Natural and Engineered Disorders of Lymphocyte Development

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Mammals have evolved complex developmental pathways to generate a large repertoire of B and T lymphocytes capable of mounting effective immune responses. Analysis of natural and engineered immunodeficiencies constitutes a powerful approach to delineating these pathways and identifying the molecular sensors that couple the survival of developing lymphocytes to the achievement of successful gene rearrangements at the loci coding for B and T cell antigen receptors. Besides identifying cytokines, growth factors, and transcription factors involved in lymphocyte development, genetic analysis also makes it possible to organize most of these protagonists into gene networks that control critical events in the life of developing lymphocytes.

The past decade has witnessed the identification of the molecular bases of many primary immunodeficiencies. In a few cases, immunodeficiencies were found to result from mutations in hitherto unknown genes (*1*). In other instances, a direct relation was established between a given immunodeficiency and a known but functionally elusive gene. Thus, analysis of patients deficient in the ligand for the lymphocyte surface protein CD40 (CD40L) revealed the role played by the CD40L-CD40 interaction during immunoglobulin (Ig) isotype switching (*2*).

However, deciphering the immunoregulatory role of altered gene products is not always straightforward. The finding that the WAS protein is absent or abnormal in patients with Wiskott-Aldrich syndrome has only provided hints as to its possible functions (*3*). The genetic analysis of human immunodeficiencies has revealed that the equation one disease = one gene product = one function is overly simple. A given immunodeficiency phenotype can be the result of distinct genetic defects of the same immunoregulatory pathway (*4*). Mutation of a single gene can be associated with a spectrum of phenotypes, as illustrated by the diverse consequences of mutations in the gene coding for the Bruton's tyrosine kinase (Btk) (*5*).

Understanding of the molecular bases of human immunodeficiencies has also greatly benefited from comparative studies with mice in which known genes have been inactivated by homologous recombination. The existence of almost identical phenotypes in immunodeficient patients and engineered mouse mutants also helped to rule out the involvement of particular gene products in certain human diseases (*6*, *7*). In this article, we review the natural and engineered mutations that perturb normal T and B cell development in primary lymphoid organs. Genetic defects that affect peripheral lymphocyte homeostasis are reviewed in a companion article (*8*).

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Genetic Disorders of T Lymphocyte Development

Intrathymic T cell development proceeds through discrete stages defined on the basis of both the configuration of T cell antigen receptor (TCR) gene loci and the expression of surface markers. Figure 1 summarizes the events in the life of developing T cells and links them with certain disorders of T cell development. Our understanding of such events would still be rudimentary without the insights provided by engineered mouse mutant strains and human primary immunodeficiencies that specifically affect T cell development.

Mutations affecting cell-fate decisions. Analysis of gene deficiencies has provided insights into the factors that commit precursor cells to a given lymphoid lineage and potential precursor-progeny relations between cell subsets. For instance, mice homozygous for a transdominant negative mutation in *Ikaros*, a gene coding for a zinc finger DNA-binding protein, lack all cells of lymphoid origin but still produce cells of myeloid and erythroid lineages (*9*). These observations suggest either the existence of a common lymphoid progenitor (*10*) or the sharing by T, B, natural killer (NK), and lymphoid dendritic cells (DC) of *Ikaros* gene family products. Similarly, an uncommon form of human severe combined immunodeficiency (SCID) called reticular dysgenesis affects both lymphoid and myeloid development and suggests the existence of a gene product shared by these lineages or required for the commitment step into lymphoid and myeloid lineages (*11*). Despite these revealing observations, the molecular events establishing lineage- and subset-associated gene expression patterns are still largely unknown (*12*). For instance, the recent observations that *Ikaros* isoforms are capable of functioning as negative regulators of transcription and are already expressed in hematopoietic stem cells are not consistent with early models in which *Ikaros* was thought to be a master regulator gene responsible for specifying lymphoid commitment (*13*).

Mutations affecting stromal environment. The thymic stromal microenvironment comprising epithelial cells, mesenchymal cells, macrophages, and dendritic cells provides developing T cells with essential extracellular matrix components, cell surface ligands, and secreted cytokines. Mutations in the transcription factors winged-helix nude (*14*) and RelB (*15*) affect stromal development and have indirect but important effects on T cell development. Furthermore, thymuses from mice deficient in the Hoxa-3 transcription factor (*16*) have a mesenchymal defect that affects thymus development in a way that is reminiscent of patients with DiGeorge syndrome with hemizygous gene deletions on the long arm of chromosome 22 (*17*).

