The Thymus as an Inductive Site for T Lymphopoiesis

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Key words: Thymus, T cell development, Notch, cytokines, morphogens, stromal cells.

Abstract

Like all hematopoietic cells, T lymphocytes are derived from bone marrow resident stem cells. However, while most blood lineages are generated within the marrow, the majority of T cell development occurs in a specialized organ, the thymus. This distinction underscores the unique capacity of the thymic microenvironment to support T lineage restriction and differentiation. Although the identity of many of the contributing thymus-derived signals are well established and rooted in highly conserved pathways involving Notch, morphogenic, and protein tyrosine kinase signals, the manner in which the ensuing cascades are integrated to orchestrate the underlying processes of T cell development remain under investigation. This review focuses on the current definition of the early stages of T cell lymphopoiesis with emphasis placed on the nature of thymus-derived signals delivered to T cell progenitors that support their commitment and differentiation towards the αβ and γδ T cell lineages.

Glossary list

FTOC: fetal thymus organ culture; an assay system for T cell development using cultured E14-E15 thymic lobes that may be depleted of endogenous thymocytes and reconstituted with donor progenitors.

OP9-DL1: A bone marrow stromal cell line (OP9) ectopically expressing the Notch ligand, Delta-like 1, which is able to support T lymphopoiesis in vitro.

β-selection: The process by which DN thymocytes that express a functionally rearranged TCRβ chain are selected to differentiate to the next stage of T cell development, giving rise to DP cells.

CCR9^{GFP}: A transgenic reporter mouse expressing GFP under the control of the endogenous *CCR9* gene promoter.

Thymus seeding progenitor: The precise identity of this cell remains controversial, but this term is used to functionally define cells that home to the thymus and possess potent T cell progenitor function.

Thymic Epithelial Cells (TECs): these are part of the non-hematopoietic stromal component of the thymus that is responsible for inducing and supporting T lymphopoiesis.

Fringe: Golgi-resident glycoslytransferases that modify Notch receptors to alter ligand specificity; include Radical, Lunatic, and Manic family members.

ADAM proteases: A family of transmembrane disintegrins and metalloproteases that proteolytically cleave the juxtamembrane region of transmembrane proteins and release their extracellular regions in a process termed ectodomain shedding.

Dominant negative: An inactive mutant or variant of a protein that interferes with the function of the wild-type form when expressed in the same cell, usually by binding and sequestering relevant interacting partners.

Abbreviation and Acronym list

APC, adenomatous polyposis coli;

bHLH, basic helix-loop-helix;

BMP, bone morphogenic protein

CMJ, cortical-medullary junction;

CSL, CBF-1/Suppressor of hairless/Lag-1;

DC, dendritic cells;

Dll, Delta-like;

dn, dominant negative

DN, double negative ($CD4^-$ $CD8^-$);

DP, double positive ($CD4^+$ $CD8^+$);

ETP, early T lineage progenitor;

FTOC, fetal thymus organ culture;

GFP, green fluorescent protein;

JAK, Janus kinase;

Hh, hedgehog;

HMG, high mobility group;

HSC, hematopoietic stem cell;

LEF, lymphocyte enhancer binding factor;

Lin, lineage markers;

LRP, low density lipoprotein receptor related protein;

MamL, mastermind-like;

NK, natural killer;

PI3K, Phosphatidylinositol-3-kinase;

RBPJ, recombining binding protein Jκ;

SCF, stem cell factor;

SCZ, subcapsular zone;

SP, single positive ($CD4^+$ or $CD8^+$);

STAT5, signal transducer and activator of transcription 5;

TCF, T cell factor;

TEC, thymus epithelial cell;

TCR, T cell receptor.

INTRODUCTION

The immune system originates from bone marrow resident hematopoietic stem cells (HSCs) that give rise to a hierarchy of progenitor populations with increasingly restricted lineage potential, ultimately leading to the generation of all lineages of mature blood cells. While the majority of hematopoietic lineages mature in the marrow, T cell development takes place in a specialized organ, the thymus. The thymus plays host to marrow-derived progenitors and supports multistage lineage commitment and differentiation steps to yield mature self-tolerant functional T cells.

In the adult, thymus-resident T cell progenitors possess limited self-renewing potential, thus, sustained T cell production requires the continual influx of blood-borne hematopoietic progenitors (Scollay et al 1986, Donskoy & Goldschneider 1992). Such progenitor seeding appears to be a gated phenomenon, occurring in a periodic manner that is dependent on the availability of limited thymic microenvironmental niches (Foss et al 2001, Prockop & Petrie 2004). Despite intense investigation by several groups, the true nature of the thymus-seeding progenitor(s) remains uncertain, largely due to the technical challenges associated with identifying these exceedingly rare cells under physiological conditions. Nevertheless, there is evidence for multiple types of thymus-seeding cells that possess varying degrees of T lineage commitment (Bhandoola et al 2007).

Two lineages of T cells are generated in the thymus, αβ and γδ, which are defined by the expression of distinct αβ- or γδ-T cell receptor (TCR) complexes. This is accomplished via a highly regulated process, during which several checkpoints function to limit the production of cells bearing nonfunctional or autoreactive TCRs. These events occur at discrete phenotypic stages, distinguished by the coordinated expression of key cell surface molecules, namely the CD4 and CD8 coreceptors. The most immature subset of thymocyte precursors lack expression of CD4 and CD8, and are thus denoted double negative (DN). It is at this critical stage that progenitors become committed to the αβ or γδ T cell lineage (Petrie et al 1992, Dudley et al 1995, Ciofani et al 2006). αβ lineage thymocytes progress to the CD4 CD8 double positive (DP)

stage where they undergo further positive and negative selection to generate major histocompatibility complex (MHC)-restricted and self-tolerant, CD4 and CD8 single positive (SP) T cells (Figure 1) (Starr et al 2003). The resulting mature γδ T cells, and αβ T cells comprising the $CD8^+$ cytotoxic, $CD4^+$ helper and regulatory lineages, form the basis of cellular immunity. Notwithstanding the central role of thymus-derived factors in supporting all stages of thymocyte development and selection, this review will focus on the influence of such elements during the early DN progenitor stages, where much progress has been made in recent years.

ONTOGENY OF THE THYMUS STROMA

During mouse embryogenesis, the thymus primordium is formed between E10.5 and E11.5 from the third pharyngeal pouch endoderm, a process that depends on interactions with the surrounding neural crest-derived mesenchyme (Auerbach 1960, Blackburn & Manley 2004, Gordon et al 2004). At approximately E12, the thymic rudiment is first colonized by lymphocyte progenitors (Jotereau et al 1987), which enter through the capsule via a chemoattractive mechanism (Liu et al 2006). At this stage, the rudiment lacks the histologically defined cortex and medulla regions characteristic of the adult organ; rather it comprises predominantly bipotent thymic epithelial cell (TEC) progenitors that subsequently undergo a poorly defined lineage commitment and differentiation program, resulting in the establishment of distinct cortical and medullary TEC subsets, the major constituents of thymic stroma (Rossi et al 2006). Thus, the first wave of T cell differentiation coincides with the late stages of thymus organogenesis.

Notably, following the initial epithelial cell patterning event, thymocyte-derived signals are essential for maintaining TEC differentiation and organization in the neonate and adult (Anderson et al 2001, Klug et al 2002). The exquisite interdependence of thymocyte and TEC populations has been termed 'thymic cross-talk' (van Ewijk et al 1994). Although its molecular basis is largely unknown, there is evidence that it may involve signals of the NFκB signaling pathway downstream of tumor necrosis factor receptor associated factor 6 and the Lymphotoxin β receptor in the medulla (Derbinski & Kyewski 2005). Finally, other non-hematopoietic stromal elements, such as fibroblast and endothelial cells, and distinct hematopoietic-derived myeloid dendritic cells (DC) and macrophages also contribute to the final thymic architecture.

ARCHITECTURAL PERSPECTIVE

The thymic epithelium is essential to T cell development. This is best evidenced by the phenotype of *nude* mice in which disruption of the transcription factor FoxN1 arrests TEC development at an immature progenitor stage and leads to loss of intrathymic T cell development and severe immunodeficiency (Nehls et al 1996, Su et al 2003, Bleul et al 2006). The specialized TEC subsets of the cortex and medulla facilitate distinct phases of T cell development. Consistent with this view, thymocytes at different stages of maturation occupy distinct regions in the adult thymus, indicating that differentiation is coupled with the highly coordinated migration between microenvironments; progenitors enter at the cortico-medullary junction (CMJ), migrate through the cortex to the outer subcapsular zone (SCZ) during the early progenitor stages, and then back towards the CMJ and into the medulla (see Figure 1) (Lind et al 2001, Petrie & Zúñiga-Pflücker 2007). Accordingly, cortical TECs support early T cell progenitor commitment and differentiation, and play a central role in the processes of β-selection (see below) and positive selection (Anderson et al 1993, Anderson et al 1994). Whereas, medullary TECs, in conjunction with DC, are essential for effective negative selection during the late stages of development (Derbinski et al 2001, Gallegos & Bevan 2004).

STAGES OF T LYMPHOPOIESIS, FROM DN TO DP

The immature DN subset is classically subdivided into four successive developmental stages based on the expression of CD44, CD117 (c-kit), and CD25 (Godfrey et al 1993, Zúñiga-Pflücker & Lenardo 1996). The most primitive intrathymic progenitors are found within the $CD44^{+}$ $CD117^{+}$ $CD25^{-}$ DN1 subset. Such canonical T cell progenitors may be defined by the ability to proliferate extensively and generate all thymocyte populations of both αβ and γδ lineages. In the adult thymus, these early T lineage progenitors (ETPs) were originally characterized as $CD4^{lo}$ $Sca\text{-}1^{+}$ $CD117^{+}$ and lineage marker negative (Lin^{-}) cells (Wu et al 1991b), and subsequently restricted to a $CD117^{hi}$ $IL\text{-}7R\alpha^{-/lo}$ population therein (Allman et al 2003). In addition to robust T cell precursor activity, when transferred intravenously into irradiated hosts, ETPs can generate other thymus-derived lineages such as DCs and natural killer (NK) cells, yet

possess only minimal B cell or myeloid potential (Wu et al 1991a, Ardavin et al 1993, Moore & Zlotnik 1995, Allman et al 2003, Sambandam et al 2005, Tan et al 2005). The heterogeneity within the ETP compartment has led to further subfractionation with the goal of identifying the most immature thymocyte progenitor or the earliest thymus-seeding cell. In this regard, rare, but largely overlapping populations have been defined as $CD24^{-}$ (DN1a) (Porritt et al 2004), $CD135^{hi}$ (Sambandam et al 2005), and *Ccr9*-EGFP reporter^{hi} (Benz & Bleul 2005) that possess greater proliferative and T cell precursor activity, although these subfractions have been reported to posses varying degrees of non-T lineage potential.

T lineage restriction is a protracted process, lasting up to two weeks, as thymocytes traverse the subsequent two developmental stages beyond the DN1 stage. Expression of CD25 on DN1 cells marks progression to the T lineage specified DN2 stage ($CD44^{+}$ $CD117^{+}$ $CD25^{+}$). This subset is characterized by active proliferation, thus expanding the rare precursor population. Although DN2 cells give rise primarily to T cells when injected into mouse recipients, or assayed *in vitro* in fetal thymic organ cultures (FTOC) and in OP9-DL1 stromal monolayer cultures (Moore & Zlotnik 1995, Zúñiga-Pflücker et al 1995, Schmitt et al 2004), this progenitor subset also retains limited potential for NK and DC lineages (Ardavin et al 1993, Wu et al 1996, Ikawa et al 1999, Shen et al 2003, Schmitt et al 2004). Uniform and irreversible T lineage commitment is achieved at the subsequent DN3 stage, concurrent with the down regulation of CD44 and CD117 expression.

Recombination-activating gene (RAG) 1- and RAG 2-mediated rearrangements of the TCRβ, TCRγ, and TCRδ loci, which are required for the assembly of the TCR, are initially detected in DN2 cells, and continue predominantly during the mostly non-cycling DN3 stage (Godfrey et al 1994, Capone et al 1998, Livak et al 1999). Implicit in this process is an obligate developmental checkpoint ensuring that only those cells that have productively rearranged their TCR loci are permitted further differentiation, while those that fail undergo cell death (Michie & Zúñiga-Pflücker 2002). Accordingly, immature thymocytes expressing a functional TCRβ chain, which associates with the invariant pre-TCRα (pTα) chain and CD3 signaling molecules to form the pre-TCR complex, are selected for αβ lineage differentiation. The pre-TCR mediates the 'β-selection' event (Dudley et al 1994), by signaling rescue from apoptosis, intensive cellular

expansion (six to eight cycles), cessation of TCRβ locus recombination (allelic exclusion), and differentiation to the DP stage.

During the pre-TCR-induced transition to the DP stage, selected cells down regulate CD25 (to become DN4 or pre-DP cells) and progress through an immature CD8 single positive (ISP) stage. The critical role of pre-TCR formation for αβ lineage development is evidenced by the severe arrest in T cell development at the DN3 stage observed in mice with deficiencies in RAG1, RAG2, or any component of the pre-TCR complex. Although the pre-TCR is considered to signal cell-autonomously in a ligand-independent manner by self-oligomerization (Yamasaki et al 2006), differentiation to the DP stage is additionally dependent on thymus-derived factors (see Notch section below).

