Genes, pathways and checkpoints in lymphocyte development and homeostasis

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Действуют несколько КПП (checkpoints) во время развития В и Т клеток, чтобы гарантировать отбор лимфоцитов с функциональными, но не само-реагирующими рецепторами, чтобы циркулировать п всему телу хозяина в поисках чужеродных антигенов. Для В клеток доминирующие КПП находятся в костном мозге на пре-В клеточной стадии и стадии незрелых В клеток, и в селезенке, где клетки отбираются в зрелый долгоживущий рециркулирующий пул. Дл Т клеток КПП действуют на пре-Е клеточной стадии при негативной и позитивной селекции при переходе от $CD4^+$ $CD8^+$ в двойных позитивных клеток к CD4+ или CD8+ позитивным клеткам. На периферии лимфоциты также находятся под плотным контролем, нуждающимся в сигналах выживания в форме ʽtonic’ стимуляции антигенных рецепторов и растворимых факторов.

Regulation of B cell development and homeostasis

Stages of B cell development

Развитие В клеток происходит преимущественно в печени плодов во время средних и поздних стадий беременности, затем в костном мозге после рождения и продолжается там всю жизнь, хотя с фозрастом с всё больше уменьшающейся скоростью. У мышей развитие конвенционных В (В2) лимфоцитов из предшественников В клеток происходит в виде множественных последовательных ступеней, характеризующихся последовательной экспрессией генов и маркеров клеточной поверхности (Рис. 1). Дифференцировка В1 клеток ненее определенна.

Hardy et al., разделяют популяции В клеток по стадиям в соответствии с достижениями соматических перестроек и экспрессии на клеточной поверхности CD43 (leukosialin S7), CD24 (HSA) и BP1 на B220+ клетках костного мозга.[1] CD43 экспрессируется рано во время В лимфопоэза, а B220+ CD43+ популяция может быть далее подразделена на субпопуляции в соответствии с экспрессией CD24 и BP1. Во фракции А оба локуса heavy chain (IgH) и light chain (IgL) находятся в germline конфигурации и клетки лишены экспрессии CB24 и BP1/ Dj фракции В клетки обнаруживают инициированную D_H-J_H рекомбинацию и экспрессируют CD24. В фракции С клетки имеют инициированную V_H-DJ_H перестройку и экспрессируют и CD24 и BP1. Вторая популяция, которая сходна с фракцией С, но экспрессирует высокие уровни CD24, обозначена фракцией С’. Клетки этой фракции экспрессируют функциональные pre-B cell receptor (BCR) и подвергаются нескольким раундам клеточных делений. Клетки, которые проходят pre-B checkpoint, фракция D, больше не экспрессируют S7 эпитоп CD43 и являются маленькими и отдыхающими. Развитие затем переходит к фракции Е, которая представлена незрелыми В клетками, которые имеют перестроившиеся гены легкой цепи и экспрессируют поверхностный IgM. В костном мозге рециркулирующие зрелые В клетки (фракция F) экспрессирует и IgM и IgD.

Далее В-клеточные популяции дифференцируются в костном мозге по размеру клеток и поверхностной экспрессией c-kit (CD117), CD25 и суррогата легкой цепи.[2] Pro/pre-BI клетки экспрессируют c-kit и суррогатную легкую цепь и имеют инициированные D_H-J_H перестройки. Pre-BII клетки подвергаются V_H-DJ_H рекомбинации и не экспрессируют больше c-kit. Pre-BII клетки имеют инициированные V_L-J_L перестройки и не экспрессируют больше суррогатную легкую цепь.

Субнабор В1 клеток отличается заметно от конвенциональных В2 клеток. Во-первых, В1 клетки преимущественно обнаруживаются в плевральной и перитонеальной полостях и составляют лишь малую часть В клеток в селезенке. [3,4] Во-вторых, В1 клетки отличаются от В2 клеток экспрессией молекул клеточной поверхности. В1 клетки являются

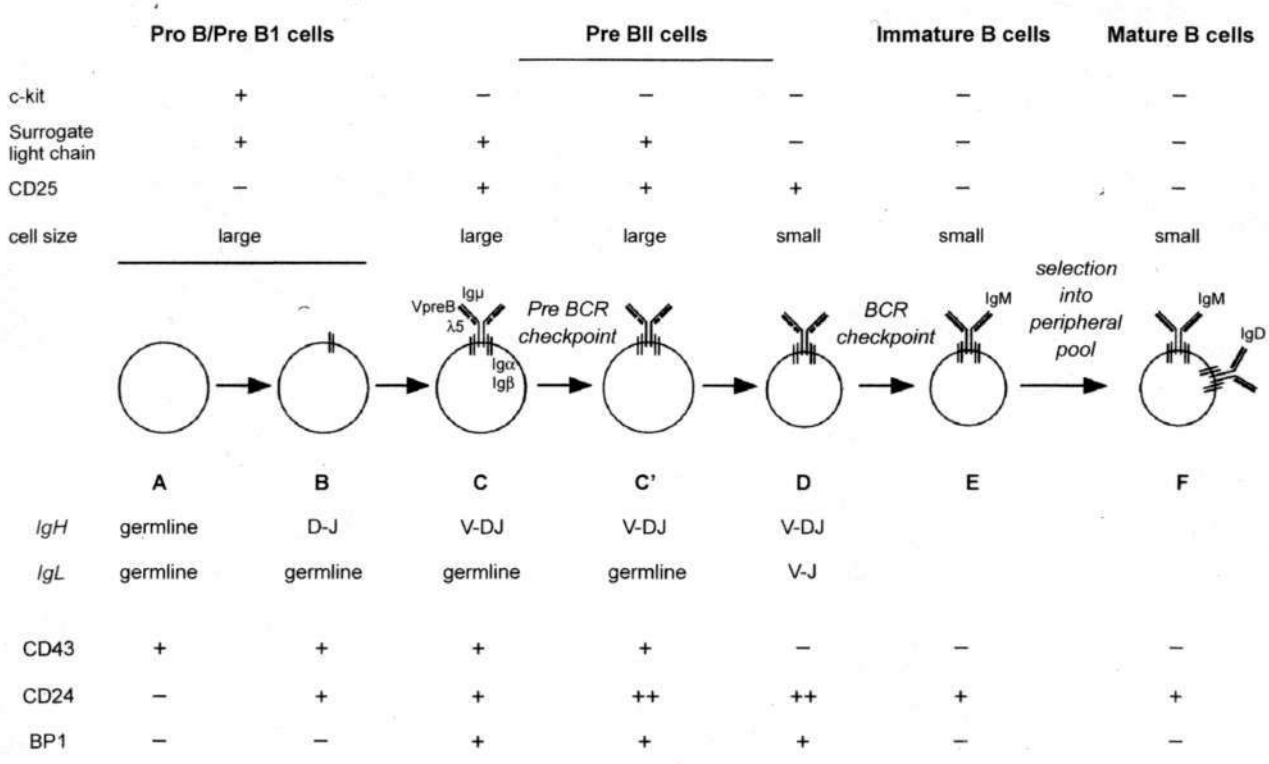


Figure 1 Murine B cell development in the bone marrow as defined by two methods to distinguish B cell populations. Stages of B cell development can be distinguished by cell size and surface expression of c-kit, surrogate light chain and CD25, and development proceeds from pro-B/pre-BI cells to pre-BII cells, to immature and then mature B cells. Alternatively, stages of B cell development can be classified into fractions A through to F according to the rearrangement status of the immunoglobulin heavy and light chain genes and cell surface expression of CD43, CD24 and BP1. The three dominant checkpoints during B lymphopoiesis occur at the pre-B cell receptor (BCR), BCR checkpoints and during selection into the long-lived peripheral pool.