Mutations affecting cytokine-mediated signaling. The intense phase of cell proliferation before the $CD44^{-/low}CD25^{+}$ triple-negative (TN) stage (Fig. 1) presumably serves to generate a pool of cells large enough for the constitution of a repertoire of diverse TCRβ chains. In humans, the molecular basis of the most frequent form of SCID (X-linked SCID or SCID-X1) highlights the role played by some cytokines in promoting survival and proliferation of early T cells before TCR gene rearrangements. SCID-X1 is characterized by the absence of mature T and NK lymphocytes (*18*). This defect is intrinsic to the hematopoietic lineage, because stem cell transplantation cures the disease (*18*). Although not precisely defined, the blockade in T cell development occurs very early and patients' thymuses are

almost completely devoid of T cells (*18*). Moreover, in hybrid fetal thymic organ culture, SCID-X1 $CD34^+$ cells do not differentiate beyond the $CD34^+CD4^{low}CD8^-CD4^-CD3^-CD1^-$ immature thymocyte stage (*19*). The SCID-X1 locus maps to Xq12-13.1 and encodes the common cytokine receptor γ chain, γc, which is shared by the receptors for interleukin-2 (IL-2), IL-4, IL-7, IL-9, and IL-15 (*18*, *20*, *21*). The γc mutations result in either the absence of the γc subunit or the expression of γc polypeptides that cannot associate with the Jak-3 protein tyrosine kinase (PTK) and thus do not transduce growth signals (*18*). Mutations in Jak-3 cause a phenotype indistinguishable from patients with SCID-X1 (*20*) and underline the functional relations between γc and Jak-3.

The defect in γc or Jak-3 appears more severe in humans than in mice. Mice deficient in γc have an incomplete intrathymic block that is similar to mice deficient in IL-7 or in the α chain of the IL-7 receptor (IL-7R α) (*22*). Thus, other γc-dependent cytokines cannot compensate for IL-7 at this early stage of T cell development in the mouse. The analyses of mice deficient in signaling through c-kit or through the stem cell factor receptor or IL-7R (or both) further highlighted the partially overlapping role played by these two receptors in the developmental sequence that precedes TCRβ rearrangement (Fig. 1). The thymuses of mice deficient in both c-kit and γc are almost alymphoid, whereas in the absence of either of these receptors, thymuses are hypocellular and display a rather normal distribution of CD4-CD8 subsets (*21*–*23*). A null mutation in GATA-3 (*24*) affects T cell development in a fashion similar to