Alternatively, generation of a γδ-TCR, resulting from the production and pairing of in-frame TCRγ and TCRδ chains, permits differentiation of DN3 progenitors along the γδ T cell lineage, whereby CD25 is down regulated and the majority of cells remain DN (Passoni et al 1997, Kang et al 1998). Notably, the γδTCR can also mediate 'β-selection'-like differentiation to the DP stage, a phenomenon associated with reduced receptor expression or strength of signal (Hayes et al 2005). Until recently, the outcomes of γδ-TCR formation have been poorly characterized, owing largely to the lack of distinguishing phenotypic markers for emerging γδ lineage cells or their immediate precursors. In this regard, an elegant study by Prinz et al employed a green fluorescent protein (GFP) reporter of the TCRδ locus transcription in combination with γδ-TCR surface expression to decipher the early events of γδ T cell selection (Prinz et al 2006). This analysis delineated GFP^{+} γδ-TCR^{lo} pre-selection cells within the DN3 subset that up regulate γδ-TCR expression, and mature contingent on γδ-TCR signaling. Similarly, using a TCRβ-deficient mouse model, Taghon *et al.* demonstrated that, analogous to β-selection, γδ-selection is accompanied by the up regulation of CD5 and CD27, allowing the definition of common pre- and post-selection DN3 subsets: DN3a and DN3b, respectively (also known as DN3E/DN3L) (Dudley et al 1994, Taghon et al 2006).

The ability to track newly emerging γδ T cells has revealed that, contrary to earlier views, γδ-selection triggers significant cellular expansion, although this remains on average two cell cycles fewer than that observed for β-selection (Prinz et al 2006, Taghon et al 2006). However, unlike αβ development, there is no evidence for a pre-antigen receptor regulated checkpoint in γδ T cell differentiation, rather selection occurs at a single TCR-dependent event (Prinz et al 2006). Although non-classical MHC ligands have been described for a few γδ-TCR specificities (Chien & Konigshofer 2007), and an obligate positive selecting thymic stromal determinant was uncovered for a subset of canonical γδ-TCR-expressing cells (Lewis et al 2006), a universal ligand-dependence for γδ T cell differentiation remains unclear.

γδ lineage cells can additionally be distinguished from αβ lineage DP cells by a γδ-biased gene expression profile, which is also shared by DN2 and DN3 thymocyte progenitors, and appears to be dependent on Lymphotoxin-induced signals provided by αβ lineage DP cells (Pennington et al 2003, Silva-Santos et al 2005). Several studies from Hayday's group form the basis of a model in which interactions between DP cells and progenitors regulate early T cell development. Such 'trans-conditioning' does not influence αβ versus γδ lineage decisions, rather it appears to impact on a progenitor's future functional capacity such that in pre-TCR-deficient mice, lacking in DP cells, γδ thymocytes exhibit an atypical regulatory T cell profile (Pennington et al 2006) (for an excellent review see (Hayday & Pennington 2007)). While the bulk of the present review addresses stromal-derived factors that regulate early T lineage differentiation in the thymus, it is worth considering that the majority of the organ constitutes the thymocytes themselves, and as such, interactions between developing T cells would likely impinge on this process.

LINEAGE DIVERSIFICATION, αβ versus γδ

In recent years, there has been renewed interest in the mechanism of αβ versus γδ lineage commitment. In fetal and adult stages, both lineages emerge from a common DN progenitor (Dudley et al 1995, Ciofani et al 2006). Establishing the timing of lineage divergence is central to understanding the context in which αβ versus γδ commitment occurs. Current models of lineage commitment at this stage distinguish between two possible roles for TCR, as instructing

commitment or reinforcing a prior commitment event. A great body of evidence supports the important role of TCR complex formation in lineage outcome, and the evidence for these competing models have been reviewed extensively (Hayday et al 1999). This debate can be resolved by defining the divergence point of the two lineages. This, however, is not an easy task due to the lack of definitive markers to distinguish αβ and γδ lineage cells prior to TCR expression. In one strategy, a clonal analysis of DN2 and DN3 potential was performed in OP9-DL1 cultures. The results reinforced the view that lineage divergence initiates at the DN2 stage and is completed at the subsequent DN3 stage (Ciofani et al 2006). Interestingly, RAG2-deficient DN3 progenitors retain a high degree of potential for both αβ and γδ lineages, as provision of functionally rearranged TCRβ or both TCRγ and TCRδ chains results in differentiation towards the cognate αβ or γδ lineage with a frequency approaching unity. These data strongly argue in support of the view that pre-TCR or γδ-TCR expression ultimately directs lineage outcome. This evidence does not rule out pre-commitment options, per se, yet suggests that any factors that predispose a particular lineage likely do so by influencing TCR rearrangement and/or expression.

Recently, support for the notion that pre-commitment events may influence lineage decisions was provided by the identification of Sox13 as a γδ T cell-specific high mobility group (HMG)-box transcription factor, and putative lineage regulator (Melichar et al 2007). While gene deficiency indicates Sox13 to be important for normal γδ T cell development, Sox13 over expression in DN thymocytes specifically impairs αβ lineage development to the DP stage (Melichar et al 2007). Notably, Sox13 displays heterogeneous expression in DN2 cells, which falls below detection by the subsequent DN3 stage, suggesting that it exerts its effects prior to TCR expression. Although compelling, several questions remain as to what factors regulate Sox13 expression, and whether it directly influences TCR rearrangement or expression.

As noted above, the TCR (pre-TCR or γδ-TCR) provides lineage-determining signals. Importantly, a recent variation of the instructive model, based on differences in TCR signal strength, helps to clarify how particular TCRs yield alternative αβ and γδ lineage outcomes (Haks et al 2005, Hayes et al 2005). Whereas relatively weaker signals are associated with the

pre-TCR, which is presumed to signal autonomously (Irving et al 1998, Yamasaki et al 2006), quantitatively larger signals are achieved by the γδ-TCR, perhaps via ligand engagement. Indeed, alterations in parameters that quantitatively affect TCR surface expression or signal transduction have been demonstrated to influence αβ versus γδ lineage outcome (Haks et al 2005, Hayes et al 2005). Differences in TCR signal strength may not only apply to this lineage choice, but also distinguish the processes of positive and negative selection, and likely influence the CD4 versus CD8 lineage decision.

While TCR signals ultimately define lineage, inputs from the thymus-derived factors have been suggested to also influence this commitment stage as will be addressed below.

NOTCH AND T CELL DEVELOPMENT

The evolutionarily conserved Notch pathway has proven to be necessary for nearly all aspects of metazoan development. The past decade has witnessed the emergence of this pathway as a defining signaling mechanism in T cell lymphopoiesis, underpinning the processes of commitment, differentiation, and transformation.

In essence, Notch receptor-ligand interactions serve to communicate signals between neighboring cells. This is reflected by a highly conserved signaling mechanism activated by interaction of Notch with cognate ligands (reviewed in (Bray 2006)). Four mammalian Notch receptors have been identified (Notch1-4), which can engage five known ligands (Delta-like 1 (Dll1), Dll3, Dll4, Jagged1, and Jagged2). The specificity of receptor-ligand binding can be modulated by Fringe family glycosyltransferases to restrict Notch activation to Delta ligands (Panin et al 1997). Canonical signaling is initiated by the ligand-induced proteolysis of Notch by ADAM proteases, which removes the majority of the extracellular domain. This generates a substrate for Presenilin-dependent γ-secretase cleavage within the transmembrane region, releasing the intracellular domain of the receptor (Notch-IC). The latter cleavage event appears to depend on ubiquitination of truncated Notch and its targeting to endocytic vesicles (Gupta-Rossi et al 2004). Once freed, Notch-IC translocates to the nucleus and directly regulates target gene expression via its interaction with the DNA-bound transcription factor CSL (CBF-

1/Suppressor of hairless/Lag-1; RBPJ in mice). In this regard, Notch-IC coordinates a repressor-activator switch, displacing a histone deacetylase corepressor complex and recruiting co-activators of the Mastermind-like (MamL) family to activate transcription (Bray 2006).

The canonical Notch signaling pathway, as described, is seemingly simple, lacking second messengers and signal amplification, however, the outcomes of Notch activation on cell fate and cellular processes are complex and cellular context dependent. Only a limited number of conventional Notch target genes have been identified, including basic helix-loop-helix (bHLH) transcriptional repressors Hairy-enhancer of split (*Hes*) 1, *Hes5*, *Herp* (Hes-related repressor protein), *Deltex*, and *Nrarp* (Jarriault et al 1995, Deftos et al 2000, Iso et al 2001, Krebs et al 2001). Although tissue-specific targets also exist, for instance *Ptcra* (pre-Tα) and *Cd25* in thymocytes (Reizis & Leder 2002, Maillard et al 2006), there is evidence that specificity in expression is achieved via transcriptional co-regulation (Cave et al 2005). This feature also extends to apparently universal targets such as *Hes* family genes, for which there are examples of co-regulation by evolutionarily related bHLH factors in multiple species (Bailey & Posakony 1995, Ikawa et al 2006).

T cell lineage commitment

The best-established function for Notch signaling in the hematopoietic system is its essential role in T lineage specification. While thymocytes express multiple Notch receptors (Notch1, 2, and 3), this event is mediated exclusively by Notch1. Induced deletion of Notch1 or RBPJ in hematopoietic progenitors results in a complete block in T cell development and ectopic differentiation of immature B cells in the thymus (Radtke et al 1999, Han et al 2002). Similar aberrations in lymphopoiesis in the thymus are observed following attenuation of Notch signaling following over expression of dominant negative (dn) MAML (Maillard et al 2004), or Deltex-1 (Izon et al 2002) in hematopoietic progenitors. Conversely, progenitors expressing constitutively active Notch-IC fail to generate B cells, and rather give rise to DP stage immature T cells extrathymically (Pui et al 1999). Employing a distinct approach, several groups have demonstrated that ectopic expression of Dll1 or Dll4 endows bone marrow stromal cell lines with the capacity to support early T lineage development, while inhibiting B cell generation from

hematopoietic progenitors in vitro (Jaleco et al 2001, Schmitt & Zúñiga-Pflücker 2002, Hozumi et al 2004). Taken together, these reciprocal findings indicate that canonical CSL-dependent Notch signals serve not only to induce T lineage specification, but also to limit B cell development in the thymus. While early models suggested that these events occur concurrently in a T-B bipotent common lymphoid progenitor in the thymus (Radtke et al 1999), it appears that this need not be the case as such thymocyte precursors are exceedingly rare (Benz & Bleul 2005), and moreover, the majority of B cell potential in the thymus fails to co-segregate with robust T cell potential characteristic of canonical T cell progenitors (Porritt et al 2004).

Although the central role of Notch in T versus B lineage specification has been recognized for nearly a decade, relatively little is understood regarding the molecular targets of Notch that are essential for T lineage development. Recent evidence suggests that Notch acts in concert with E2A to induce a T lineage-specific program of gene expression (Ikawa et al 2006). Such transcriptional cooperativity, may account for why developmentally relevant targets have remained elusive (Weerkamp et al 2006b). In addition to activation of targets, repression via Hes-1 also appears to be required for normal T cell development. However, Hes-1 deficiency has a greater effect on cell expansion and survival rather than T lineage commitment (Tomita et al 1999, Kaneta et al 2000). In line with this finding, over expression of Hes-1 in thymocytes enhances proliferation (Kaneta et al 2000). On the other hand, although over expression of Hes-1 and Hes-5 has been shown to limit B cell development (Kawamata et al 2002), the mechanism of Notch-mediated opposition of B lineage commitment remains to be characterized.

The generation and/or maintenance of the earliest intrathymic ETP subset and its subsequent differentiation through to the DN3 stage is highly dependent on Notch signals (Schmitt et al 2004, Sambandam et al 2005, Tan et al 2005). As discussed above, T lineage commitment is a protracted process, not uniformly achieved until the DN3 stage. Accordingly, continued Notch ligand interactions at the DN1 and DN2 stages are necessary to maintain T lineage specification such that discontinuation of signal in vitro allows differentiation to an alternative lineage, such as NK cells (Schmitt et al 2004) and DCs (Cheng et al 2003) or plasmacytoid-DCs (Dontje et al 2006). It is unclear to what extent intrathymic niches of limited Notch ligand availability in fact contribute to the generation of small numbers of non-T lineage cells. Importantly, different

Notch signaling thresholds are associated with various cellular outcomes: relatively low levels are sufficient for suppression of B lineage potential, while higher levels are required for loss of NK potential and promotion of T lineage specification (Schmitt et al 2004, Tan et al 2005). However, super-physiological levels of Notch signaling appear to be detrimental, as ETPs lacking Mint, an inhibitor of Notch/RBPJ-mediated transcription, display reduced transition to the DN2 stage (Tsuji et al 2007). This fine balance likely results from the stringent regulation of Notch-IC turnover, and may explain the absence of intrathymic development from progenitors overexpressing active Notch-IC (Pui et al 1999).