B220[10] IgM^{hl} IgD~ CD21~ CD23 , and a fraction of these, the Bla subset, are $CD5^{+}$, whereas the Bib cell subset is CD5.[5] Third, Bl cells have a restricted immunoglobulin repertoire and have self-renewing capabilities.[45] The developmental relationship between Bl and B2 cells remains controversial. It has been suggested that Bl and B2 subsets derive from the same lineage and development into either subset is driven by receptor specificity.[5,6] Alternatively, Bl and B2 cells have been proposed to develop from separate lineages altogether.[7] In support of the latter hypothesis, IL-7 has been shown to be critical for the development of conventional B cells (see 'Survival of B cell precursors' section), whereas the Bl cell subset is unaffected in *IL-* 7-deficient mice.[8]

Commitment to the B cell lineage and survival of precursors

B cell commitment

Transcription factors EBF and E2A are required early during B lymphopoiesis. EBF is expressed during the early stages of B cell development and in non-lymphoid tissues. Mice lacking *EBF* only have cells in fraction A (Table l).[9] Similarly, mice lacking the ubiquitously expressed *E2A* gene, which generates the two transcription factors E12 and E47 through differential splicing, exhibit a similar block in development prior to the onset of *IgH* rearrangement.[10] The majority of *E2A*-deficient mice die shortly after birth; however, a small proportion of mice survive and can live for up to 10 months.[11] Interestingly, of the 13 mice that survived, six individuals developed rumours of immature thymocyte origin.[11] This finding may be due in part to the interaction of E2A with the T cell-specific Notch-1 pathway (see 'T cell commitment' section).

The transcription factor Pax5 is required for B cell commitment and maturation. Pax5 is expressed exclusively in the B cell lineage and has been shown to activate B cell lineage genes *CD19, Iga* and *blnk/slp-65,* which are molecules that are critical for pre-BCR signalling.[12-14] In addition, Pax5 has been shown to suppress genes of other lineages, including the T cell-specific transcription factor Notch.[15] B cells in *pax5*-deficient mice undergo arrest at the pro-B/pre-BI stage.[1216] Interestingly, poxJ-deficient pro-B cells are uncommitted to the B lineage and are able to differentiate into monocytes, granulocytes and NK cells *in vitro.*[15] Additionally, conditional /?ox5-deficient mice have demonstrated a role for Pax5 in mature B cell survival and function.[14] Thus, the transcription factors EBF, E2A and Pax5 are required for commitment to the B cell lineage,

Survival of B cell precursors

Once committed to the B cell lineage, the survival of early B cell progenitors is dependent upon contact with stroma and

Table 1 Mutations causing defects in pre-B cell development

Molecule	Mutation	B cell development	References
EBF	Knockout	Fr. A to B block	9
E2A	Knockout	Fr. A to B block	10
Pax5	Knockout	Fr. B to C block	12,16
IL-7	Knockout	Fr. C to D block/Fr. A to B block	8,19
IL-7Rcc	Knockout	Fr. A to B block/Fr. C to D block	18,26
yc	Knockout	Fr. C to D block	30
JAK3	Knockout	Fr. C to D block	33
RAG1	Knockout	Fr. C to C block	40
RAG2	Knockout	Fr. B to C block	39
DNA-PK_{CS}	*scid* mouse	Fr. C to D block	39,45
Ku70	Knockout	Fr. C to D block	46,47
Ku80	Knockout	Fr. C to D block	48,49
Igu	uMT mice	Large pre-B to small pre-B block	51
	JH deletion	Large pre-B to small pre-B block	50
Iga-IgP	Iga^{Ac}	Fr. C to C partial block	57
	Igp^{Ac}/Iga^{Ac}	Fr. C to C block	56
	IgP knockout	Fr. C to D block	55
^5	Knockout	Large to small pre-B partial block	237
VpreBl/B2	Knockout	Large to small pre-B partial block	54
Syk	Knockout	Pro-B to pre-B partial block	61
Syk-ZAP-70	Double knockout	Pro-B to pre-B block	61
p85a	Knockout	Fr. C to D partial block	65,66
BLNK	Knockout	Fr. C to D partial block	67,68

Fr, fraction.

growth factors. Stromal cells provide an essential signal for cells in fraction A, which are strongly dependent on direct physical contact with stroma; fraction B cells are moderately dependent on contact with stromal cells, whereas cells in fraction C are equally dependent on interactions with stroma and soluble factors.[1]

In 1998, IL-7 was identified and cloned on the basis of its ability to induce B cell proliferation in the absence of stromal cells and was consequently shown to be required for the survival of B cell, T cell and myeloid progenitors.[17-20] The importance of IL-7 in B cell development was underscored by studies in mice with excessive amounts of IL-7, either by injection with IL-7 or in ZL-7-overexpressing transgenic mice, which develop B cell lymphomas.[20-23]

Interleukin-7 is one of the critical growth factors produced by stromal cells in a number of non-hemopoietic and haemopoietic tissues, including fetal liver, bone marrow, thymus **and spleen.**[24] The IL-7 receptor (IL-7R) is expressed on early B lineage cells then lost after *IgL* rearrangement. Fractions B and C depend on IL-7, whereas cells in fraction A and the latest stages of development do not. • Although discrepancies exist in results as to the B cell developmental stages that are **blocked in** mice lacking IL-7 signalling either as a result of treatment with anti-IL-7 monoclonal antibody or because of an *IL-7* or *IL-7Ra* deficiency, it is not surprising that B cell development is arrested after fraction A, a stage that is unresponsive to IL-7.[8,18,19,25,26] In some studies, mice deficient in *IL-7* or *IL-7Ra* exhibited a block in B cell differentiation at the A-to-B transition, whereas others demonstrated a C-to-D defect.[8-18,19,25,26] Th_e developmental block in *IL-7-* or *IL-Ra-*deficient mice is incomplete, as indicated by the presence of some peripheral B cells. The development of these B cells may be because of the presence of other B cell growth factors, such as haemokinin 1 and thymic stromal lymphopoietin.[27,28]

The IL-7R is composed of the IL-7Rct chain and common gamma (yc) chain shared with IL-2, IL-4, IL-9, IL-15 and IL-21. Similar to the situation in *IL-7-* and 7Z-7?a-deficient mice, in mice deficient in *yc* or *jak3* (a tyrosine kinase that associates with and is activated by yc), B cell development is partially blocked at the pre-B cell stage.[29-33] Thus, the survival of early B cell precursors depends on IL-7-JAK3-mediated survival signals.

Checkpoints during B cell development

Pre-BCR checkpoint

The pre-BCR checkpoint selects against self-reactive cells and those with unsuccessful VDJ rearrangement, and promotes the survival and differentiation of cells that have successfully rearranged *IgH* and can express a pre-BCR complex composed of an IgM heavy chain (Igu) associated with surrogate light chain and an Iga-Igp heterodimer on the cell surface. Successful signalling through the pre-BCR leads to cell proliferation, allelic exclusion and the initiation of *IgL* rearrangements. Approximately 75% of cells die at the pre-BCR checkpoint.[34]

Rearrangement of the IgH locus Unique to B and T cells is the process of somatic gene rearrangement of the immunoglobulin and T cell receptor (TCR) loci, which generate a diverse antibody and T cell repertoire, respectively. In the germline, genes encoding the B and T cell receptors are spatially separated into multiple segments, but during B and T cell development V, D and J gene segments are made contiguous by the process of somatic rearrangement. The recombination-activating genes (RAG) 1 and 2 have an integral role in somatic rearrangement by generating double-strand breaks in DNA.[35,37] To repair double-strand breaks, DNA-dependent protein kinase (DNA-PK) and DNA ligase proteins are required.

The expression of RAG1 and RAG2 is restricted to lymphocytes undergoing somatic rearrangement. Mutant mouse strains that lack either *ragl* or *rag!* are unable to rearrange the *IgH* locus, leading to a complete block at the pre-B cell stage of development.[38,39] The arrested development in *ragl'*-B cells can be overcome by the expression of heavy and light chain transgenes, which permits the development of immature and mature B cells.[40] *scid* mice with a point mutation in the gene encoding the catalytic component of DNA-PK (DNA-PK^) lack the ability to repair double-strand breaks and thus cannot rearrange *IgH,* leading to a developmental block in B lymphopoiesis at the pre-BCR checkpoint.[39,41,,45] Additionally, mice deficient in the *Ku70* and *Ku80* DNA-binding subunits of DNA-PK lack peripheral B cells because of a comparable block in development at the pre-B cell stage.[46-49] Thus, B cell progenitors lacking the ability to rearrange their heavy chain arrest at an early stage in development.