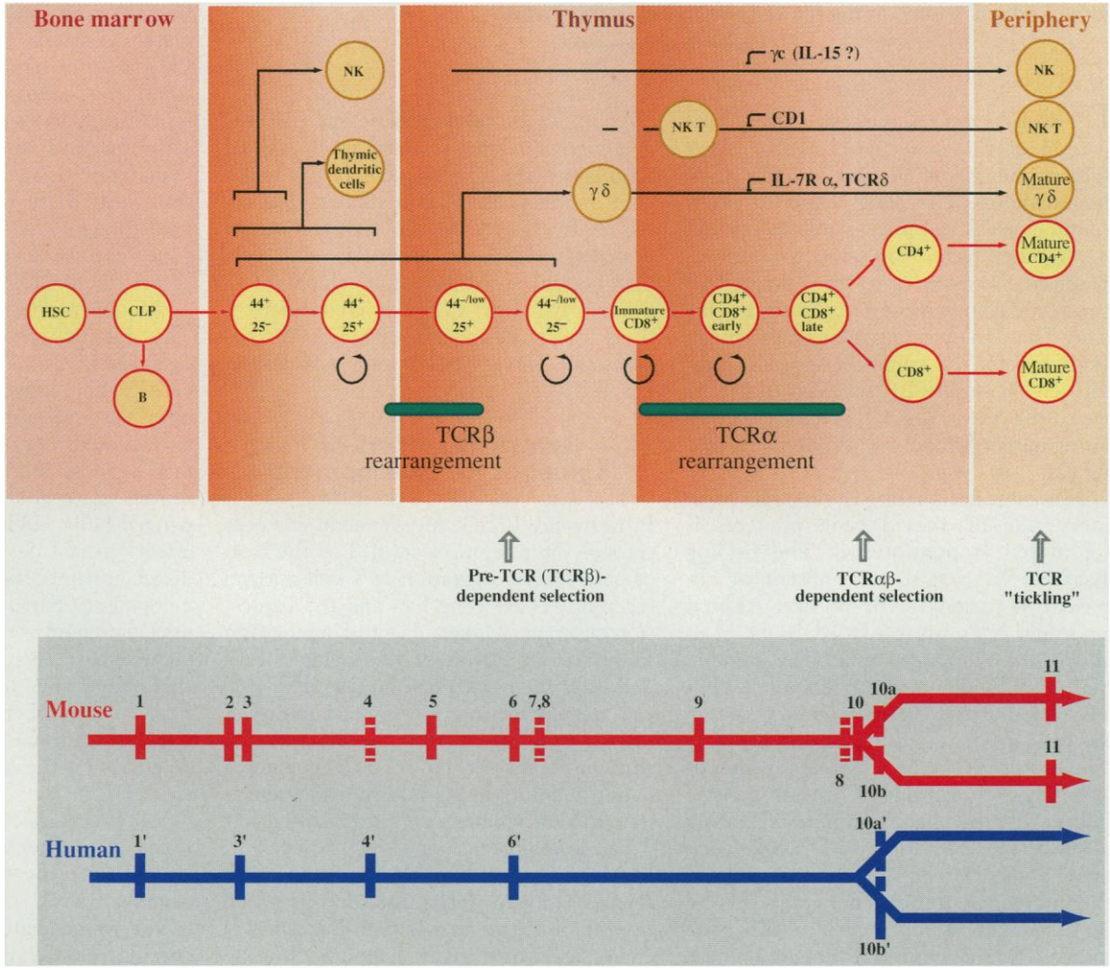

Fig. 1. Genetic dissection of the T lymphocyte developmental pathway. (**Top**) In adult mice, bone marrow contains hematopoietic stem cells (HSC), which can give rise to all lymphoid populations through common lymphoid progenitors (CLP). Upon thymus colonization, these progenitors develop into cells that express low CD4 ($CD4^{low}$). Next, these $CD4^{low}$ precursors lose CD4 expression to become TN ($CD4^-CD8^-CD3^-$) cells. On the basis of the expression of CD25 and CD44, mouse TN cells have been subdivided in three different subsets. Late TN cells can mature into $CD4^+CD8^+$ (DP) cells, some of which develop into $CD4^+CD8^-$ or $CD4^-CD8^+$ (SP) cells that exit from the thymus. During intrathymic differentiation, the genes encoding the TCR variable region are assembled by site-specific DNA recombination reactions. TCRβ gene rearrangements start around the transition to the $CD44^{-/low}$ $CD25^+$ TN stage, whereas the first TCRα rearrangements are measurable close to the transition to the DP stage. The $CD4^{low}$ precursors can also produce NK cells and thymic DCs. In contrast, cells belonging to the next developmental stage ($CD44^+CD25^+$) cannot generate NK cells but still give rise to thymic DCs. Commitment to the T cell lineage occurs at the next TN stage ($CD44^{-/low}CD25^+$), coincident with the onset of TCRβ, TCRγ, and TCRδ gene rearrangements. Finally, the irreversible decision to become an αβ rather than a γδ T cell may not take place until both TCRα loci have rearranged and concurrently excised the TCRδ loci. The phases during which thymocytes are undergoing cell division are highlighted by curved arrows. The developmental stages shown correspond to those found in the mouse. 44, CD44; 25, CD25. (**Bottom**) The extent of αβ T cell development in thymus of mice (red time line) or humans (blue time line) (*85*) deficient for a few selected genes is shown. Stringent developmental blocks are depicted as continuous bars, whereas leaky mutations are depicted as dashed bars. The bars interrupting the mouse αβ T cell development time line correspond to mutations in the following: 1, *Ikaros*; 2, c-kit + γ_c and GATA-3; 3, winged-helix nude; 4, IL-7, IL-7Rα, γ_c, JAK-3, and c-kit; 5, γ_c + pTα; 6, RAG-1, RAG-2, SCID (DNA-PK catalytic subunit), Ku80, TCRβ enhancer, CD3-$\varepsilon^{\Delta 5}$ + CD3-ζ/η, TCRβ + TCRδ, Lck + Fyn, and ZAP-70 + Syk; 7, pTα; 8, CD3-ζ/η, Vav, Lck, CD45, and TCF-1 + LEF-1; 9, TCF-1 + LEF-1; 10 (affecting both $CD4^+$ and $CD8^+$ T cells), TCRα enhancer, CD3δ, and ZAP-70; 10a (affecting only $CD4^+$ T cells), MHC class II, CD4, and H-2M; 10b (affecting only $CD8^+$ T cells), MHC class I, β2m, TAP-1, and CD8; and 11, LKLF. The bars interrupting the human αβ T cell development time line correspond to mutations in the following: 1′, reticular dysgenesis; 3′, Di George syndrome; 4′, SCID-X1 (γ_c) and SCID JAK3; 6′, RAG-1, RAG-2, and other SCID; 10a′, MHC class II; and 10b′, ZAP-70 and TAP-2.

that in mice made doubly deficient for c-kit and γc. Apart from its acting as a survival or proliferation factor before and during TCRβ rearrangement, no evidence suggests that IL-7 triggers the TCRβ recombination process (*25*). B cell development proceeds normally in SCID-X1– and Jak-3–deficient patients (*18*), but mice made deficient in γc have severely reduced numbers of B cells that disappear with age (*21*).