Notch signaling during αβ and γδ lineage commitment

Among DN thymocyte subsets, Notch1 and Notch3 receptor expression and activity appear to be maximal in DN3 cells (Huang et al 2003, Duncan et al 2005, Taghon et al 2006), consistent with the prominent role of this pathway both prior to and during β-selection. At this stage, the classic function of Notch as a commitment factor gives way to a more unconventional role as a trophic factor, maintaining the survival of pre-T cells (Ciofani & Zúñiga-Pflücker 2005). Notch signals support Akt activation and c-Myc expression in pre-selection DN3 cells (Ciofani & Zúñiga-Pflücker 2005, Weng et al 2006), pathways central to the regulation of glucose and protein metabolism. This feature of Notch likely provides an essential metabolic competence for the energy-intensive requirements of β-selection, highlighting a parameter not typically considered, yet critical for cellular responsiveness during developmental transitions associated with a rapid and extensive burst of proliferation.

The requirement for Notch activity during the DN to DP transition is supported by numerous studies. Conditional inactivation of Notch1 or RBPJ during the DN2-DN3 transition results in impaired β-selection (Wolfer et al 2002, Tanigaki et al 2004). This developmental arrest has been attributed, in part, to inefficient Vβ to DJβ rearrangement in the absence of Notch1 (Wolfer et al 2002), although interestingly, this defect has not been observed in other deficiency models. Nevertheless, expression of the pre-TCR or its downstream signaling mediators in RAG-deficient DN3 cells differentiated in OP9/OP9-DL1 cultures revealed that cooperative signaling provided by Notch is additionally necessary for the functional outcomes of β-selection (Ciofani

et al 2004). The absolute requirement for Notch signals in this process was recently confirmed in vivo via inhibition of Notch activity in thymocytes through transgenic expression of dnMAML (Maillard et al 2006). In vitro studies indicate that Notch signals continue to be important in supporting cellular expansion at later stages of DN to DP transition (Garbe et al 2006). Data to date support the conclusion that this developmental transition is intrinsically dependent on Notch signals irrespective of the selection complex, be it pre-, αβ-, or γδTCR (Ciofani et al 2006, Garbe et al 2006), suggesting that Notch fulfills fundamental roles in this process. Importantly, transition to the DP stage is accompanied by attenuation of Notch receptor expression and activity (Huang et al 2003, Duncan et al 2005, Taghon et al 2006); this represents an obligatory event, as dysregulation of Notch at this stage is highly oncogenic (see side box).

αβ/γδ lineage divergence is completed at the DN3 stage of development. As the quintessential commitment factor, early studies suggested that Notch functions in directing the αβ versus γδ lineage decision. Indeed, reduced Notch1 gene dosage favored γδ T cell development from Notch1$^{+/-}$ HPC when compared to wild-type precursors in mixed bone marrow reconstitution chimeras (Washburn et al 1997). More recently, the nominal increase in γδ cellularity coupled with impaired αβ development following conditional deletion of RBPJ prior to the DN3 stage was considered to further support this model (Tanigaki et al 2004). As issues of commitment are difficult to assess in steady state systems, several recent studies have examined the differentiation of defined DN precursor populations in OP9 cultures in the presence and absence of Notch signals (reviewed in (Hayday & Pennington 2007)). Notably, analysis of αβ and γδ precursor frequency among DN2 and DN3 subsets in this context indicate that Notch-Dll1 interactions do not direct lineage choice, rather TCR signals represent the major determinant at this bifurcation (Ciofani et al 2006). Studies in which Notch signals are attenuated or lost due to Notch1 or RBPJ gene deletion (Wolfer et al 2002, Tanigaki et al 2004), use of γ-secretase inhibitors in FTOC (Doerfler et al 2001), and more recently, following conditional expression of dn-MAML (Maillard et al 2006), or culture of DN3 cells with OP9 cells lacking Delta-like ligands (Lehar et al 2005, Ciofani et al 2006, Garbe et al 2006, Taghon et al 2006), are consistent with a differential requirement for the Notch pathway during αβ and γδ lineage development, a trend easily mistaken for a role for Notch in lineage choice. Moreover, in vitro analysis in which the timing of TCR receptor expression and Dll1 availability are precisely controlled indicates

that while Notch serves as a critical competence factor during β-selection, this is not the case for γδ-selection (Ciofani et al 2006). In fact, γδ lineage differentiation becomes relatively independent of Notch signals once γδTCR expression is attained. Nevertheless, while not obligatory, Notch signals can enhance γδ differentiation by supporting proliferation and/or survival of γδ-selected cells or their immediate precursors (Lehar et al 2005, Ciofani et al 2006, Garbe et al 2006).

Side Box: **Notch and T cell acute lymphoblastic leukemia (T-ALL)**

Among thymus-derived signals, dysregulation of the Notch pathway is most associated with transformation in the T lineage. Indeed, the clearest example of oncogenic Notch signaling is observed in T-ALL, an aggressive immature T cell neoplasm. In fact, the first mammalian Notch homolog (NOTCH1) was identified due to its involvement in a (7;9) chromosomal translocation occurring in ~1% of T-ALL cases (Ellisen et al 1991). Recently, a prominent role for aberrant Notch signaling in leukemia pathogenesis was revealed with the identification of activating mutations of NOTCH1 in greater than 50% of T-ALLs (Weng et al 2004). Notably, in mouse models, efficient thymocyte transformation by Notch-IC is dependent on intact pre-TCR formation and signaling (Allman et al 2001, Bellavia et al 2002, Campese et al 2006), underscoring the importance of the observed attenuation of Notch activation following β-selection. Thus far, the most promising targets of the Notch pathway (direct and indirect) that may contribute to cell autonomous survival and growth in T-ALL include c-myc (Palomero et al 2006, Weng et al 2006), and the serine/threonine kinase Akt (Ciofani & Zúñiga-Pflücker 2005). Both of these pathways regulate cellular metabolism and stimulate protein biogenesis and growth, and thus represent potential targets for future therapies.

Role of Notch at Late Stages of T cell development

The involvement of Notch signaling during CD4 and CD8 T cell lineage commitment remains unclear. Indeed, early studies employing transgenic expression of Notch-IC in thymocytes produced disparate outcomes: constitutive Notch activation promoted CD8 over CD4 lineage differentiation in one case (Robey et al 1996), while enhancing MHC-independent positive selection to both lineages in another (Deftos et al 1998, Deftos et al 2000); this discrepancy was

subsequently shown to be due to the confounding effects of transformation in the later (Fowlkes & Robey 2002). Nevertheless, conditional thymocyte deficiencies in Notch1 or RBPJ do not alter intrathymic CD4/CD8 ratios or differentiation (Wolfer et al 2001, Tanigaki et al 2004), arguing against a requirement for canonical Notch signaling at this stage. However, selection events have not been fully addressed in these models as the effects of Notch signals may not be apparent in a diverse repertoire. Clarification of this issue will necessitate further investigation.

Notch Ligands

While numerous studies have identified transcripts for all Notch ligands in the thymus (reviewed by (Radtke et al 2004)), presently, a detailed expression topography is lacking. Both the paucity of ligand-specific antibodies and differences in the limit of detection among assays has contributed to conflicting reports on this issue (see Table I) (Schmitt et al 2004, Lehar et al 2005, Heinzel et al 2007). Notwithstanding theses difficulties, the expression of multiple Notch ligands on TECs raises the question as to which interactions are physiologically relevant for T lineage commitment and maturation. Although gene inactivation studies have failed to reveal a non-redundant role for Jagged1, Jagged2 or Dll1 in the thymus (Jiang et al 1998, Hozumi et al 2004, Mancini et al 2005), members of the Delta-like family appear to be crucial in this regard, as ectopic expression of Dll1 or Dll4, but not Jagged1 or Jagged2, on bone marrow stromal cell monolayers confers the capacity to support T lineage specification events in vitro (Jaleco et al 2001, Schmitt & Zúñiga-Pflücker 2002, Hozumi et al 2004). The apparent inefficiency of Jagged ligands likely stems from the observed expression of Lunatic Fringe by thymic progenitors (Visan et al 2006), which restricts Notch activation to Delta-family ligands. The importance of Delta-induced signals is highlighted in a recent study demonstrating that loss of Dll1 and Dll4 expression accounts for the inability of fresh-ex vivo thymic stromal monolayers to support T lineage specification upon disruption of their three-dimensional organization (Mohtashami & Zúñiga-Pflücker 2006).

Recent work from Radtke and colleagues proposes that Dll4 may trigger the physiological Notch1 signal for T lineage specification (Besseyrias et al 2007). Notch1$^{-/-}$ hematopoietic progenitor cells, which fail to differentiate towards the T cell lineage in vivo, undergo Notch2-dependent T cell development in OP9-DL1, but not OP9-DL4 cultures, suggesting that Notch1

signaling to early thymocyte progenitors is restricted to Dll4 in the thymus. Consistent with this view, Dll4 binds immature thymocytes, including ETPs, with high affinity (Heinzel et al 2007). Conditional deletion of Dll4 in thymic stroma, alone and in combination with Dll1 thymic deficiency, will provide a definitive test of this hypothesis. Currently, it is unknown whether different ligands regulate activation in the CMJ and SCZ, or rather, at different stages of T cell development.

CYTOKINES: EARLY T CELL LYMPHOTROPHIC FACTORS

Among the numerous cytokines produced by the thymic stroma, stem cell factor (SCF) and IL-7 are notable, long recognized for serving non-redundant essential functions during early T lymphopoiesis. SCF signals via the c-kit receptor protein-tyrosine kinase (CD117), which is highly expressed on HSCs, lymphoid progenitors, and canonical T cell precursors at the DN1/ETP and DN2 stages (Ogawa et al 1991, Wu et al 1991b, Godfrey et al 1993). Early studies revealed that while permissive to T cell development, CD117 deficiency results in a severe reduction in DN1 cellularity (Di Santo & Rodewald 1998). A similar defect is noted in SCF-mutant fetal thymus grafts transplanted into wild-type mice, emphasizing that thymus-expressed SCF is essential for T cell progenitor expansion (Rodewald et al 1995). However, the location of SCF-producing cells in the adult thymus has yet to be reported. Recent studies examining the effects of attenuated CD117 signals for purified DN subsets in OP9-DL1 cultures have extended this requirement to DN2, yet not DN3 stage cells (Massa et al 2006). Signaling through CD117 is pleiotropic, and the full range of functions relevant to rare precursors remains to be assessed. In this regard, neutralization of SCF, not only inhibits proliferation, yet also accelerates T lineage maturation from lymphoid progenitors, suggesting that SCF can maintain cells in an undifferentiated state (Wang et al 2006). This property likely contributes to the progressive age-dependent loss of lymphoid and thymocyte progenitors in adult CD117-deficient viable mice (Waskow et al 2002).

Defects in IL-7 signaling results in one of the most dramatic phenotypes among cytokine deficiencies and accounts for severe combined immunodeficiency in humans (Puel et al 1998). In the thymus, the IL-7 receptor (IL-7R) is expressed on DN2 and DN3 progenitors, and is down

regulated concurrent with β-selection, after which DP thymocytes remain refractory to IL-7 until positive selection (Sudo et al 1993, Van De Wiele et al 2004, Yu et al 2006). IL-7R signals are transmitted via the Janus kinase (JAK) 1 and JAK3/STAT5 (signal transducer and activator of transcription 5), and Phosphatidylinositol-3-kinase (PI3K)/Akt pathways. Unlike Notch, IL-7 signals are not considered to directly regulate T lineage commitment. In line with this, ETPs express little to no surface IL-7R (Allman et al 2003, Porritt et al 2004). Nevertheless, restricting IL-7 signaling in such uncommitted progenitors may contribute to limiting B cell potential in the thymus. Indeed, constitutive activation of STAT5 in immature thymocytes can upregulate the master regulator Pax-5 and promote adoption of the B cell fate (Goetz et al 2005). However, low level IL-7 signaling is important at or prior to the ETP stage as complete T lymphopenia in CD117-mutant mice is contingent on concomitant loss of IL-7R signaling (Rodewald et al 1997). Thus, CD117 and IL-7R appear to synergize in supporting the establishment of the T cell compartment.

Deficiencies in IL-7 or either or its receptor components, IL-7Rα (CD127) or IL-2Rγc (CD132; common receptor γ chain) result in an severe developmental block at the DN2 stage, reduced thymic cellularity, and defects in DN2 survival and proliferation (Peschon et al 1994, Cao et al 1995, von Freeden-Jeffry et al 1995, Moore et al 1996). In DN thymocytes, a major function of IL-7 signaling via PI3K is to overcome the inhibitory effects of phosphatase and tensin homologue-deleted on chromosome 10 (PTEN), as PTEN deficiency permits IL-7-independent cell differentiation (Hagenbeek et al 2004). Similarly, transgenic over expression of Bcl-2 or deletion of pro-apoptotic Bax or Bim can partially restore T lymphopoiesis in young IL-$7^{-/-}$ or $CD127^{-/-}$ mice, indicating that the capacity of IL-7 to prevent thymocyte apoptosis contributes substantially to its effects on early T cell development (reviewed in (Ciofani & Zúñiga-Pflücker 2006)). Among Bcl-2 family proteins, inactivation of Mcl-1 phenocopies the effects of abrogated IL-7 signaling, implicating it as the physiological anti-apoptotic mediator of this pathway (Opferman et al 2003). In keeping with its central role as a lymphotrophic factor, current data suggest a model in which access to limiting amounts of IL-7 regulates the size of the thymocyte progenitor pool, thus contributing to thymus homeostasis. Consistent with this view, the highest density of IL-7-producing stromal cells in the adult thymus is localized to the

medulla and CMJ, with only sparse distribution in the cortex and SCZ where the majority of IL-7R-expressing DN progenitors reside (Zamisch et al 2005).