Expression of the pre-BCR complex Following the generation of an inframe rearranged IgH, pre-B cells are tested for the ability to bind surrogate light chain and deposition into the cell membrane in association with Ig*a*-Ig*b*. Cells that express a pre-BCR complex are positively selected, enter the cell cycle and proliferate.

In JHT mice that do not express surface Ig*mu*, and in *mu***MT** mice in which the membrane region has been disrupted and therefore cannot be expressed on the cell surface, B cell development arrests at the pre-BCR checkpoint.[5051]

An inframe Ig*mu* chain associates with surrogate light chain, the latter being expressed in fractions A to C. In mice, three genes encode the components of the surrogate light chain: A5, *VpreBl* and *VpreB2.* Testing the ability of Ig*mu* to bind surrogate light chain results in the removal of Ig*mu* chains that would not be able to bind rearranged IgL and would subsequently not be useful. This is an important control mechanism, because it has been estimated that half of all heavy chains are unable to associate with surrogate light chain.[52] In the absence of *X5* or *VpreBl* and *VpreB2,* mice exhibit defects in B cell development that is most notable at the transition from fraction C to D.[53,54]

The BCR is non-catalytic and lacks cytoplasmic signalling capacity, requiring the formation of a complex with signal transducers Ig*a* (CD79a, mb-1) and Ig*b* (CD70b, B29). Ig*a* and Ig*b* contain immunoreceptor tyrosine activation motifs (ITAM) within the cytoplasmic tail and are the signal transducers of the BCR complex. The importance of Ig*mu* association with a catalytically active Iga and Igp heterodimer at the pre-BCR checkpoint is demonstrated in mice deficient in *Igp,* and mice where the cytoplasmic tails of Iga and Igb have been deleted (Igb^{AC}/Iga^{AC}), because B cell development does not progress beyond the pre-BCR checkpoint.[5556] Additionally, mice with a cytoplasmic deletion of Iga or where the Iga ITAM tyrosine residues are mutated to phenylalanine have a partial defect at the transition from pro-B to pre-B.[57,58] Thus, rearranged heavy chains that lack the ability to associate with surrogate light chain or Ig*a*-Ig*b* and thus cannot express an intact pre-BCR complex on the cell surface do not pass the pre-BCR checkpoint.

Signalling through the pre-BCR complex Once the pre-BCR complex is formed on the cell surface, the cell signals to cease rearrangement of the ***IgH*** locus and initiates rearrangement of the *IgL* locus. This achieves allelic exclusion whereby one allele generates one antibody receptor and thus each cell has a single antibody specificity. Mice lacking certain components of the BCR signalling pathway have a block or partial block at the pre-BCR checkpoint.

Phosphorylation of Ig*a*-Ig*b* ITAM following BCR crosslinking leads to the recruitment and consequent phosphorylation of the tyrosine kinase Syk. Activation of Syk leads to further signal transduction via multiple proteins, generation of second messengers and gene regulation. The Syk family of tyrosine kinases contains two members, Syk and ZAP-70, which have critical roles in signalling, predominantly downstream of the BCR and TCR, respectively.[5960] rag-deficient mice reconstituted with *Syk*-deficient bone marrow have a partial block in B cell development at the transition from fraction C to D, and have no mature B cells because of a further and complete block in development, because cells are not selected into a long-lived peripheral pool.[59,60] Interestingly, Schweighoffer *et al.* have recently shown that mice deficient in both *Syk* and *zap-70* have a complete block in B cell development at the pre-B cell stage.[61] ZAP-70 was thought to be only expressed in T cells and NK cells, but has recently been shown to be expressed in primary B cells at pro-B, pre-B and mature B cell stages of development.[6162] ZAP-70 is critical for T cell development, whereas B cell development was intact in *zap*-70-deficient mice.[6364] Thus, the block in B cell development at the pre-B cell stage in the absence of both Syk and ZAP-70 demonstrates that both kinases can support signalling and selection of the pre-BCR.

Mice lacking additional components of the BCR signalling pathway exhibit defects at the pre-BCR checkpoint. Mice deficient in *p85a,* the regulatory subunit of PI3K, and *blnk*-deficient mice also demonstrate a partial block in B cell development at the pre-BCR checkpoint.[65-68] Additionally *p85a-/-* and *blnk-*[1]- mice have further defects in peripheral B cell maturation (see 'BCR signalling pathway' section). Thus, pre-BCR signalling is critical for cells to pass the pre-BCR checkpoint

BCR checkpoint

Cells in fraction C or pre-BII large cells, which express the pre-BCR complex, proliferate prior to rearrangement of ***IgL,*** thereby increasing the repertoire diversity by permitting numerous light chains to associate with a single heavy chain in different daughter cells. Once a light chain has been successfully rearranged and cells express conventional IgM on the cell surfaces, cells receive signals to stop further rearrangements of the *IgL* locus and continue differentiation. The nature of these signals is unclear. Immature B cells at this stage undergo selection prior to export into the periphery.

It has been estimated that the number of immature B cells generated in the bone marrow of mice is about 2×10^7 cells per day, and that 2×10^6 immature cells are exported into the periphery.[34,69] Thus, only 10% of immature B cells produced in the bone marrow enter the periphery. The dramatic decrease in immature B cell numbers from the pre-B pool is likely to be due to the low probability of producing inframe rearrangements, the inability of IgH and IgL chains to pair, or inefficient signalling through the BCR. Furthermore, cells are screened for self-reactivity in the bone marrow. Binding of antigen in the bone marrow can lead to a number of cell fates: (i) deletion; (ii) anergy, whereby cells are rendered inactive; or (iii) receptor editing, where secondary *IgL* gene rearrangement has the potential to produce a new non-self-reacting light chain.[70"75]

Entry into and maintenance of the peripheral B cell pool

Regulation of peripheral B cell numbers is achieved by a combination of regulating B cell export from the bone marrow, selection into the long-lived recirculating repertoire and death of recirculating B cells. These selection and survival processes involve signalling through the BCR and BAFF pathways. Mutant mice with defects in peripheral B cell populations are summarized in Table 2.

If 2×10^7 immature B cells are produced in the bone marrow of mice each day, and the peripheral B cell pool consists of an estimated 1.5×10^8 cells, then the entire

Table 2 Mutations causing defects in peripheral B cell populations

Molecule	Immature B cells	Mature B cells	MZB cells	Bl cells	References
Igot^{AX}	Decreased	Decreased	NR	NR	56,57
Igfjax	Normal	Absent	NR	NR	56
IgpF	Normal	Decreased	Decreased	Decreased	$*
CD45	Increased	Decreased	NR	Absent	91
CD19	Normal	Normal	Absent	Decreased	101,102
Syk	Decreased	Absent	NR	Absent	59,60
BLNK	Decreased	Decreased	Normal	Absent	67,68
PLCy2	Increased	Decreased	Decreased	Absent	96.97
PKC(3	Normal	Normal	NR	Decreased	105
p85a	Decreased	Decreased	NR	Absent	65,66
Btk	Increased	Decreased	Decreased	Absent	93-95
BCAP	Increased	Decreased	Normal	Absent	92
VAV	Normal	Normal	NR	Decreased	103,104
VAV1/2/3	Increased	Decreased	Decreased	NR	98
OCT2	Normal	Decreased	NR	Absent	99
OBF1	Decreased	Decreased	NR	NR	108
BAFF	Normal	Absent	Absent	Normal	111,112
BAFF-R	Normal/Decreased	Decreased	Decreased	Normal	113,125
IKKa	NR	Decreased	NR	NR	126
NIK	NR	Decreased	Absent	Increased	116,127
NFKB2	Normal	Decreased	Decreased	Increased	117

MZB, marginal zone B cell; NR, not reported.

