)	- [5,9] (Thy Sk)	+ [5] (Thy)	+ [9] (Sk)	ND	+ [5,48] (Thy Sk)	+ [5] (Thy)	- [12] (Sk)	ND	- [5,13] (Thy Sk)
NKT-like γδ cells (Vγ1-biased)	+ [11] (Thy)	ND	- [11] (Thy)	ND	- [16] (Thy)	+ [11] (Thy)	- [11] (Thy)	+ [47] (Thy)	Het [11] (Thy)	- [17] (Thy)	ND	ND
TL-specific γδ cells (on TL^b background)	+ [7] (Spl)	- [7]	ND	- [7] (Spl)	ND	+ [7] (Spl)	ND	+ [7] (Spl)	Het [7] (Spl)	ND	- [20] (Thy)	ND
Gut γδ IELs (Vγ7-biased)	lo [16]	- [19,63]	ND	- [16]	+ [16]	lo [63]	ND	lo [19]	ND	- [17]	+ [19]	ND
Lymphoid γδ^{27+} cells (CD122$^-$NK1.1$^-$)	+ [16]	- [16]	- [16]	- [16]	+ [16]	- [16]	+ [16]	- [16]	- [16]	- [17]	ND	ND
Lymphoid γδ^{27-} cells	- [16]	+ [16]	- [16]	lo [16]	- [16]	+ [16]	- [16]	- [16]	ND	Het [17] (LN)	Het [17]	Het [13] (LN)
Reproductive tract and peritoneal γδ cells (Vγ6-biased)	- [14]	+ [14]	ND	lo [14]	- [15]	+ [14]	ND	- [14]	- [16]	ND	ND	ND
Dermal γδ cells (Vγ4-biased)	ND	+ [9,12]	ND	lo [9]	ND	+ [9]	ND	ND	ND	+ [12]	ND	Het [13]

Expression of markers and cytokines by γδ subsets

"+" indicates expression of marker by a subset; "-" indicates absence of marker; "lo" indicates low expression of marker; "Het" indicates heterogeneous expression of marker; "ND" indicates expression not determined. Brackets indicate organ where expression was specifically observed; "Thy" is thymus; "Spl" is spleen; "LN" is lymph nodes; "Sk" is skin. References are provided in square brackets.


Agonist ligand TCRγδ signalling
Pre-TCR signalling
Ligand-independent TCRγδ signalling
TCR signal strength in DN thymocytes
Favours αβ fate
• Older thymus
• DN4 >DN2
• No CD127/Sox13
etc
Elements of pre-commitment to an αβ or γδ fate
• Younger thymus
• DN2 >DN4
• CD127+/Sox13+
etc
Favours γδ fate
αβ fate
γδ fate
Negative selection?