Unlike αβ lineage differentiation, development of γδ T cells is strictly dependent on IL-7-mediated signals (Maki et al 1996b, Moore et al 1996, Malissen et al 1997). This differential has largely been attributed to the direct regulation of TCRγ locus accessibility and rearrangement by IL-7 (Maki et al 1996a, Durum et al 1998). However, the inability of a γδTCR transgene to restore lasting development in CD132- or IL-7-deficient adult mice supports the notion that IL-7 provides additional survival and/or differentiation signals to emerging γδ T cells (Malissen et al 1997, Laky et al 2003). Thus, although αβ and γδ lineages share common DN progenitors, β-selection requires Notch signals, whereas γδ-selection appears more dependent on IL-7.

MORPHOGENIC PATHWAYS

The Wnt and Hedgehog (Hh) family proteins, and the bone morphogenetic proteins (BMPs) function as morphogens to regulate tissue patterning and organogenesis during vertebrate embryogenesis. These secreted molecules typically act in a concentration gradient-dependent manner, providing positional signals to specify cell fate. However, such factors may not adhere to this classical definition in the maintenance of adult tissues. Indeed, as discussed below, morphogen proteins appear to regulate thymocyte proliferation and survival, rather than cell fate specification. This viewpoint may reflect the challenge in directly examining morphogen factor function during T cell development as traditional gene deletion strategies are complicated by high incidences of either embryonic lethality or factor redundancy among multiple ligands and receptors. Moreover, analyses have been further confounded by effects of morphogens in both stromal and thymocyte compartments.

Wnt

Wnts are a family of secreted lipid-modified glycoproteins known to play a broad role in development. Similar to the Notch pathway, canonical Wnt signaling enforces a transcriptional switch centered on regulation of the key effector, β-catenin (reviewed in (Staal & Clevers

2005)). In the absence of Wnt signaling, β-catenin is sequestered in a cytoplasmic inhibitory complex where it is continuously phosphorylated and targeted for proteasomal degradation. Upon Wnt binding to its receptor complex, consisting of a member of the Frizzled (Fz) family of proteins and the low density lipoprotein receptor related protein (LRP)5/6 co-receptor, β-catenin phosphorylation is prevented, allowing its accumulation and translocation into the nucleus. Here, it forms a bipartite transcription factor complex with HMG-box T cell factor (TCF)/lymphocyte enhancer binding factor (LEF) family proteins, and activates transcription of otherwise repressed target genes.

In the thymus, Wnt proteins are produced primarily by the thymic epithelium (see Table I), with limited expression by thymocytes; while Fz receptors are expressed by thymocytes and stromal cells (Staal et al 2001, Balciunaite et al 2002, Pongracz et al 2003, Weerkamp et al 2006a). This pattern suggests that Wnt proteins exert biological effects in both stromal and thymocyte compartments. Indeed, Wnt signals can regulate expression of FoxN1 in TEC cell lines (Balciunaite et al 2002), although an essential role for Wnts in TEC development remains to be demonstrated.

Several lines of evidence support the view that Wnt signals provide essential proliferation signals to DN thymocytes. Among thymocytes, the highest levels of Wnt signaling are detected in early progenitors at the DN1 to DN3 stages (Weerkamp et al 2006a). Moreover, mice deficient in both Wnt1 and Wnt4 display a two fold reduction in thymus cellularity, attributed to reduced proliferation of DN and ISP cells (Mulroy et al 2002). This mild phenotype likely reflects molecular redundancy with other thymus-expressed Wnt proteins, as inhibition of Wnt signaling by other means substantially influences developmental transitions. For instance, expression of the secreted inhibitor of Wnt signaling, Dickkopf, in fetal liver-derived progenitors blocks differentiation in FTOC in a dose-dependent fashion, with a complete arrest prior to the DN2 stage at high levels (Weerkamp et al 2006a). Additionally, a DN to DP block was observed in previous studies in which inhibition was mediated by soluble Fz receptor ectodomains (Staal et al 2001).

Additional evidence supporting a role for Wnt in T cell development is provided by genetic studies of downstream pathway components. TCF1-deficient mice display a progressive age-dependent impairment in thymocyte proliferation and differentiation at multiple stages, including an incomplete arrest at the ISP stage in young mice, and an apparent block at the earliest DN1 stage in mice of several months of age (Verbeek et al 1995, Schilham et al 1998). Among subsets, proliferative DN2 progenitors and post-β-selection DN4 and ISP cells are extremely reduced in *Tcf1*$^{-/-}$ mice (Schilham et al 1998). Defects in TCF1 mutant mice are compounded by loss of related LEF1, indicating functional redundancy among these factors during T lymphopoiesis (Okamura et al 1998). While the phenotype of TCF1-deficient mice may also rest in Wnt-independent activity, for instance in the loss of repressive function, only isoforms of TCF containing the N-terminal β-catenin-interacting domain are capable of restoring αβ T cell development in *Tcf1*$^{-/-}$ mice (Ioannidis et al 2001). Although, not excluding the possibility of other binding partners, this suggests that Tcf1 functions in concert with β-catenin downstream of Wnt signaling.

The requirement for β-catenin during T lymphopoiesis is controversial. Conditional deletion of β-catenin prior to the DN3 stage impaired β-selection and expansion of DN4 stage cells (Xu et al 2003), while induced deletion in HSCs failed to produce any thymic defects following transplantation (Cobas et al 2004). This discrepancy has been suggested to result from compensation by the related γ-catenin (plakoglobin) in a reconstitution setting. In support of this premise, differentiation of thymocytes expressing an inhibitor of TCF/LEF binding by β- and γ-catenin, ICAT, are arrested at the ISP stage (Pongracz et al 2006), reminiscent of the effects of TCF1-deficiency or Wnt inhibition by soluble Fz receptors.

In addition to evidence that Wnt-mediated signals are necessary during the DN to DP transition, it appears that this pathway must be tightly regulated at this stage. Deficiencies in factors linked to the degradation of β-catenin, such as adenomatous polyposis coli (APC) or the E3 ubiquitin ligase SIP, have revealed a role for these molecules in preventing maturation of DN3 cells in the absence of functional TCRβ rearrangements (Gounari et al 2005, Fukushima et al 2006). This suggests that attenuation of β-catenin-, and by extension, Wnt-mediated signals is critical to maintaining the DN3 checkpoint status, such that this pathway otherwise furnishes elements of

the subsequent developmental program. In support of this view, constitutive stabilization of β-catenin in *Rag2*$^{-/-}$ thymocytes also results in aberrant pre-TCR-independent maturation to the DP stage (Gounari et al 2001). Notably, while β-catenin is the key effector of Wnt signals, it is important to consider its function in regulating E-cadherin-mediated adhesion, also known to impact thymocyte development (Muller et al 1997).

While the Wnt pathway is critical to the αβ T cell lineage, loss of Wnt signaling via deficiency in TCF1/LEF1 (Verbeek et al 1995, Ohteki et al 1996, Okamura et al 1998), or β-catenin (Xu et al 2003) does not appear to significantly affect γδ thymocyte development. This differential may reflect a reduced dependence of γδ T cell development on Wnt-mediated proliferation signaling, or simply the reliance on separate pathways, such as IL-7. Alternatively, differential Wnt/TCF1 signals have recently been proposed to contribute to αβ versus γδ lineage commitment (Melichar et al 2007). This view emerges from work by the Kang lab that identified the HMG-box factor Sox13 as a specific regulator of γδ T cell differentiation and a potential antagonist of TCF1 activity (Melichar et al 2007). While intriguing, the latter hypothesis remains to be tested in a developmental context.

Taken together, the data suggest that Wnt signals are critical mediators of early T cell differentiation and proliferation. This coincides nicely with the function of genes that have been proposed as targets of Wnt signaling in developing T cells, which by and large include cell cycle and cell adhesion regulators (for instance, c-*fos,* c-*jun*, and integrins) (Staal et al 2004), and the recent finding that TCF controls CD4 gene expression in DP cells (Huang et al 2006). Wnt/TCF signals also appear to influence Bcl-xL expression in DP thymocytes, which is in line with its predominant role in promoting survival at this stage (Ioannidis et al 2001), a function that becomes relevant at developmental stages following pre-TCR expression (Goux et al 2005).

Hedgehog

In mammals, three secreted Hh proteins have been identified, Sonic Hh (SHh), Indian Hh (IHh), and Desert Hh, which bind to a bipartite receptor comprised of the transmembrane proteins Patched (Ptc) and Smoothened (Smo). Hh binding relieves the negative inhibition of Smo

exerted by Ptc, permitting activation of the downstream effector Gli family of zinc finger transcription factors (Ingham & McMahon 2001).

Among Hh proteins, SHh and IHh are expressed in the mouse thymus (Outram et al 2000, El Andaloussi et al 2006). In particular, SHh expression is localized to TECs at the CMJ and medulla (El Andaloussi et al 2006) (see Table I). Hh pathway components are expressed at the highest levels in ETP and DN2 thymocytes, and are progressively downregulated by the DP stage of αβ development (Outram et al 2000, El Andaloussi et al 2006). Consistent with this trend, *Shh*$^{-/-}$ embryos exhibit diminished thymic cellularity due to defects in DN1 and DN2 progenitor expansion (Shah et al 2004), similar to that observed with Wnt signaling deficiency (Verbeek et al 1995, Schilham et al 1998). An essential role for Hh during early T lymphopoiesis has recently been confirmed using a conditional deletion strategy in which Smo, the sole non-redundant component of the pathway, is inactivated in HSCs and at sequential stages of T cell development (El Andaloussi et al 2006). The findings suggest that thymocyte progenitors continuously depend on Hh signals for survival, expansion, and differentiation throughout the ETP to DN3 stages prior to pre-TCR signaling, during and after which Hh signals appear to no longer be required.

Interestingly, the distribution of SHh expression in the thymus (Table I) suggests that, in line with the Hh-dependence of early DN cells, thymus-seeding progenitors encounter high levels of Shh and subsequently migrate to regions of low Hh expression as differentiation proceeds through the late DN stages and β-selection. In fact, limiting concentrations of SHh may be optimal for transition to the DP stage, as high levels of exogenous SHh have been demonstrated to antagonize this developmental progression (Outram et al 2000).

Bone Morphogenic Proteins (BMPs)

The bone morphogenic proteins (BMPs) are secreted proteins that belong to the Transforming growth factor (TGF-β) superfamily of growth factors that include TGF-β and Activins. Binding of BMP dimers to members of type I and type II serine-threonine kinase cell-surface receptors initiates a cascade resulting in the phosphorylation of receptor-regulated Smad proteins (R-

Smads) (Mehra & Wrana 2002). Phosphorylated R-Smads associate with the common Smad 4, and translocate to the nucleus where they function coordinately with other transcription factors to regulate gene expression. The effective concentration of active extracellular BMP is regulated, in part by secreted antagonists including Noggin, Chordin, Twisted gastrulation (Tsg), and Cerberus.

Among TGF-β family members, BMPs appear to be the most relevant to thymus and T cell development. While TGF-β exerts pleiotropic effects on mature T cells, genetic manipulations of TGF-β signaling, via the overexpression of a dominant interfering form of the TGF-β receptor or by deletion of TGF-β1 or its receptor, have failed to reveal major perturbations in thymocyte development (Shull et al 1992, Lucas et al 2000, Leveen et al 2005). In addition, immature DN thymocytes express several components of Activin signaling and appear to activate the TGF-β/Activin pathway in vivo, however, the relevance of this pathway to T lymphopoiesis remains to be assessed (Rosendahl et al 2003, Licona et al 2006).

In the thymus, BMP7 is expressed by both thymocytes and stroma, while BMP2 and BMP4 are expressed exclusively by the thymic stroma predominantly in the SCZ and medulla (Graf et al 2002, Hager-Theodorides et al 2002). BMP antagonists, Chordin and Tsg are also expressed by thymic stroma, with expression of Tsg in thymocytes as well. The role of BMPs during development has been assessed mainly via exogenous addition of factors to FTOC. Treatment of FTOC with BMP2/BMP4 appears to block T cell development at the DN1 stage, and impairs progression through the later stages of differentiation, notably at the DN to DP transition (Graf et al 2002, Hager-Theodorides et al 2002). These effects are reversed by the addition of either Noggin or both Chordin and Tsg. BMP signaling appears to enhance survival of the most immature thymocyte progenitors, while limiting proliferation and differentiation. Consistent with this trend, expression of the BMP antagonist, Tsg, is relatively low in early progenitor subsets and up regulated following β-selection, perhaps to permit transient withdrawal from the inhibitory effects of BMP signals that may otherwise be incompatible with DN to DP transition (Graf et al 2002). Accordingly, mice deficient for Tsg have an atrophic thymus and significantly reduced thymus cellularity (Nosaka et al 2003).