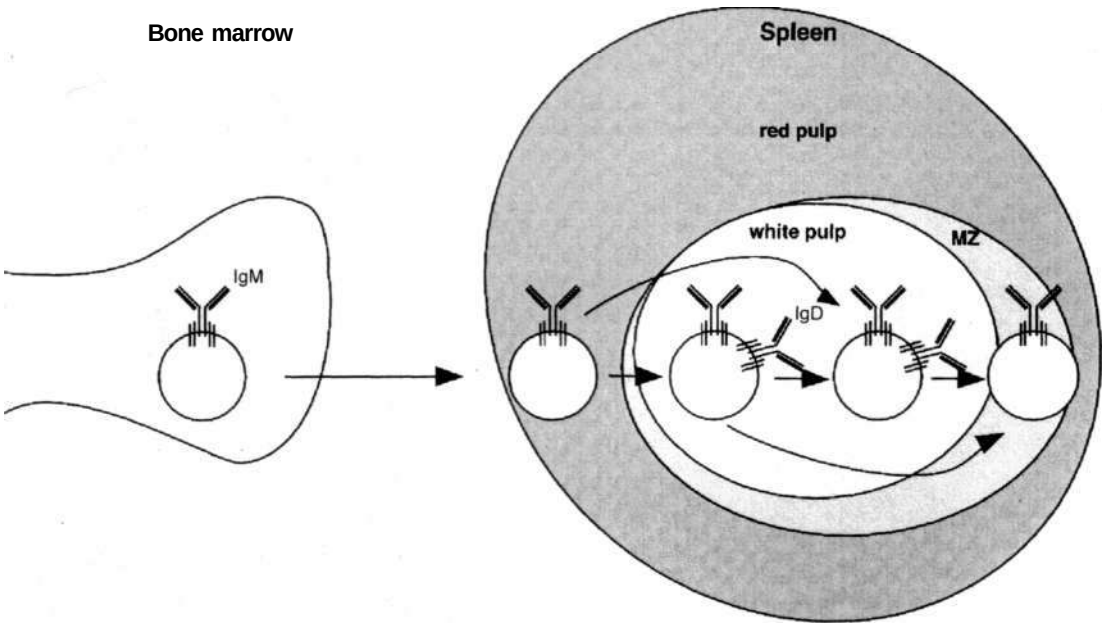
recirculating pool could be replaced within days.[76] However, of the immature B cells that are produced, an estimated 10% of these reach the spleen, and only 1-3% enter the long-lived population.[7778] Additionally, Rolink and co-workers estimated that more than half the immature B cells in the spleen develop into mature B cells.[79] Thus, although estimates vary, it is apparent that only a fraction of immature B cells enter the mature recirculating compartment.

Peripheral B cell maturation Immature B cells exit the bone marrow and enter the spleen via the blood, where they develop into mature naive recirculating B cells through a transitional phase (Fig. 2).[78] Recent bone marrow emigrants, also called newly formed transitional type 1 (Tl) or fraction III cells, enter the red pulp of the spleen. After about 1 day, Tl cells move into the B cell follicles where IgD and CD23 are acquired, marking T2/fraction II cells. Mature B cells (fraction I) downregulate IgM and HSA to low expression and express lymph node-homing receptor L selectin (CD62L), allowing recirculation through lymph nodes. Mature B cells also lack staining with the antibody 493 (ClqRp) that recognizes AA4.1, which is present on all earlier B cell subsets.[79] Mature B cells can develop from both Tl and T2 cells.[78,80] An additional B cell population, the marginal zone B (MZB) cells, reside in the marginal zone of the spleen.

Peripheral B cell lifespan When considering how homeostasis is maintained, the lifespan of the peripheral pool must be considered. Accordingly, B cell lifespan has been the subject of numerous investigations over several decades. Although it is generally agreed that immature B cells are short lived, on average 3-4 days, the lifespan of follicular B cells has been more difficult to define.[7981] Labelling studies, deletion of precursors and cell-transfer experiments have estimated that follicular B cells have a lifespan ranging from just a few days to an 'almost indefinite' period.[81,82] By generating *rag2* conditional knockout mice, where B cell development in the bone marrow of adult mice was blocked, thus preventing replenishment of the peripheral pool by newly generated immature cells, Hao and Rajewsky showed that the follicular B cell population slowly decreased over the period of 1 year with an average half-life of 4.5 months.[83] Thus, with this approach the follicular B cell population can be considered relatively long lived.

The BCR signalling pathway The BCR signalling pathway is initiated by the cross-linking of the BCR by antigen (Fig. 3). Through mechanisms that remain unclear, BCR ligation induces the phosphorylation of tyrosine residues within ITAM of Iga-Igp by the Src kinases Lyn, Fyn and Blk.[84] Phosphorylation of Iga-Igp results in the recruitment and subsequent activation of Syk, followed by the formation of an early signalosome.[85] Adapter protein BLNK is critical for signalling through the pre-BCR as well as the BCR complex. BLNK acts to recruit and localize multiple proteins following phosphorylation by Syk.[86] Recruitment of the Tec kinase Btk and the lipid-metabolizing enzyme PLCy2 to the adapter complex leads to phosphorylation and activation of PLCy2 by Btk and Syk.[8788] PLCy2 cleaves phophatidylinositol-4,5-bisphosphate (PIP2), generating diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP3), which results in the activation of protein kinase C (PKC) and the release of intracellular Ca^{2+} from the endoplasmic reticulum, respectively. Additionally, the constitutive binding of adapter protein Grb2 and nucleotide exchange factor Sos with BLNK, together with the induced binding of guanine nucleotide exchange factor VAV1, leads to activation of the mitogen-activated protein kinase (MAPK) cascade.[89] As a consequence of increased intracellular Ca^{2+} and MAPK signalling, transcription factors such as NFAT and NFKB translocate into the nucleus and regulate gene transcription.

To assess the role of BCR expression in the maintenance of the mature B cell pool, Lam *et al.* generated a mouse strain with inducible deletion of BCR expression.[90] The deletion of



Immature B cells	T1	T2	Mature B cells	MZB
IgM^{hi}	IgM^{hi}	IgM^{hj}	IgM^{10}	IgM^{hi}
IgD"	IgD^{10}	igD^{+}	igD+	IgD
HSA^{hi}	HSA^{hj}	HSA^{hi}	HSA^{10}	HSA+
CD21"	$CD21^{10}$	$CD21^{int}$	$CD21^{jnt}$	$CD21^{hi}$
CD23"	CD23"	CD23+	CD23+	CD23-
493+	493+	493+	493"	493"

Figure 2 Murine peripheral B cell maturation in the spleen. Transitional type 1 (Tl) cells, the recent emigrants from the bone marrow, enter the red pulp of the spleen and upregulate the expression of IgD and CD21 to become transitional type 2 (T2) cells. In the white pulp, T2 cells differentiate into mature naive follicular B cells following the downregulation of IgM, HSA and 493. Mature B cells can also develop from Tl cells and marginal zone B (MZB) cells are thought to develop from both T2 and mature B cells.

the BCR in adulthood resulted in the rapid depletion of follicular, MZB and B1 cells.[90] Thus, this experimental approach demonstrated that the BCR provides survival signals for all peripheral B cell populations.

Disruption of the BCR signalling cascade by the absence of critical signalling components such as CD45, Syk, BLNK, BCAP, Btk, p85a, PLCy2, Vavl/2/3 as well as the transcription factor OCT2 and its coactivator OBF1 exhibit mature B cell deficiencies.[59,60,65,68-91,100] Significantly, the Bl population is more severely affected as a result of disrupting the BCR pathway. Additionally, mice deficient in CD 19, VAV and PKCp have normal numbers of mature B cells, but a diminished or absent population of Bl cells.[101-105] Furthermore, a BCR signal strength model for the development of follicular, MZB and Bl cells has been proposed whereby weak BCR signals lead to the development of MZB cells, intermediate BCR signals to the development of follicular B cells and strong BCR signals lead to the development of Bl cells.[80,106] Thus, BCR expression and signal transduction are critical for the survival of peripheral B cells and the strength of signal determines the selection of cells into specific B cell subsets.