Notably, BMPs are necessary for normal development of the thymic stroma (Bleul & Boehm 2005), and can induce changes in stomal expression of FoxN1 and of chemokines (Tsai et al 2003), therefore thymocyte-specific genetic ablation of pathway components will be necessary to solidify a cell-autonomous role for BMPs during early T cell development.

CHEMOKINE-DRIVEN REGULATION OF INTRA-THYMIC MIGRATION

Chemokines are responsible for regulating thymocyte localization and motility at multiple stages of T cell development (reviewed in (Takahama 2006)). Chemokines are important for recruitment of progenitors to the thymus, and thereafter play specific roles in directing the movement of thymocytes as they progress through T cell development.

Blood-borne progenitor cells enter the thymus at near the junction between the cortex and the medulla (the CMJ). The thymus seeding cells have been recently shown to express CCR9 (Benz & Bleul 2005), and CCR9-deficient bone marrow progenitors are not as efficient in settling the thymus as their wild-type counterparts (Schwarz et al 2007). The potential role for CCR9 in recruitment of progenitors to the thymus is further supported by the strong expression pattern of its ligand, CCL25, by TECs (Wurbel et al 2000). Of note, Flt3 signals were shown to induce CCR9 expression among potential thymus seeding cells, which belong to the $Flt3^+$ subset of $CD117^+$ $Sca1^+$ Lin^- bone marrow stem cells (Schwarz et al 2007).

Through the early DN stages, thymocytes migrate towards the outer cortex under the influence of several chemokines. In particular, CXCL12 (SDF-1), CCL19, and CCL21 appear to be essential for this process. Thymocytes deficient for CXCR4, the receptor for CXCL12 (SDF-1) have revealed several defects in early thymocyte development (Ara et al 2003, Plotkin et al 2003). Interestingly, CXCR4-deficient progenitor thymocytes fail to migrate into the cortex from the CMJ, and as a result are blocked at the DN1 stage of T cell development (Plotkin et al 2003). Likewise, CCR7 deficient mice have been used to demonstrate a similar role for CCL19 and CCL21 at the early DN stages of T cell development (Misslitz et al 2004). Furthermore, CCR9-dependent signals appear to be required for the localization of DN3 cells at the SCZ prior to β-selection of DN3 thymocytes (Benz et al 2004).

The migration of thymocytes in response to chemokines likely involves changes in the expression and activation state of multiple integrins (reviewed in (Petrie 2003)). In particular, interactions between α4 integrins on DN thymocytes and VCAM1 expressed by cortical stromal cells appear to be essential for thymocyte migration (Prockop et al 2002), suggesting that thymocytes migrate along a scaffold of $VCAM1^+$ stromal cells rather than extracellular matrix during T cell development. The thymic medulla provides a specialized microenvironment for efficient negative selection and maturation of SP thymocytes. Thus, several chemokines have been implicated in regulating the migration of T cells into the medulla following positive selection.

Following β-selection, DN thymocytes migrate back through the thymic cortex as they progress to the DP stage. Early DP thymocytes undergo positive selection through interactions with self-peptide/MHC complexes expressed by cortical epithelial cells near the CMJ. The TCR signals associated with positive selection induce several changes in integrin and chemokine receptor gene expression, signaling positively selected thymocytes to migrate into the medulla. In particular, the chemokine receptors CCR7 (Ueno et al 2004), and CCR9 (Norment et al 2000, Uehara et al 2002) are upregulated by DP T cells following TCR-dependent positive selection. Importantly, the chemokine ligands for CCR7, CCL19 and CCL21, are expressed primarily by medullary epithelial cells in the thymus (Ueno et al 2002). Takahama and colleagues made use of CCR7-deficient mice to demonstrate that signaling through CCR7 is essential for migration into the thymic medulla following positive selection (Ueno et al 2004). This notion is supported by another study, in which ectopic expression of CCR7 in thymocytes prior to positive selection resulted in the localization of pre-selection DP thymocytes to the medulla of transgenic mice (Kwan & Killeen 2004). Taken together, it is clear that chemokine-directed movement within the thymus ensures that developing thymocytes are positioned at the appropriate microenvironments to support specific stages of T cell differentiation.

CONCLUSIONS: SIGNALING INTEGRATION

The coordinated migration of immature T cell progenitors through the thymus cortex likely serves to provide access to stage-dependent differentiation, survival, and proliferation signals, such as those addressed in this review. Indeed, there is good evidence for the existence of limiting stromal niches that specialize in the support of early T cell precursors (Prockop & Petrie 2004). While these have been functionally defined, there is little known regarding their exact composition. In this regard, the site of production of various factors in the thymus have been delineated (Table I), however, it remains to be determined which factors colocalize to define specific progenitor niches. The perturbation of DN thymocyte development observed in model systems in which DP thymocytes are engineered to aberrantly bind IL-7 or Delta-like ligands (Munitic et al 2004, Visan et al 2006), factors normally restricted predominantly to immature DN thymocytes in the cortex, suggest that such signals are in limited availability. Thus, migration-dependent accessibility to limiting trophic and differentiation and/or commitment factors represents a primary mechanism by which the thymus not only provides stage-specific signals, yet also regulates homeostasis and size.

Specialized niches may facilitate signaling integration among pathways required for T lymphopoiesis. Indeed, as depicted in Table II, multiple pathways may provide non-redundant inputs into the regulation of proliferation and survival at individual DN stages. Now that the majority of contributors have been identified, deciphering the manner in which their signals are integrated represents an important goal for future studies. This necessarily includes the construction of transcriptional regulatory networks involving the effector nuclear factors of key signaling pathways: Notch, IL-7 (STAT5), Hh (Gli), Wnt (β-catenin/TCF), and BMP (Smads). While there is great precedence for cross-talk among these evolutionarily conserved signaling mechanisms in many organ systems, this remains to be elucidated for T cell lymphopoiesis in the thymus.

While all of the aforementioned pathways provide important inputs during T lymphopoiesis, their roles at different developmental stages are not equivalent. In this regard, Notch ligands and essential cytokines, such as IL-7, which play a primary role in T lineage commitment, differentiation and survival, are absolutely required for the development of αβ and γδ T cell lineages. In addition to these preeminent factors, a supporting role is provided by morphogens

that enable the T cell developmental program by regulating progenitor cell proliferation and survival, influences that may reflect, in part, a secondary role in maintaining stromal homeostasis and function. With this in mind, it is clear that the complexity of the thymus will continue to inspire research aimed at further elucidating the function of this specialized site of lymphopoiesis.

SUMMARY POINTS

1. The thymus represents the primary site for T cell lymphopoiesis, providing a coordinated set of critical factors to induce and support lineage commitment, differentiation, and survival of thymus seeding cells.
2. Essential roles for Notch receptor-ligand interactions in inducing and reinforcing T cell lineage commitment, and promoting a favorable cellular trophic state have been demonstrated to predominate at distinct stages of T cell development.
3. IL-7 provides essential competences to T lineage committed progenitors, and is uniquely important for γδ T cell differentiation. Conversely, Notch signals, while not required for γδ lineage selection, represent an obligate co-factor for β-selection of αβ lineage cells.
4. Although specific thymus-derived factors are vital to αβ and γδ T cell selection events, the pre-TCR and γδ-TCR expressed by DN3 thymocytes constitute and provide the lineage-determining signals.
5. Different thymus stromal elements comprise defined niches that provide morphogen signals to developing thymocytes. These signals may not only impact on the developing thymocytes, but may also serve to maintain stromal homeostasis and function.

FUTURE ISSUES

1. Determining the precise role and composition of unique niches within the thymus remains a challenging endeavor, as is pinpointing the precise cellular identity and lineage potential of "the" thymus-seeding cell.
2. While numerous non-redundant thymus-derived signals have been identified, the manner in which these signals cooperate in the execution of common processes during T cell progenitor commitment, survival, proliferation, and differentiation requires further definition.

3. The demonstration of which Notch ligands, alone or in combination, within the thymus are required to support distinct T cell developmental events have yet to be fully revealed.
4. Deciphering the contribution of thymus-derived signals to the differentiation of thymocytes as opposed to the development and maintenance of the stromal compartment will require a more rigorous evaluation using conditional genetic inactivation studies involving specific deletion in both cell types.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Michele K. Anderson (Sunnybrook Research Institute) for critical reading of the manuscript. JCZP is supported by a Canada Research Chair in Developmental Immunology.

REFERENCES

Allman D, Karnell FG, Punt JA, Bakkour S, Xu L, et al. 2001. Separation of Notch1 promoted lineage commitment and expansion/transformation in developing T cells. *J Exp Med* 194: 99-106

Allman D, Sambandam A, Kim S, Miller JP, Pagan A, et al. 2003. Thymopoiesis independent of common lymphoid progenitors. *Nat Immunol* 4: 168-74

Anderson G, Jenkinson EJ, Moore NC, Owen JJ. 1993. MHC class II-positive epithelium and mesenchyme cells are both required for T-cell development in the thymus. *Nature* 362: 70-3

Anderson G, Owen JJ, Moore NC, Jenkinson EJ. 1994. Thymic epithelial cells provide unique signals for positive selection of CD4+CD8+ thymocytes in vitro. *J Exp Med* 179: 2027-31

Anderson G, Pongracz J, Parnell S, Jenkinson EJ. 2001. Notch ligand-bearing thymic epithelial cells initiate and sustain Notch signaling in thymocytes independently of T cell receptor signaling. *Eur J Immunol* 31: 3349-54

Ara T, Itoi M, Kawabata K, Egawa T, Tokoyoda K, et al. 2003. A role of CXC chemokine ligand 12/stromal cell-derived factor-1/pre-B cell growth stimulating factor and its receptor CXCR4 in fetal and adult T cell development in vivo. *J Immunol* 170: 4649-55

Ardavin C, Wu L, Li CL, Shortman K. 1993. Thymic dendritic cells and T cells develop simultaneously in the thymus from a common precursor population. *Nature* 362: 761-3

Auerbach R. 1960. Morphogenetic interactions in the development of the mouse thymus gland. *Dev Biol* 2: 271-84

Bailey AM, Posakony JW. 1995. Suppressor of hairless directly activates transcription of enhancer of split complex genes in response to Notch receptor activity. *Genes Dev* 9: 2609-22

Balciunaite G, Keller MP, Balciunaite E, Piali L, Zuklys S, et al. 2002. Wnt glycoproteins regulate the expression of FoxN1, the gene defective in nude mice. *Nat Immunol* 3: 1102-8

Bellavia D, Campese AF, Checquolo S, Balestri A, Biondi A, et al. 2002. Combined expression of pTalpha and Notch3 in T cell leukemia identifies the requirement of preTCR for leukemogenesis. *Proc Natl Acad Sci U S A* 99: 3788-93

Benz C, Bleul CC. 2005. A multipotent precursor in the thymus maps to the branching point of the T versus B lineage decision. *J Exp Med* 202: 21-31

Benz C, Heinzel K, Bleul CC. 2004. Homing of immature thymocytes to the subcapsular microenvironment within the thymus is not an absolute requirement for T cell development. *Eur J Immunol* 34: 3652-63

Besseyrias V, Fiorini E, Strobl LJ, Zimber-Strobl U, Dumortier A, et al. 2007. Hierarchy of Notch-Delta interactions promoting T cell lineage commitment and maturation. *J Exp Med* 204: 331-43

Bhandoola A, von Boehmer H, Zúñiga-Pflücker JC, Petrie HT. 2007. Commitment and developmental potential of extrathymic and intrathymic T cell
precursors: plenty to choose from. *Immunity* in press

Blackburn CC, Manley NR. 2004. Developing a new paradigm for thymus organogenesis. *Nat Rev Immunol* 4: 278-89

Bleul CC, Boehm T. 2005. BMP signaling is required for normal thymus development. *J Immunol* 175: 5213-21

Bleul CC, Corbeaux T, Reuter A, Fisch P, Monting JS, Boehm T. 2006. Formation of a functional thymus initiated by a postnatal epithelial progenitor cell. *Nature* 441: 992-6

Bray SJ. 2006. Notch signalling: a simple pathway becomes complex. *Nat Rev Mol Cell Biol* 7: 678-89

Campese AF, Garbe AI, Zhang F, Grassi F, Screpanti I, von Boehmer H. 2006. Notch1-dependent lymphomagenesis is assisted by but does not essentially require pre-TCR signaling. *Blood* 108: 305-10

Cao X, Shores EW, Hu-Li J, Anver MR, Kelsall BL, et al. 1995. Defective lymphoid development in mice lacking expression of the common cytokine receptor gamma chain. *Immunity* 2: 223-38

Capone M, Hockett RD, Jr., Zlotnik A. 1998. Kinetics of T cell receptor beta, gamma, and delta rearrangements during adult thymic development: T cell receptor rearrangements are present in CD44(+)CD25(+) Pro-T thymocytes. *Proc Natl Acad Sci U S A* 95: 12522-7

Cave JW, Loh F, Surpris JW, Xia L, Caudy MA. 2005. A DNA transcription code for cell-specific gene activation by notch signaling. *Curr Biol* 15: 94-104

Cheng P, Nefedova Y, Miele L, Osborne BA, Gabrilovich D. 2003. Notch signaling is necessary but not sufficient for differentiation of dendritic cells. *Blood* 102: 3980-8

Chien YH, Konigshofer Y. 2007. Antigen recognition by gammadelta T cells. *Immunol Rev* 215: 46-58

Ciofani M, Knowles GC, Wiest DL, von Boehmer H, Zúñiga-Pflücker JC. 2006. Stage-specific and differential notch dependency at the alphabeta and gammadelta T lineage bifurcation. *Immunity* 25: 105-16

Ciofani M, Schmitt TM, Ciofani A, Michie AM, Cuburu N, et al. 2004. Obligatory role for cooperative signaling by pre-TCR and Notch during thymocyte differentiation. *J Immunol* 172: 5230-9

Ciofani M, Zúñiga-Pflücker JC. 2005. Notch promotes survival of pre-T cells at the beta-selection checkpoint by regulating cellular metabolism. *Nat Immunol* 6: 881-8

Ciofani M, Zúñiga-Pflücker JC. 2006. A survival guide to early T cell development. *Immunol Res* 34: 117-32

Cobas M, Wilson A, Ernst B, Mancini SJ, MacDonald HR, et al. 2004. Beta-catenin is dispensable for hematopoiesis and lymphopoiesis. *J Exp Med* 199: 221-9

Deftos ML, He YW, Ojala EW, Bevan MJ. 1998. Correlating notch signaling with thymocyte maturation. *Immunity* 9: 777-86

Deftos ML, Huang E, Ojala EW, Forbush KA, Bevan MJ. 2000. Notch1 signaling promotes the maturation of CD4 and CD8 SP thymocytes. *Immunity* 13: 73-84

Derbinski J, Kyewski B. 2005. Linking signalling pathways, thymic stroma integrity and autoimmunity. *Trends Immunol* 26: 503-6

Derbinski J, Schulte A, Kyewski B, Klein L. 2001. Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. *Nat Immunol* 2: 1032-9

Di Santo JP, Rodewald HR. 1998. In vivo roles of receptor tyrosine kinases and cytokine receptors in early thymocyte development. *Curr Opin Immunol* 10: 196-207

Doerfler P, Shearman MS, Perlmutter RM. 2001. Presenilin-dependent gamma-secretase activity modulates thymocyte development. *Proc Natl Acad Sci U S A* 98: 9312-7

Donskoy E, Goldschneider I. 1992. Thymocytopoiesis is maintained by blood-borne precursors throughout postnatal life. A study in parabiotic mice. *J Immunol* 148: 1604-12

Dontje W, Schotte R, Cupedo T, Nagasawa M, Scheeren F, et al. 2006. Delta-like1-induced Notch1 signaling regulates the human plasmacytoid dendritic cell versus T-cell lineage decision through control of GATA-3 and Spi-B. *Blood* 107: 2446-52

Dudley EC, Girardi M, Owen MJ, Hayday AC. 1995. Alpha beta and gamma delta T cells can share a late common precursor. *Curr Biol* 5: 659-69

Dudley EC, Petrie HT, Shah LM, Owen MJ, Hayday AC. 1994. T cell receptor beta chain gene rearrangement and selection during thymocyte development in adult mice. *Immunity* 1: 83-93

Duncan AW, Rattis FM, DiMascio LN, Congdon KL, Pazianos G, et al. 2005. Integration of Notch and Wnt signaling in hematopoietic stem cell maintenance. *Nat Immunol* 6: 314-22

Durum SK, Candeias S, Nakajima H, Leonard WJ, Baird AM, et al. 1998. Interleukin 7 receptor control of T cell receptor gamma gene rearrangement: role of receptor-associated chains and locus accessibility. *J Exp Med* 188: 2233-41

El Andaloussi A, Graves S, Meng F, Mandal M, Mashayekhi M, Aifantis I. 2006. Hedgehog signaling controls thymocyte progenitor homeostasis and differentiation in the thymus. *Nat Immunol* 7: 418-26

Ellisen LW, Bird J, West DC, Soreng AL, Reynolds TC, et al. 1991. TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* 66: 649-61

Foss DL, Donskoy E, Goldschneider I. 2001. The importation of hematogenous precursors by the thymus is a gated phenomenon in normal adult mice. *J Exp Med* 193: 365-74

Fowlkes BJ, Robey EA. 2002. A reassessment of the effect of activated Notch1 on CD4 and CD8 T cell development. *J Immunol* 169: 1817-21

Fukushima T, Zapata JM, Singha NC, Thomas M, Kress CL, et al. 2006. Critical function for SIP, a ubiquitin E3 ligase component of the beta-catenin degradation pathway, for thymocyte development and G1 checkpoint. *Immunity* 24: 29-39

Gallegos AM, Bevan MJ. 2004. Central tolerance to tissue-specific antigens mediated by direct and indirect antigen presentation. *J Exp Med* 200: 1039-49

Garbe AI, Krueger A, Gounari F, Zúñiga-Pflücker JC, von Boehmer H. 2006. Differential synergy of Notch and T cell receptor signaling determines {alpha}{beta} versus {gamma}{delta} lineage fate. *J Exp Med* 203: 1579-90

Godfrey DI, Kennedy J, Mombaerts P, Tonegawa S, Zlotnik A. 1994. Onset of TCR-beta gene rearrangement and role of TCR-beta expression during CD3-CD4-CD8- thymocyte differentiation. *J Immunol* 152: 4783-92

Godfrey DI, Kennedy J, Suda T, Zlotnik A. 1993. A developmental pathway involving four phenotypically and functionally distinct subsets of CD3-CD4-CD8- triple-negative adult mouse thymocytes defined by CD44 and CD25 expression. *J Immunol* 150: 4244-52

Goetz CA, Harmon IR, O'Neil JJ, Burchill MA, Johanns TM, Farrar MA. 2005. Restricted STAT5 activation dictates appropriate thymic B versus T cell lineage commitment. *J Immunol* 174: 7753-63

Gordon J, Wilson VA, Blair NF, Sheridan J, Farley A, et al. 2004. Functional evidence for a single endodermal origin for the thymic epithelium. *Nat Immunol* 5: 546-53

Gounari F, Aifantis I, Khazaie K, Hoeflinger S, Harada N, et al. 2001. Somatic activation of beta-catenin bypasses pre-TCR signaling and TCR selection in thymocyte development. *Nat Immunol* 2: 863-9

Gounari F, Chang R, Cowan J, Guo Z, Dose M, et al. 2005. Loss of adenomatous polyposis coli gene function disrupts thymic development. *Nat Immunol* 6: 800-9

Goux D, Coudert JD, Maurice D, Scarpellino L, Jeannet G, et al. 2005. Cooperating pre-T-cell receptor and TCF-1-dependent signals ensure thymocyte survival. *Blood* 106: 1726-33

Graf D, Nethisinghe S, Palmer DB, Fisher AG, Merkenschlager M. 2002. The developmentally regulated expression of Twisted gastrulation reveals a role for bone morphogenetic proteins in the control of T cell development. *J Exp Med* 196: 163-71

Gupta-Rossi N, Six E, LeBail O, Logeat F, Chastagner P, et al. 2004. Monoubiquitination and endocytosis direct gamma-secretase cleavage of activated Notch receptor. *J Cell Biol* 166: 73-83

Hagenbeek TJ, Naspetti M, Malergue F, Garcon F, Nunes JA, et al. 2004. The loss of PTEN allows TCR alphabeta lineage thymocytes to bypass IL-7 and Pre-TCR-mediated signaling. *J Exp Med* 200: 883-94

Hager-Theodorides AL, Outram SV, Shah DK, Sacedon R, Shrimpton RE, et al. 2002. Bone morphogenetic protein 2/4 signaling regulates early thymocyte differentiation. *J Immunol* 169: 5496-504

Haks MC, Lefebvre JM, Lauritsen JP, Carleton M, Rhodes M, et al. 2005. Attenuation of gammadeltaTCR signaling efficiently diverts thymocytes to the alphabeta lineage. *Immunity* 22: 595-606

Han H, Tanigaki K, Yamamoto N, Kuroda K, Yoshimoto M, et al. 2002. Inducible gene knockout of transcription factor recombination signal binding protein-J reveals its essential role in T versus B lineage decision. *Int Immunol* 14: 637-45

Hayday AC, Barber DF, Douglas N, Hoffman ES. 1999. Signals involved in gamma/delta T cell versus alpha/beta T cell lineage commitment. *Semin Immunol* 11: 239-49

Hayday AC, Pennington DJ. 2007. Key factors in the organized chaos of early T cell development. *Nat Immunol* 8: 137-44

Hayes SM, Li L, Love PE. 2005. TCR signal strength influences alphabeta/gammadelta lineage fate. *Immunity* 22: 583-93

Heinzel K, Benz C, Martins VC, Haidl ID, Bleul CC. 2007. Bone marrow-derived hemopoietic precursors commit to the T cell lineage only after arrival in the thymic microenvironment. *J Immunol* 178: 858-68

Hozumi K, Negishi N, Suzuki D, Abe N, Sotomaru Y, et al. 2004. Delta-like 1 is necessary for the generation of marginal zone B cells but not T cells in vivo. *Nat Immunol* 5: 638-44

Huang EY, Gallegos AM, Richards SM, Lehar SM, Bevan MJ. 2003. Surface expression of Notch1 on thymocytes: correlation with the double-negative to double-positive transition. *J Immunol* 171: 2296-304

Huang Z, Xie H, Ioannidis V, Held W, Clevers H, et al. 2006. Transcriptional regulation of CD4 gene expression by T cell factor-1/beta-catenin pathway. *J Immunol* 176: 4880-7

Ikawa T, Kawamoto H, Fujimoto S, Katsura Y. 1999. Commitment of common T/Natural killer (NK) progenitors to unipotent T and NK progenitors in the murine fetal thymus revealed by a single progenitor assay. *J Exp Med* 190: 1617-26

Ikawa T, Kawamoto H, Goldrath AW, Murre C. 2006. E proteins and Notch signaling cooperate to promote T cell lineage specification and commitment. *J Exp Med* 203: 1329-42

Ingham PW, McMahon AP. 2001. Hedgehog signaling in animal development: paradigms and principles. *Genes Dev* 15: 3059-87

Ioannidis V, Beermann F, Clevers H, Held W. 2001. The beta-catenin--TCF-1 pathway ensures CD4(+)CD8(+) thymocyte survival. *Nat Immunol* 2: 691-7

Irving BA, Alt FW, Killeen N. 1998. Thymocyte development in the absence of pre-T cell receptor extracellular immunoglobulin domains. *Science* 280: 905-8

Iso T, Sartorelli V, Chung G, Shichinohe T, Kedes L, Hamamori Y. 2001. HERP, a new primary target of Notch regulated by ligand binding. *Mol Cell Biol* 21: 6071-9

Izon DJ, Aster JC, He Y, Weng A, Karnell FG, et al. 2002. Deltex1 redirects lymphoid progenitors to the B cell lineage by antagonizing Notch1. *Immunity* 16: 231-43

Jaleco AC, Neves H, Hooijberg E, Gameiro P, Clode N, et al. 2001. Differential effects of Notch ligands Delta-1 and Jagged-1 in human lymphoid differentiation. *J Exp Med* 194: 991-1002

Jarriault S, Brou C, Logeat F, Schroeter EH, Kopan R, Israel A. 1995. Signalling downstream of activated mammalian Notch. *Nature* 377: 355-8

Jiang R, Lan Y, Chapman HD, Shawber C, Norton CR, et al. 1998. Defects in limb, craniofacial, and thymic development in Jagged2 mutant mice. *Genes Dev* 12: 1046-57

Jotereau F, Heuze F, Salomon-Vie V, Gascan H. 1987. Cell kinetics in the fetal mouse thymus: precursor cell input, proliferation, and emigration. *J Immunol* 138: 1026-30

Kaneta M, Osawa M, Sudo K, Nakauchi H, Farr AG, Takahama Y. 2000. A role for pref-1 and HES-1 in thymocyte development. *J Immunol* 164: 256-64

Kang J, Coles M, Cado D, Raulet DH. 1998. The developmental fate of T cells is critically influenced by TCRgammadelta expression. *Immunity* 8: 427-38

Kawamata S, Du C, Li K, Lavau C. 2002. Overexpression of the Notch target genes Hes in vivo induces lymphoid and myeloid alterations. *Oncogene* 21: 3855-63

Klug DB, Carter C, Gimenez-Conti IB, Richie ER. 2002. Cutting edge: thymocyte-independent and thymocyte-dependent phases of epithelial patterning in the fetal thymus. *J Immunol* 169: 2842-5

Krebs LT, Deftos ML, Bevan MJ, Gridley T. 2001. The Nrarp gene encodes an ankyrin-repeat protein that is transcriptionally regulated by the notch signaling pathway. *Dev Biol* 238: 110-9

Kwan J, Killeen N. 2004. CCR7 directs the migration of thymocytes into the thymic medulla. *J Immunol* 172: 3999-4007