BAFFpathway In addition to the BCR providing a survival signal to peripheral B cells, BAFF, which is also known as BLyS, TALL-1, THANK and zTNF4, is specifically required for the regulation of mature recirculating B2 cells. BAFF, a TNF family member, has been shown to be an essential molecule in promoting mature B cell survival *in vitro,* in *baff* over-expressing transgenic mice and in mice deficient in *baff.*[101-U2] Immature B cells develop normally in *baff*-deficient mice, but they lack mature B cells.[111,112] Similarly, the A/WySnJ mouse strain, in which the BAFF Receptor (BAFF-R, BR3) is naturally mutated, also exhibits deficiencies in mature B cells.[112-115] Mice lacking *baff, baff-R* or downstream signalling molecules have normal or elevated numbers of peritoneal Bl cells, indicating that BAFF-BAFF-R signalling is essential for mature B2, but not Bl cell survival.[112,113,116,117]

The signalling mechanisms downstream of BAFF are only beginning to be defined. To date three receptors have been identified that bind BAFF; these are TACI, BCMA and BAFF-R.[114118120] Although APRIL, a molecule closely related to BAFF, can also bind TACI and BCMA, BAFF-R

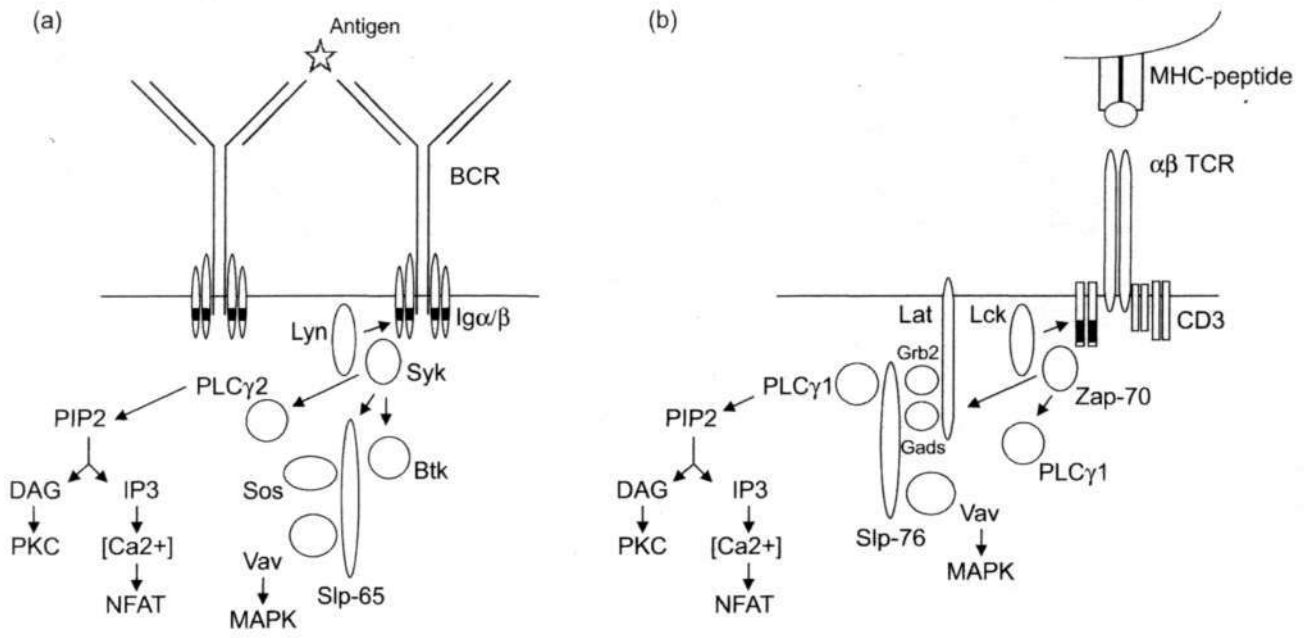


Figure 3 Simplified schematic diagram of antigen receptor signalling. Upon (a) crosslinking of the pre-B cell receptor (BCR) and (b) T eel 1 receptor (TCR) ligation with peptide-MHC, IT AM within the coreceptor Iga/p and CD3 molecules are phosphorylated by the Src kinases Lyn and Lck in B cells and T cells, respectively. Syk and ZAP-70 are recruited to the coreceptor molecules and activated, leading to further phosphorylation and activation of several downstream targets and pathways, ultimately leading to the regulation of gene transcription.

specifically binds BAFF.[114] Mice deficient in *bema* have normal numbers of peripheral B cells, whereas mice deficient in *taci* have an increased number of B cells, which suggests a negative regulatory role for TACI in B cell homeostasis.[115121122] Together, these findings suggest that BAFF, signalling predominantly through BAFF-R, has a role in maintaining peripheral B cell numbers.

The details of the BAFF-BAFF-R signalling pathway are only partially defined. Two independent groups have demonstrated that BAFF stimulation resulted in the cleavage of the NFKB2 precursor pi00 to the active p52 subunit; a process shown to be dependent on NIK and IKKa.[123124] Interestingly, pi00 processing by BAFF occurs 2-3 h after treatment and was dependent upon protein synthesis, because pi00 cleavage was not observed when cells were treated with cycloheximide, raising the question of what genes must be transcribed in order to observe the generation of p52.[123] It is also interesting to note that some follicular B cells are formed in A/WySnJ mice and mice lacking *ikka, nik* or *nfrd>2,* whereas this subset is almost entirely absent in mice lacking *baff,* raising the question of what additional pathways mediate BAFF signals.[111-113,116,117-125-130]

As mentioned earlier, NIK is required for BAFF-BAFF-R signalling and one model that has been proposed recently is that activation of BAFF-R allows the NIK protein to accumulate and become active in signalling to IKK by inhibiting TRAF3-promoted ubiquitination and degradation of NIK.[43] Although overexpression of TRAF3 inhibits BAFF-R signalling, and only TRAF3 is found to interact directly with BAFF-R, TRAF2 also plays a critical role in repressing NFKB2 signalling.[131132] A conditional deficiency of TRAF2 enhances the processing of NFKB2 and increases mature B cell numbers, especially MZB cells, similar to BAFF transgenic mice.[132] How BAFF signalling is initiated and regulated in B cells by crosstalk with other receptors is clearly an important area for future work.

Although both BAFF and BCR signalling are required to maintain the mature recirculating pool, it is not understood how these two inputs are integrated. It is possible that the two input signals act in parallel to promote the expression of prosurvival genes such as Bcl2. Mature B cells lacking Bcl2 fail to accumulate in the periphery.[133134] Constitutive Bcl2 expression dramatically expands the pool of follicular B cells and can partially correct the peripheral B cell deficiency caused by defects in BCR and BAFF signalling.[135-139] BCR signalling can activate the classical NFKB transcription factors comprising NFtcBl-c-rel and NFKBl-rel-A, and combined deficiency of these factors leads to defects in mature B cells.[140,141] In contrast to the reduction in B cells in *nfabl*-deficient mice, a combined deficiency of NFKB1 and NFKB2 completely eliminates the mature B cell pool, consistent with action in parallel pathways.[117128-130,142] Finally, NFKBI, NFKB2, c-rel and rel-A are all capable of regulating Bcl2 expression.[123,140,143]

Regulation of <xp T cell development and homeostasis

Stages ofT cell development

T cell development begins in the fetal liver during early embryogenesis and continues within the newly developed thymus that forms around embryonic days (E) 10-13.5.[144] Following commitment to the T cell lineage, thymocytes develop through stages differentiated by the expression of cell surface markers and rearrangement of the *TCR* loci (Fig. 4). The four predominant thymocyte populations are distinguished