Laky K, Lewis JM, Tigelaar RE, Puddington L. 2003. Distinct requirements for IL-7 in development of TCR gamma delta cells during fetal and adult life. *J Immunol* 170: 4087-94

Lehar SM, Dooley J, Farr AG, Bevan MJ. 2005. Notch ligands Delta 1 and Jagged1 transmit distinct signals to T-cell precursors. *Blood* 105: 1440-7

Leveen P, Carlsen M, Makowska A, Oddsson S, Larsson J, et al. 2005. TGF-beta type II receptor-deficient thymocytes develop normally but demonstrate increased CD8+ proliferation in vivo. *Blood* 106: 4234-40

Lewis JM, Girardi M, Roberts SJ, S DB, Hayday AC, Tigelaar RE. 2006. Selection of the cutaneous intraepithelial gammadelta+ T cell repertoire by a thymic stromal determinant. *Nat Immunol* 7: 843-50

Licona P, Chimal-Monroy J, Soldevila G. 2006. Inhibins are the major activin ligands expressed during early thymocyte development. *Dev Dyn* 235: 1124-32

Lind EF, Prockop SE, Porritt HE, Petrie HT. 2001. Mapping precursor movement through the postnatal thymus reveals specific microenvironments supporting defined stages of early lymphoid development. *J Exp Med* 194: 127-34

Liu C, Saito F, Liu Z, Lei Y, Uehara S, et al. 2006. Coordination between CCR7- and CCR9-mediated chemokine signals in prevascular fetal thymus colonization. *Blood* 108: 2531-9

Livak F, Tourigny M, Schatz DG, Petrie HT. 1999. Characterization of TCR gene rearrangements during adult murine T cell development. *J Immunol* 162: 2575-80

Lucas PJ, Kim SJ, Melby SJ, Gress RE. 2000. Disruption of T cell homeostasis in mice expressing a T cell-specific dominant negative transforming growth factor beta II receptor. *J Exp Med* 191: 1187-96

Maillard I, Tu L, Sambandam A, Yashiro-Ohtani Y, Millholland J, et al. 2006. The requirement for Notch signaling at the beta-selection checkpoint in vivo is absolute and independent of the pre-T cell receptor. *J Exp Med* 203: 2239-45

Maillard I, Weng AP, Carpenter AC, Rodriguez CG, Sai H, et al. 2004. Mastermind critically regulates Notch-mediated lymphoid cell fate decisions. *Blood* 104: 1696-702

Maki K, Sunaga S, Ikuta K. 1996a. The V-J recombination of T cell receptor-gamma genes is blocked in interleukin-7 receptor-deficient mice. *J Exp Med* 184: 2423-7

Maki K, Sunaga S, Komagata Y, Kodaira Y, Mabuchi A, et al. 1996b. Interleukin 7 receptor-deficient mice lack gammadelta T cells. *Proc Natl Acad Sci U S A* 93: 7172-7

Malissen M, Pereira P, Gerber DJ, Malissen B, DiSanto JP. 1997. The common cytokine receptor gamma chain controls survival of gamma/delta T cells. *J Exp Med* 186: 1277-85

Mancini SJ, Mantei N, Dumortier A, Suter U, MacDonald HR, Radtke F. 2005. Jagged1-dependent Notch signaling is dispensable for hematopoietic stem cell self-renewal and differentiation. *Blood* 105: 2340-2

Massa S, Balciunaite G, Ceredig R, Rolink AG. 2006. Critical role for c-kit (CD117) in T cell lineage commitment and early thymocyte development in vitro. *Eur J Immunol* 36: 526-32

Mehra A, Wrana JL. 2002. TGF-beta and the Smad signal transduction pathway. *Biochem Cell Biol* 80: 605-22

Melichar HJ, Narayan K, Der SD, Hiraoka Y, Gardiol N, et al. 2007. Regulation of gammadelta versus alphabeta T lymphocyte differentiation by the transcription factor SOX13. *Science* 315: 230-3

Michie AM, Zúñiga-Pflücker JC. 2002. Regulation of thymocyte differentiation: pre-TCR signals and beta-selection. *Semin Immunol* 14: 311-23

Misslitz A, Pabst O, Hintzen G, Ohl L, Kremmer E, et al. 2004. Thymic T cell development and progenitor localization depend on CCR7. *J Exp Med* 200: 481-91

Mohtashami M, Zúñiga-Pflücker JC. 2006. Three-dimensional architecture of the thymus is required to maintain delta-like expression necessary for inducing T cell development. *J Immunol* 176: 730-4

Moore TA, von Freeden-Jeffry U, Murray R, Zlotnik A. 1996. Inhibition of gamma delta T cell development and early thymocyte maturation in IL-7 -/- mice. *J Immunol* 157: 2366-73

Moore TA, Zlotnik A. 1995. T-cell lineage commitment and cytokine responses of thymic progenitors. *Blood* 86: 1850-60

Muller KM, Luedecker CJ, Udey MC, Farr AG. 1997. Involvement of E-cadherin in thymus organogenesis and thymocyte maturation. *Immunity* 6: 257-64

Mulroy T, McMahon JA, Burakoff SJ, McMahon AP, Sen J. 2002. Wnt-1 and Wnt-4 regulate thymic cellularity. *Eur J Immunol* 32: 967-71

Munitic I, Williams JA, Yang Y, Dong B, Lucas PJ, et al. 2004. Dynamic regulation of IL-7 receptor expression is required for normal thymopoiesis. *Blood* 104: 4165-72

Nehls M, Kyewski B, Messerle M, Waldschutz R, Schuddekopf K, et al. 1996. Two genetically separable steps in the differentiation of thymic epithelium. *Science* 272: 886-9

Norment AM, Bogatzki LY, Gantner BN, Bevan MJ. 2000. Murine CCR9, a chemokine receptor for thymus-expressed chemokine that is up-regulated following pre-TCR signaling. *J Immunol* 164: 639-48

Nosaka T, Morita S, Kitamura H, Nakajima H, Shibata F, et al. 2003. Mammalian twisted gastrulation is essential for skeleto-lymphogenesis. *Mol Cell Biol* 23: 2969-80

Ogawa M, Matsuzaki Y, Nishikawa S, Hayashi S, Kunisada T, et al. 1991. Expression and function of c-kit in hemopoietic progenitor cells. *J Exp Med* 174: 63-71

Ohteki T, Wilson A, Verbeek S, MacDonald HR, Clevers H. 1996. Selectively impaired development of intestinal T cell receptor gamma delta+ cells and liver CD4+ NK1+ T cell receptor alpha beta+ cells in T cell factor-1-deficient mice. *Eur J Immunol* 26: 351-5

Okamura RM, Sigvardsson M, Galceran J, Verbeek S, Clevers H, Grosschedl R. 1998. Redundant regulation of T cell differentiation and TCRalpha gene expression by the transcription factors LEF-1 and TCF-1. *Immunity* 8: 11-20

Opferman JT, Letai A, Beard C, Sorcinelli MD, Ong CC, Korsmeyer SJ. 2003. Development and maintenance of B and T lymphocytes requires antiapoptotic MCL-1. *Nature* 426: 671-6

Outram SV, Varas A, Pepicelli CV, Crompton T. 2000. Hedgehog signaling regulates differentiation from double-negative to double-positive thymocyte. *Immunity* 13: 187-97

Palomero T, Lim WK, Odom DT, Sulis ML, Real PJ, et al. 2006. NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting leukemic cell growth. *Proc Natl Acad Sci U S A* 103: 18261-6

Panin VM, Papayannopoulos V, Wilson R, Irvine KD. 1997. Fringe modulates Notch-ligand interactions. *Nature* 387: 908-12

Passoni L, Hoffman ES, Kim S, Crompton T, Pao W, et al. 1997. Intrathymic delta selection events in gammadelta cell development. *Immunity* 7: 83-95

Pennington DJ, Silva-Santos B, Shires J, Theodoridis E, Pollitt C, et al. 2003. The inter-relatedness and interdependence of mouse T cell receptor gammadelta+ and alphabeta+ cells. *Nat Immunol* 4: 991-8

Pennington DJ, Silva-Santos B, Silberzahn T, Escorcio-Correia M, Woodward MJ, et al. 2006. Early events in the thymus affect the balance of effector and regulatory T cells. *Nature* 444: 1073-7

Peschon JJ, Morrissey PJ, Grabstein KH, Ramsdell FJ, Maraskovsky E, et al. 1994. Early lymphocyte expansion is severely impaired in interleukin 7 receptor-deficient mice. *J Exp Med* 180: 1955-60

Petrie HT. 2003. Cell migration and the control of post-natal T-cell lymphopoiesis in the thymus. *Nat Rev Immunol* 3: 859-66

Petrie HT, Scollay R, Shortman K. 1992. Commitment to the T cell receptor-alpha beta or -gamma delta lineages can occur just prior to the onset of CD4 and CD8 expression among immature thymocytes. *Eur J Immunol* 22: 2185-8

Petrie HT, Zúñiga-Pflücker JC. 2007. Zoned Out: Functional Mapping of Stromal Signaling Microenvironments in the Thymus. *Annu Rev Immunol*

Plotkin J, Prockop SE, Lepique A, Petrie HT. 2003. Critical role for CXCR4 signaling in progenitor localization and T cell differentiation in the postnatal thymus. *J Immunol* 171: 4521-7

Pongracz J, Hare K, Harman B, Anderson G, Jenkinson EJ. 2003. Thymic epithelial cells provide WNT signals to developing thymocytes. *Eur J Immunol* 33: 1949-56

Pongracz JE, Parnell SM, Jones T, Anderson G, Jenkinson EJ. 2006. Overexpression of ICAT highlights a role for catenin-mediated canonical Wnt signalling in early T cell development. *Eur J Immunol* 36: 2376-83

Porritt HE, Rumfelt LL, Tabrizifard S, Schmitt TM, Zúñiga-Pflücker JC, Petrie HT. 2004. Heterogeneity among DN1 prothymocytes reveals multiple progenitors with different capacities to generate T cell and non-T cell lineages. *Immunity* 20: 735-45

Prinz I, Sansoni A, Kissenpfennig A, Ardouin L, Malissen M, Malissen B. 2006. Visualization of the earliest steps of gammadelta T cell development in the adult thymus. *Nat Immunol* 7: 995-1003

Prockop SE, Palencia S, Ryan CM, Gordon K, Gray D, Petrie HT. 2002. Stromal cells provide the matrix for migration of early lymphoid progenitors through the thymic cortex. *J Immunol* 169: 4354-61

Prockop SE, Petrie HT. 2004. Regulation of thymus size by competition for stromal niches among early T cell progenitors. *J Immunol* 173: 1604-11

Puel A, Ziegler SF, Buckley RH, Leonard WJ. 1998. Defective IL7R expression in T(-)B(+)NK(+) severe combined immunodeficiency. *Nat Genet* 20: 394-7

Pui JC, Allman D, Xu L, DeRocco S, Karnell FG, et al. 1999. Notch1 expression in early lymphopoiesis influences B versus T lineage determination. *Immunity* 11: 299-308

Radtke F, Wilson A, Mancini SJ, MacDonald HR. 2004. Notch regulation of lymphocyte development and function. *Nat Immunol* 5: 247-53

Radtke F, Wilson A, Stark G, Bauer M, van Meerwijk J, et al. 1999. Deficient T cell fate specification in mice with an induced inactivation of Notch1. *Immunity* 10: 547-58

Reizis B, Leder P. 2002. Direct induction of T lymphocyte-specific gene expression by the mammalian Notch signaling pathway. *Genes Dev* 16: 295-300

Robey E, Chang D, Itano A, Cado D, Alexander H, et al. 1996. An activated form of Notch influences the choice between CD4 and CD8 T cell lineages. *Cell* 87: 483-92

Rodewald HR, Kretzschmar K, Swat W, Takeda S. 1995. Intrathymically expressed c-kit ligand (stem cell factor) is a major factor driving expansion of very immature thymocytes in vivo. *Immunity* 3: 313-9

Rodewald HR, Ogawa M, Haller C, Waskow C, DiSanto JP. 1997. Pro-thymocyte expansion by c-kit and the common cytokine receptor gamma chain is essential for repertoire formation. *Immunity* 6: 265-72

Rosendahl A, Speletas M, Leandersson K, Ivars F, Sideras P. 2003. Transforming growth factor-beta- and Activin-Smad signaling pathways are activated at distinct maturation stages of the thymopoeisis. *Int Immunol* 15: 1401-14

Rossi SW, Jenkinson WE, Anderson G, Jenkinson EJ. 2006. Clonal analysis reveals a common progenitor for thymic cortical and medullary epithelium. *Nature* 441: 988-91

Sambandam A, Maillard I, Zediak VP, Xu L, Gerstein RM, et al. 2005. Notch signaling controls the generation and differentiation of early T lineage progenitors. *Nat Immunol* 6: 663-70

Schilham MW, Wilson A, Moerer P, Benaissa-Trouw BJ, Cumano A, Clevers HC. 1998. Critical involvement of Tcf-1 in expansion of thymocytes. *J Immunol* 161: 3984-91

Schmitt TM, Ciofani M, Petrie HT, Zúñiga-Pflücker JC. 2004. Maintenance of T cell specification and differentiation requires recurrent notch receptor-ligand interactions. *J Exp Med* 200: 469-79