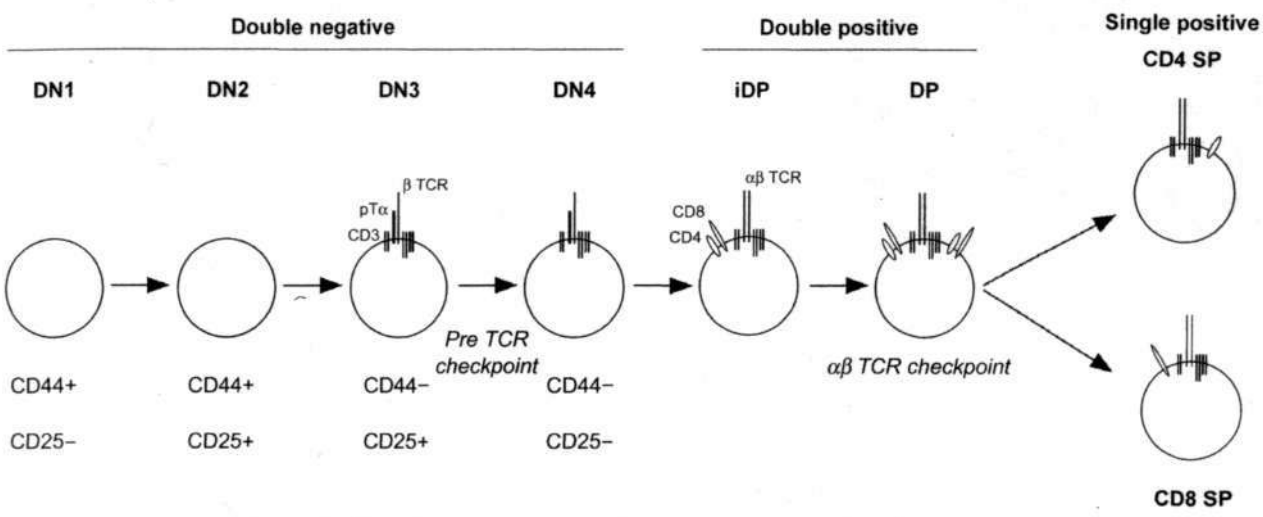


Figure 4 Murine T cell development in the thymus. Early stages of T cell development in the thymus are distinguished by CD44 and CD25 expression on double-negative (DN) cells. Selection of a functional pre-T cell receptor (TCR) is followed by the upregulation of the coreceptors CD4 and CD8 to become double-positive (DP) cells. Following *ab* TCR selection, DP cells downregulate CD4 or CD8 to become CD8 SP or CD4 SP cells, respectively, prior to export into the periphery.

by expression of the TCR coreceptors CD4 and CD8. The earliest of these stages are the CD4~ CD8" double-negative (DN) cells that rearrange and express TCR p. Coexpression of CD4 and CD8 and an *ab* TCR occurs at the $CD4^+$ $CD8^+$ double-positive (DP) stage of development and subsequent downregulation of either CD4 or CD8 leads to the development of $CD8^+$ single-positive (SP) or $CD4^+$ SP cells, respectively, following selection for a TCR able to recognize self-peptide-MHC complexes without being self-reactive.

Stages of early thymocyte development within the DN population can be delineated by expression of CD25 (IL-2Ra) and CD44 (phagocyte glycoprotein, Pgpl).[145146] The DN1 population ($CD25^-$ $CD44^+$) develops from common lymphoid progenitors entering the thymus from the bone marrow. Development then proceeds to the DN2 stage ($CD25^+$ $CD44^+$) with the upregulation of CD25 and the initiation of rearrangement at the *TCR p, y* and *5* loci. DN3 cells ($CD25^+$ CD44) undergo selection for a successfully rearranged *TCR ft* gene. Cells that pass this checkpoint downregulate CD25 to become rapidly dividing DN4 cells (CD25- CD44). DN4 cells differentiate into DP cells, initiate the rearrangement of the *TCR a* gene locus and subsequently express an *ab* TCR. Similar to ap T cells, y5 T cells undergo VDJ rearrangement of the y and 8 loci. y5 T cells differ from conventional ap T cells because they are not MHC-restricted, can recognize pathogens directly, and are rare in the blood (instead they predominate in **the skin** and gut).[147]

Commitment to the T cell lineage and survival of precursors

***T cell** commitment*

The role for Notch-1 in the commitment to the T cell lineage has been elegantly demonstrated by two studies. Overexpression of constitutively active *notch-1* by retroviral transduction led to the development of DP cells in the bone marrow and a concomitant block in B lymphopoiesis.[148] The converse experiment, where Notch-1 expression was conditionally ablated after birth, overcoming embryonic lethality in *notch-1* null mice, showed that the absence of Notch-1 caused an early block in T cell development and resulted in the development of phenotypically normal immature B cells in the thymus.[149] Therefore, when Notch-1 is present, T cells develop, and in the absence of Notch-1, B cells develop. Pui *et al.* also demonstrated the specific inhibition of E2A transcriptional activity, which is required for B cell development, by the Notch-1 pathway.[148] This result suggests a possible mechanism whereby the Notch-1 pathway inhibits a transcription factor that is critical for B cell development, but at the same time promotes the development of the T cell lineage.[150]

GATA-3 is also specifically required for the T cell lineage. GATA-3 is a zinc finger-containing transcription factor that is widely expressed, but within the haemopoietic compartment, expression is restricted to the T cell lineage.[151] Additionally, GATA-3-binding sites are found in a number of T cell-specific genes, including *TCR a, p, 8* and *CD8a.*[152A53] To overcome neonatal lethality in *gata-3*-deficient mice, Ting *et al.* utilized the *rag2-deficient* blastocyst complementation technique and found that chimeric mice lacked mature T cells, but had normal erythroid, myeloid and B cell development.[154] Analysis of thymocyte subsets revealed the. presence of DN cells, and because development in *rag2*-deficient mice is blocked at this stage, the authors were only able to conclude that thymocyte development was blocked at or before the DN stage.[154] These findings were extended by Hendriks *etal.,* who transferred *gata-3-deficient* lacZ-expressing embryonic stem (ES) cells into wild-type blastocysts, which permitted the detection of cells derived from the *gata-3-deficient* blastocyst.[155] Analysis of thymocyte development showed an absence of thymocytes, including the DN1 stage, that were derived from the *gata-3*-deficient ES cells (Table 3).[155] Together, these studies demonstrate a requirement for GATA-3 in the survival and/or proliferation of early T cell progenitors.

Table 3 Mutationsi causing defects in T cell development

Molecule	Mutation	Effect on T cell development	References
GATA3	Knockout	Absence of DN1 cells	155
IL-7	Knockout	Reduced thymocytes, normal CD4 and CD8 expression	19
IL-7R	Knockout	Reduced thymocytes, normal CD4 and CD8 expression DN to DP block	18
yc	Knockout	Reduced thymocytes, normal CD4 and CD8 expression	29,30
JAK3	Knockout	Reduced thymocytes, normal CD4 and CD8 expression	31,33
RAG1	Knockout	DN3 to DN4 block	38
RAG2	Knockout	DN to DP block	39
DNA-PK$_{CS}$	*scid* mouse	DN to DP block	43,44
Ku70	Knockout	Partial DN to DP block	46,47
Ku80	Knockout	DN to DP block	*49
TCRp	Knockout	DN to DP block	167
	Enhancer knockout	DN to DP block	169
	ER retrieval signal	DN to DP block	170
pTa	Knockout	DN3 to DN4 block	112,173
CD3e	Knockout	DN3 to DN4 block	175,176
CD3<;	Knockout	Partial DN to DP block	177-179
CD3y	Knockout	Partial DN to DP block	180
Lck	Knockout	Partial DN to DP block	1X6
	Point mutant	Partial DN to DP block	117
Lck/Fyn	Double knockout	DN to DP block	190,191
ZAP-70/Syk	Double knockout	DN3 to DN4 block	193
LAT	Knockout	DN3 to DN4 block	194
	Knockin	DN3 to DN4 block	197
SLP-76	Knockout	DN3 to DN4 block	195,196
GADS	Knockout	Partial DN3 to DN4 block	198
VAV1/2/3	Triple knockout	Partial DN3 to DN4 block	98
TCRa	Knockout	DP to SP block	167,.199
CD3$	Knockout	DP to SP block	181,182
ZAP-70	Knockout	DP to SP block	*53,64*
	R464C mutant	DP to SP block	214

ER, endoplasmic reticulum; DN, double-negative; DP, double-positive; SP, single-positive.