Schmitt TM, Zúñiga-Pflücker JC. 2002. Induction of T cell development from hematopoietic progenitor cells by delta-like-1 in vitro. *Immunity* 17: 749-56

Schwarz BA, Sambandam A, Maillard I, Harman BC, Love PE, Bhandoola A. 2007. Selective thymus settling regulated by cytokine and chemokine receptors. *J Immunol* 178: 2008-17

Scollay R, Smith J, Stauffer V. 1986. Dynamics of early T cells: prothymocyte migration and proliferation in the adult mouse thymus. *Immunol Rev* 91: 129-57

Shah DK, Hager-Theodorides AL, Outram SV, Ross SE, Varas A, Crompton T. 2004. Reduced thymocyte development in sonic hedgehog knockout embryos. *J Immunol* 172: 2296-306

Shen HQ, Lu M, Ikawa T, Masuda K, Ohmura K, et al. 2003. T/NK bipotent progenitors in the thymus retain the potential to generate dendritic cells. *J Immunol* 171: 3401-6

Shull MM, Ormsby I, Kier AB, Pawlowski S, Diebold RJ, et al. 1992. Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. *Nature* 359: 693-9

Silva-Santos B, Pennington DJ, Hayday AC. 2005. Lymphotoxin-mediated regulation of gammadelta cell differentiation by alphabeta T cell progenitors. *Science* 307: 925-8

Staal FJ, Clevers HC. 2005. WNT signalling and haematopoiesis: a WNT-WNT situation. *Nat Rev Immunol* 5: 21-30

Staal FJ, Meeldijk J, Moerer P, Jay P, van de Weerdt BC, et al. 2001. Wnt signaling is required for thymocyte development and activates Tcf-1 mediated transcription. *Eur J Immunol* 31: 285-93

Staal FJ, Weerkamp F, Baert MR, van den Burg CM, van Noort M, et al. 2004. Wnt target genes identified by DNA microarrays in immature CD34+ thymocytes regulate proliferation and cell adhesion. *J Immunol* 172: 1099-108

Starr TK, Jameson SC, Hogquist KA. 2003. Positive and negative selection of T cells. *Annu Rev Immunol* 21: 139-76

Su DM, Navarre S, Oh WJ, Condie BG, Manley NR. 2003. A domain of Foxn1 required for crosstalk-dependent thymic epithelial cell differentiation. *Nat Immunol* 4: 1128-35

Sudo T, Nishikawa S, Ohno N, Akiyama N, Tamakoshi M, et al. 1993. Expression and function of the interleukin 7 receptor in murine lymphocytes. *Proc Natl Acad Sci U S A* 90: 9125-9

Taghon T, Yui MA, Pant R, Diamond RA, Rothenberg EV. 2006. Developmental and molecular characterization of emerging beta- and gammadelta-selected pre-T cells in the adult mouse thymus. *Immunity* 24: 53-64

Takahama Y. 2006. Journey through the thymus: stromal guides for T-cell development and selection. *Nat Rev Immunol* 6: 127-35

Tan JB, Visan I, Yuan JS, Guidos CJ. 2005. Requirement for Notch1 signals at sequential early stages of intrathymic T cell development. *Nat Immunol* 6: 671-9

Tanigaki K, Tsuji M, Yamamoto N, Han H, Tsukada J, et al. 2004. Regulation of alphabeta/gammadelta T cell lineage commitment and peripheral T cell responses by Notch/RBP-J signaling. *Immunity* 20: 611-22

Tomita K, Hattori M, Nakamura E, Nakanishi S, Minato N, Kageyama R. 1999. The bHLH gene Hes1 is essential for expansion of early T cell precursors. *Genes Dev* 13: 1203-10

Tsai PT, Lee RA, Wu H. 2003. BMP4 acts upstream of FGF in modulating thymic stroma and regulating thymopoiesis. *Blood* 102: 3947-53

Tsuji M, Shinkura R, Kuroda K, Yabe D, Honjo T. 2007. Msx2-interacting nuclear target protein (Mint) deficiency reveals negative regulation of early thymocyte differentiation by Notch/RBP-J signaling. *Proc Natl Acad Sci U S A* 104: 1610-5

Uehara S, Song K, Farber JM, Love PE. 2002. Characterization of CCR9 expression and CCL25/thymus-expressed chemokine responsiveness during T cell development: CD3(high)CD69+ thymocytes and gammadeltaTCR+ thymocytes preferentially respond to CCL25. *J Immunol* 168: 134-42

Ueno T, Hara K, Willis MS, Malin MA, Hopken UE, et al. 2002. Role for CCR7 ligands in the emigration of newly generated T lymphocytes from the neonatal thymus. *Immunity* 16: 205-18

Ueno T, Saito F, Gray DH, Kuse S, Hieshima K, et al. 2004. CCR7 signals are essential for cortex-medulla migration of developing thymocytes. *J Exp Med* 200: 493-505

Van De Wiele CJ, Marino JH, Murray BW, Vo SS, Whetsell ME, Teague TK. 2004. Thymocytes between the beta-selection and positive selection checkpoints are nonresponsive to IL-7 as assessed by STAT-5 phosphorylation. *J Immunol* 172: 4235-44

van Ewijk W, Shores EW, Singer A. 1994. Crosstalk in the mouse thymus. *Immunol Today* 15: 214-7

Verbeek S, Izon D, Hofhuis F, Robanus-Maandag E, te Riele H, et al. 1995. An HMG-box-containing T-cell factor required for thymocyte differentiation. *Nature* 374: 70-4

Visan I, Yuan JS, Tan JB, Cretegny K, Guidos CJ. 2006. Regulation of intrathymic T-cell development by Lunatic Fringe- Notch1 interactions. *Immunol Rev* 209: 76-94

von Freeden-Jeffry U, Vieira P, Lucian LA, McNeil T, Burdach SE, Murray R. 1995. Lymphopenia in interleukin (IL)-7 gene-deleted mice identifies IL-7 as a nonredundant cytokine. *J Exp Med* 181: 1519-26

Wang H, Pierce LJ, Spangrude GJ. 2006. Distinct roles of IL-7 and stem cell factor in the OP9-DL1 T-cell differentiation culture system. *Exp Hematol* 34: 1730-40

Washburn T, Schweighoffer E, Gridley T, Chang D, Fowlkes BJ, et al. 1997. Notch activity influences the alphabeta versus gammadelta T cell lineage decision. *Cell* 88: 833-43

Waskow C, Paul S, Haller C, Gassmann M, Rodewald HR. 2002. Viable c-Kit(W/W) mutants reveal pivotal role for c-kit in the maintenance of lymphopoiesis. *Immunity* 17: 277-88

Weerkamp F, Baert MR, Naber BA, Koster EE, de Haas EF, et al. 2006a. Wnt signaling in the thymus is regulated by differential expression of intracellular signaling molecules. *Proc Natl Acad Sci U S A* 103: 3322-6

Weerkamp F, Luis TC, Naber BA, Koster EE, Jeannotte L, et al. 2006b. Identification of Notch target genes in uncommitted T-cell progenitors: No direct induction of a T-cell specific gene program. *Leukemia* 20: 1967-77

Weng AP, Ferrando AA, Lee W, Morris JPt, Silverman LB, et al. 2004. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science* 306: 269-71

Weng AP, Millholland JM, Yashiro-Ohtani Y, Arcangeli ML, Lau A, et al. 2006. c-Myc is an important direct target of Notch1 in T-cell acute lymphoblastic leukemia/lymphoma. *Genes Dev* 20: 2096-109

Wolfer A, Bakker T, Wilson A, Nicolas M, Ioannidis V, et al. 2001. Inactivation of Notch 1 in immature thymocytes does not perturb CD4 or CD8T cell development. *Nat Immunol* 2: 235-41

Wolfer A, Wilson A, Nemir M, MacDonald HR, Radtke F. 2002. Inactivation of Notch1 impairs VDJbeta rearrangement and allows pre-TCR-independent survival of early alpha beta Lineage Thymocytes. *Immunity* 16: 869-79

Wu L, Antica M, Johnson GR, Scollay R, Shortman K. 1991a. Developmental potential of the earliest precursor cells from the adult mouse thymus. *J Exp Med* 174: 1617-27

Wu L, Li CL, Shortman K. 1996. Thymic dendritic cell precursors: relationship to the T lymphocyte lineage and phenotype of the dendritic cell progeny. *J Exp Med* 184: 903-11

Wu L, Scollay R, Egerton M, Pearse M, Spangrude GJ, Shortman K. 1991b. CD4 expressed on earliest T-lineage precursor cells in the adult murine thymus. *Nature* 349: 71-4

Wurbel MA, Philippe JM, Nguyen C, Victorero G, Freeman T, et al. 2000. The chemokine TECK is expressed by thymic and intestinal epithelial cells and attracts double- and single-positive thymocytes expressing the TECK receptor CCR9. *Eur J Immunol* 30: 262-71

Xu Y, Banerjee D, Huelsken J, Birchmeier W, Sen JM. 2003. Deletion of beta-catenin impairs T cell development. *Nat Immunol* 4: 1177-82

Yamasaki S, Ishikawa E, Sakuma M, Ogata K, Sakata-Sogawa K, et al. 2006. Mechanistic basis of pre-T cell receptor-mediated autonomous signaling critical for thymocyte development. *Nat Immunol* 7: 67-75

Yu Q, Park JH, Doan LL, Erman B, Feigenbaum L, Singer A. 2006. Cytokine signal transduction is suppressed in preselection double-positive thymocytes and restored by positive selection. *J Exp Med* 203: 165-75

Zamisch M, Moore-Scott B, Su DM, Lucas PJ, Manley N, Richie ER. 2005. Ontogeny and regulation of IL-7-expressing thymic epithelial cells. *J Immunol* 174: 60-7

Zúñiga-Pflücker JC, Di J, Lenardo MJ. 1995. Requirement for TNF-alpha and IL-1 alpha in fetal thymocyte commitment and differentiation. *Science* 268: 1906-9

Zúñiga-Pflücker JC, Lenardo MJ. 1996. Regulation of thymocyte development from immature progenitors. *Curr Opin Immunol* 8: 215-24

Table I. Location of Factor-Producing Thymic Stromal Cells

	Zone	SCZ	Cortex	CMJ	Medulla	References
Predominant Thymocyte Population		Late DN3 pre-DP	DN2/DN3 DP	DN1 DP	SP	(Lind et al 2001)
Notch Ligand	Dll1	- (a) - (b)	++ -	+++ -	n/a -	(Schmitt et al 2004) (Heinzel et al 2007)
	Dll4	+++ (b)	+++	+++	+	(Heinzel et al 2007)
	Jag1	+ (a) - (b)	+ -	+ -	+++ -	(Lehar et al 2005) (Heinzel et al 2007)
	Jag2	+ (b)	+	+	+	(Heinzel et al 2007)
Cytokine	SCF	n/a	FT + (c)	n/a	n/a	(Moore et al 1993)
	IL-7	+ (b) NT +++	+	+++	++	(Zamisch et al 2005)
Wnt	Wnt4, 7a, 7b, 10a, 10b	n/a	FT + (c)	n/a	n/a	(Pongracz et al 2003)
Hedgehog	SHh	n/a - (d)	+ (a) -	n/a +	n/a +	(Outram et al 2000) (El Andaloussi et al 2006)
	Ihh	- (a)	+	+	+++	(Outram et al 2000)
BMP	BMP2/4 BMP4	+++ (d) + (a)	+ +	n/a n/a	+++ -	(Graf et al 2002) (Hager-Theodorides et al 2002)
Chemokine	CXCL12 (SDF1)	+ (b, c) + (a)	+ +	+ ++	- ++	(Plotkin et al 2003) (Misslitz et al 2004)
	CCL19	- (a)	-	-	+	(Misslitz et al 2004) (Ueno et al 2004)
	CCL21	- (a)	+	++	+++	(Misslitz et al 2004) (Ueno et al 2004)
	CCL25	++ (b)	++ (a)	++ (a)	+++ (a)	(Misslitz et al 2004) (Wurbel et al 2000)

a, Immunofluorescence
b, In situ hybridization
c, Reverse transcriptase-polymerase chain reaction
d, Immunohistochemistry
n/a, data not available; FT, fetal thymus TEC; NT, neonatal thymus;
+, sparse distribution to +++, high density distribution; - below detection

Table II: Summary of Factor Influence on Early T lymphopoiesis

	Differentiation Lineage Commitment	Survival Trophic Effects	Proliferation
Notch Ligand	DN1/ETP-DN2	DN3a	DN3b
SCF	?	DN1	DN2
IL-7	DN2-DN3 (γδ)	DN2-DN3, SP DN3b (γδ)	DN2-DN3
Wnt	DN3	DN1-DN3 DN4/pre-DP-DP	DN2-ISP
Hedgehog	?	DN1/ETP-DN3	DN2
BMP	?	DN1	DN3
Chemokine	DN1-SP	?	?

?, not known or unclear role
(γδ) indicates γδ-lineage specific role
See text for references

FIGURE LEGEND

Figure 1. Schematic view of T lymphopoiesis within the thymus.