Survival of T cell precursors

Proliferation and survival of early thymocyte progenitors depends largely on signals from IL-7-IL-7R and the tyrosine kinase receptor c-kit and its ligand, stromal cell factor (SCF). Specifically, the DN population is dependent upon c-kit-SCF and IL-7-IL-7R, whereas during later stages of development, pre-T cells and DP cells depend on cytokines and signals through pre-TCR and ap TCR, respectively.[156]

A great deal of information regarding the importance of c-kit and SCF in a number of biological processes has been gained from the study of the spontaneous mutant mouse strains *steel (SI)* and *dominant white spotting (W),* which have dominant mutations in *c-kit* and *scf,* respectively.[157]

Despite the expression of c-kit on DN1 and DN2 thymocytes and early B cell progenitors, peripheral lymphocyte numbers are not altered in *Sl* and *W* mice.[146157] The most notable defect in fetal thymi of *W/W* mice and mice engrafted with *Sl/Sl* thymus was a reduction in DN cells, although the proportion of DP and SP cells was maintained.[158] Additionally, DN thymocytes derived from the *Sl/Sl* thymus grafts had a twofold reduction in proliferation as assessed by BrdU labelling.[158] Thus, c-kit and SCF appear to play a role in the proliferation of DN thymocytes, but not in their differentiation, although it remains possible that the phenotype of mice deficient in *c-kit* or *SCF* is masked due to compensation of other molecules, such as IL-7.

In contrast to the strict requirement for IL-7-JAK3 signalling in B cell development, T cell development in the absence of *IL-7, IL-7Ra, yc and jak-3* results in more general defects in thymocyte survival. Mice deficient in *IL-7, IL-7Ra, yc* or *jak-3* have dramatically reduced thymic cellularity, but maintain relatively normal CD4 and CD8 expression on thymocytes.[18,19,29-31,33] An exception occurs in the absence *of IL-7Ra,* where individual mice differ in their thymocyte defects. In this strain, some mice exhibit a phenotype similar to *IL-7-, yc-* and *jak-3*-deficient mice, whereas the thymus of other mice consists entirely of DN cells.[18] Additionally, ZL-7-deficient mice have been shown to have a small defect at the transition from DN2 to DN3.[159] Interestingly, c-kit expression on DN1 and DN2 cells was reduced in /*L-7*-deficient mice suggesting that IL-7 may regulate c-kit expression.[159]

The survival effect of IL-7 on thymocytes is mediated by the antiapoptotic protein Bcl2.[160] Bcl2 overexpression in *IL-7Ra*-deficient or *yc*-deficient mice rescues them from T cell developmental defects.[161-163] Interestingly, defects in the B cell compartment are not rescued by Bcl2 overexpression, suggesting different mechanisms by which IL-7 regulates B and T cell development.[163]

To assess possible compensation by IL-7 and SCF, mice were made deficient in both *c-kit* and *yc.*[164] In the absence of *c-kit* and *yc,* thymocytes could not be detected by cell counting nor by flow cytometry.[164] This dramatic phenotype demonstrated that IL-7 (and possibly other cytokines that bind yc) and SCF can functionally compensate in the absence of one another during thymocyte development. Again, in contrast to T cell development, B cell development in *c-kit/yc*

double-deficient mice was no more severe than *yc* deficiency alone.[164] Thus, signals mediated through c-kit-SCF and IL-7 -IL-7R are critical for the survival of early thymocytes prior to the pre-TCR checkpoint.

Checkpoints during T cell development

Pre-TCR checkpoint

Similar to the processes involved at the pre-BCR checkpoint, the pre-TCR checkpoint, also deferred to as '*b* selection', selects cells that have successfully rearranged the first receptor chain. Additionally, the TCR p chain must be expressed on the cell surface in a complex formed with the T cell equivalent of the surrogate light chain and signal transduction molecules, the pre-T*a* (pT*a*) chain and CD3 molecules, respectively. Again, similar to the pre-BCR checkpoint, the pre-TCR checkpoint signals allelic exclusion, proliferative expansion, further differentiation and initiation of the ***TCR a*** chain rearrangement. Thus, to pass the pre-TCR checkpoint, efficient signalling through the pre-TCR complex is required**.**

Rearrangement of the TCR (3 locus In the absence of *TCR b* rearrangement, T cell development arrests at the pre-TCR checkpoint. The inability to initiate rearrangement of the *TCR* loci in ***rag1-*** and rag2-deficient mice results in a lack of peripheral T cells because of a developmental block at the pre-TCR checkpoint as indicated by the predominance of $CD25^+$ DN thymocytes and the absence of DP cells in these mice.[3839] As expected, *scid* mice, like rag-deficient mice, have few peripheral T cells and lack DP thymocytes, because of an inability to complete *TCR p* rearrangement.[43,44,165,166] Moreover, mice deficient in *Ku80* have a complete block at the transition from DN to DP thymocytes, whereas *Ku70*-deficient mice have a partial block at the pre-T cell stage.[46-49]

Rearrangement of the *TCR p* chain is sufficient to pass the pre-TCR checkpoint. By crossing a rearranged *TCR p* transgene onto a rag-deficient background, Mombaerts *et al.* and Shinkai *et al.* demonstrated that the presence of the *TCR p* transgene rescued thymocyte numbers and the development of DP thymocytes in rag-deficient mice.[167168] Moreover, a rearranged *TCR a* transgene in addition to the *TCR p* transgene leads to the development of DP and SP cells in rag-deficient mice.[168] This experiment clearly demonstrates the importance of both the TCR *b* and TCR a chains at the two major selection steps during thymocyte development.

Expression of the pre-TCR complex In the DN3 thymocyte subset, rearranged *TCR b* chains are expressed on the cell surface in a complex with the pTa chain and CD3 proteins.

Mice that are unable to express TCR p chain on the cell surface as a result of the deletion of the *TCR b* loci or deletion of the *TCR p* transcriptional enhancer have a T cell developmental block at the pre-T cell stage.[167,169] Also, mice in which the TCR p chain cannot exit the endoplasmic reticulum, because of the insertion of an endoplasmic reticulum retrieval signal into a rearranged *TCR b* transgene, exhibit developmental defects at the DN-to-DP transition.[170] Thus, the cell surface expression of TCR *b* is critical for the development of pre-T cells.

The TCR b chain is expressed on the cell surface with pTa, the surrogate a chain.[171] The essential role of pT*a* within the pre-TCR complex has been demonstrated by mice deficient in *pTa,* which have a selective deficiency in DN4 cells, while maintaining normal numbers of DN1, DN2 and DN3 cells.[172,173] Therefore, *pTa* is indispensable for thymocyte differentiation beyond the pre-TCR checkpoint.

Association of TCR p and pTa with IT AM containing CD3 proteins is essential for the selection of pre-T cells. The CD3 components of the pre-TCR complex are thought to consist of CD3e-*y* and CD3*e*-5 heterodimers where each of these molecules contain a single ITAM and a *CD3?,* (TCR?) homodimer containing three ITAM.[174]

CD3 molecules are essential, as demonstrated by the phenotype of mice lacking these signalling components. Mice deficient in CD3e completely lack DN4 cells, and mice deficient in CD3£ and CD3y have partial defects in the development of DP and SP cells.[175,180] In contrast, CD35 is dispensable for pre-TCR signalling.[181182] It is interesting to note that the administration of anti-CD3e monoclonal antibody *in vitro* and *in vivo* is sufficient to restore the development of DP cells from *scid*- and rag-deficient thymocytes.[183-185] These studies demonstrate that in the absence of a rearranged P chain, activation of the signalling pathway via the CD3 molecules was sufficient to stimulate the proliferation and differentiation of pre-T cells. Thus, signals mediated by the CD3 proteins are required at the pre-TCR checkpoint.

Signalling through the pre-TCR complex Expression of the pre-TCR complex signals proliferation, allelic exclusion of the *TCR b* locus, and initiation of *TCR a* rearrangement. Mice deficient in several key signalling molecules located downstream of the *ab* TCR exhibit defects in thymocyte differentiation from DN to DP cells, indicating that these molecules are critical for signalling through the pre-TCR complex.

Activation of the Src tyrosine kinases Lck and Fyn is one of the first events following TCR ligation. Mice deficient in *lck* and mice transgenic for the dominant negative (catalytically inactive) form of Lck have a partial block at the pre-T cell stage.[186187] T cell development in mice deficient *in fyn* appears normal, whereas thymocyte development in mice deficient in *lck and fyn* is completely arrested at the DN stage, which is indicative of a redundant role for the Src kinases in signalling through pre-TCR.[188-191] Additionally, overexpression of a gain-of-function *fyn* transgene in *lck*-deficient mice permits the development of DP cells, but not SP cells.[190] Overexpression of a transgene encoding a constitutively active form of Lck in *rag1* -deficient mice normalized DP cell numbers in a manner similar to the effect of the expression of a rearranged *TCR b* chain into rag-deficient hosts.[192] These findings highlight the critical role for the Src kinases in pre-TCR signalling.

The redundancy of the Syk kinases observed during B cell development also occurs during thymocyte differentiation. Mice deficient in either *zap-70* or *Syk* alone have normal numbers of DN3 and DN4 cells, and mice deficient in both kinases have a complete block at the pre-TCR stage.[5960,63,193] Thus, even though Syk acts predominantly during B cell development, it can functionally compensate in the absence of ZAP-70 during pre-T cell signalling.

Adapter proteins LAT and SLP-76 play critical roles downstream of the TCR, because T cell development in mice deficient in *lat* or *slp-76* is completely blocked at the pre-TCR stage.[194-196] Similarly, *lat* knockin mice, which lack the four cytoplasmic tyrosine residues involved in protein-protein interactions following TCR stimulation, also have a thymocyte developmental arrest at the pre-TCR stage.[197] Additionally, mice lacking the small adapter protein GADS, which recruits SLP-76 to LAT, have a partial defect at the pre-TCR checkpoint.[198] Thus, deficiency in certain TCR signalling molecules results in defects at the transition from DN3 to DN4, which highlights the requirement for effective signalling through the pre-TCR complex during T cell development.

ab TCR checkpoint

The critical events at the transition from the DP to SP stages of thymocyte development are: (i) the rearrangement and expression of the TCR **a** chain; followed by (ii) positive and negative selection of cells expressing a mature a(3 TCR prior to export into the periphery of mature T cells that are expressing receptors able to recognize self-MHC, but that lack the capacity to be self-reactive.

Formation of the ab TCR Thymocytes that have successfully rearranged *TCR p* proliferate extensively prior to the rearrangement of *TCR a,* broadening the lymphocyte repertoire by allowing multiple TCR a chains to pair with the same TCR *b* chain**.** *TCR a*-deficient mice have normal production of DP cells, but due to an inability to be positively selected, thymocyte development is blocked at the DP-to-SP cell transition.[167199]

ab TCR signalling Crosslinking of the TCR leads to the rapid activation of Lck and Fyn, which act to phosphorylate tyrosine residues within ITAM of the CD3 molecules (Fig. 3).[200] ZAP-70 binds to ITAM within CD3? via its tandem Src homology 2 (SH2) domains, leading to the activation of ZAP-70 by the phosphorylation of specific tyrosine residues by the Src kinases.[20o-205] Targets of ZAP-70 tyrosine phosphorylation include LAT, PLCyl and SLP-76. The phosphorylation of cytoplasmic residues within the transmembrane adapter protein LAT following TCR crosslinking permits the binding of a number of proteins, including PLCyl, Grb2, GADS and p85a, all via their SH2 domains.[206207] The induced association of LAT with GADS leads to the recruitment of SLP-76, which is constitutively associated with GADS, thereby recruiting SLP-76 and its associated proteins to the LAT complex near the plasma membrane.[208209] The multiprotein complex formed between SLP-76, LAT and associated proteins results in the activation of multiple signalling cascades. VAV, Nck and ADAP are involved in the reorganization of the cytoskeleton and actin polymerization.[210-212] Additionally, VAV is involved in the activation of the MAPK pathway.[213] Phosphorylation and activation of PLCyl cleaves PIP2, yielding IP3, which in turn increases cytoplasmic calcium and NFAT activation, while production of DAG leads to PKC activation and activation of the Ras pathway.

The strength of the signal transmitted through the TCR is thought to mediate selection events, thus perturbations in the TCR signalling pathway lead to defects in selection. For example, CD3*d* is not required at the pre-TCR checkpoint, but is required at the a(3 TCR checkpoint, because mice deficient in *CD3d* exhibit a block in differentiation at the DP-to-SP transition.[181,182] Mice deficient in *zap-70* have normal numbers of DP thymocytes, but completely lack CD4 and CD8 SP cells and therefore lack peripheral ap T cells.[63,64,214] Furthermore, mice deficient in a number of key TCR signalling molecules have blocks in T cell development at the pre-TCR stage, which precludes analysis of ap TCR selection, although they are also likely to be critical for ap TCR signalling.

Selection of the ab TCR The DP thymocytes that express rearranged *a* and *b* chains are selected by the recognition of self-peptide in the context of MHC, thus reflecting an antigen receptor that may be useful in binding foreign peptide-MHC complexes in the periphery.

It is generally considered that there are three main outcomes for thymocytes undergoing selection. First, thymocytes unable to bind self-MHC undergo death by neglect. Second, recognition of self-peptides in the context of MHC through weak binding of receptor and peptide-MHC complexes rescues cells from death and they are positively selected to differentiate into SP cells. Thymocytes recognizing MHC class I differentiate into CD8 SP cells, whereas DP cells able to recognize MHC class II molecules differentiate into CD4 SP thymocytes. Third, negative selection of cells that bind strongly to self-MHC complexes prevents the differentiation and export of potentially autoreactive cells into the periphery. It has been estimated that fewer than 5% of thymocytes pass *ab* TCR selection.[215]

Factors required for T cell homeostasis

Comparable to the requirements for BCR and BAFF in the maintenance of the mature B cell compartment, peripheral T cells require survival signals mediated through the antigen receptor and cytokines.

TCR-MHC interactions Studies in M//C-deficient mice have demonstrated the dependence of naive CD4 and CD8 T cell populations on TCR-MHC interactions for survival.[216-219] Furthermore, the conditional deletion of *TCR a* in mature T cells resulted in the depletion of naive CD4 and CD8 T cells with a half-life of 16 and 46 days, respectively, in one study compared with the 27 and 19 days, respectively, found in another study.[220221] By contrast, memory CD4 and CD8 T cells are less dependent on TCR-MHC interactions for survival.[219,220,222,223]

Cytokines Naive CD4 and CD8 T cells require IL-7 for survival and CD8 memory T cells depend on IL-15 for survival.[224-233] However, the survival factors for memory CD4 T cells are less well defined. The homeostasis of memory CD4 T cells is unaffected by the absence of *yc, IL-7* or *IL-*y 5 233235 However, a recent report suggests that memory CD4 T cells have a combined requirement for TCR-MHC interactions and IL-7.[236] Conditional deletion of *lck* on a *fyn*-deficient background, resulting in the absence of TCR signalling, did not affect the memory CD4 T cell pool.[236] However, when purified *Ic k/fy n-deficient* memory CD4 T cells were transferred into *rag/IL-7* double-deficient mice, the memory

CD4 cells could not proliferate nor survive.[236] These results are consistent with previous studies whereby a deficiency in either TCR-MHC interactions or IL-7-*y*c alone did not alter the number of memory CD4 T cells, whereas the study discussed here demonstrates that in the absence of both TCR-MHC and IL-7 signals, memory CD4 T cells cannot survive.

Concluding remarks

There has been remarkable progress in the last few years in identifying paired pathways for lymphocyte homeostasis. There is an interesting symmetry between B and T cells both using the IL-7-JAK3 pathway in primary lymphoid organs in concert with the highly analogous BCR and TCR signalling pathways. The fact that most of the signalling components in the TCR pathway are related to the components of the BCR pathway, but they are not shared, may reflect fundamental differences in the sensitivity and regulation of antigen recognition by these two kinds of antigen receptor. On the other hand, it is striking that B and T cells diverge in their survival requirements in the periphery: T cells continue to use the IL-7-JAK3 pathways, whereas B cells use the BAFF-BAFF-R - N F K B 2 pathway. Presumably this dissociation is necessary for independent homeostasis of B cells and T cells.

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