Mutations affecting the pre-TCR and TCR sensors. Developing T cells that do not rearrange their TCR genes, rearrange them nonproductively, or express TCRαβ combinations with inappropriate specificities are arrested at discrete developmental control points. Molecular sensors have evolved to couple the transition through these control points to the attainment of productive rearrangements at the TCRα and TCRβ loci. One of these sensors, known as the pre-TCR complex, controls the transition from the TN to the double-positive (DP) stage, whereas another is made of the αβ TCR and controls the transition from the DP to the single-positive (SP) stage (Fig. 2A). Several classes of mutations (6 to 10b, Fig. 1) have helped to resolve the structure and function of these two sensors.

Pre-TCR. Mutations preventing TCRβ gene rearrangements (*26*) or proper intracellular distribution of TCRβ polypeptides (*27*) have emphasized the unique role the TCRβ chain plays in the pre-TCR, irrespective of TCRβ's later function as a component of the αβ TCR. For instance, mutations in genes encoding the lymphoid-specific components required for effecting the double strand breaks (DSB) associated with the initiation of V(D)J recombination (that is, the recombination-activating genes *RAG-1* and *RAG-2*) or in some general DSB repair factors [for example, the catalytic and Ku80 subunits of DNA-dependent protein kinase (DNA-PK)] impair the completion of the V(D)J recombination reactions and prevent T cell development beyond the $CD44^{-/low}$-$CD25^{+}$ stage (*28*). Some patients have a phenotype similar to that of mice deprived of functional DNA-PK (SCID mice) (*6*, *7*). However, in contrast to SCID mice, all patients express functional DNA-PK, which points to the probable existence of unidentified effectors of the V(D)J recombination or DSB repair processes (or both) (*7*).

The different phenotypes observed for null mutations in either the TCRβ or TCRα loci (*26*, *29*, *30*) suggest that productive TCRβ rearrangement is a rate-limiting step in the progression beyond the $CD44^{-/low}$ $CD25^{+}$ TN stage. Identification of a substitute for TCRα, denoted as the pre-TCRα (pTα) chain, resulted in the hypothesis that newly formed TCRβ chains must associate with pTα to form a functional pre-TCR complex that then triggers the transition to the DP stage (*31*). This hypothesis was directly tested by generation of mice lacking a functional pTα gene (*32*). Adult $pT\alpha^{-/-}$ mice lacked most DP thymocytes. The small numbers of mature αβ T cells still found in $pT\alpha^{-/-}$ mice probably result from the few constitutive TCRα rearrangements in $CD44^{-/low}CD25^{+}$ TN cells before pre-TCR assembly (*33*). Likewise, in mice lacking the λ5 subunit of the pre-B cell receptor (pre-BCR), premature Ig light (IgL) chain gene rearrangements may account for the few mature B cells present (*34*).

The pTα-TCRβ heterodimers associate with CD3-γ, CD3-ε, CD3-ζ, and CD3-δ (*35*). These CD3 components are likely to contribute to both the efficient assembly of the pre-TCR complex and its signaling function. Studies of mice with a targeted mutation of the CD3-ε gene (referred to as $CD3\text{-}\varepsilon^{\Delta 5}$; Fig. 1) have shown that some CD3 components are essential for the assembly or function of the pre-TCR. By simultaneously eliminating the CD3-γ, -δ, and -ε subunits, the $CD3\text{-}\varepsilon^{\Delta 5}$ mutation totally prevented the progression beyond the $CD44^{-/low}CD25^{+}$ stage (*36*). In contrast, disruption of the CD3-ζ/η gene incompletely blocked the DN to DP transition (*37*). DP cells found in $CD3\text{-}\zeta/\eta^{-/-}$ mice can be distinguished from bona fide wild-type DP cells because they may contain more than one productive TCRβ chain gene rearrangement and limited TCRα rearrangement, they have reduced sensitivity to dexamethasone-induced apoptosis, and some still express CD25. This split pattern of phenotypic changes suggests that the role of the CD3-ζ/η module is to strengthen signals elicited by the pre-TCR complex and allow them to reach the higher activation thresholds plausibly required by some demanding cellular responses associated with progression beyond the $CD44^{-/low}$ $CD25^{+}$ stage (*38*). Moreover, when considered together with the phenotypes associated with knockout of various transcription factors [for example, Ets-1 (*39*), TCF-1 + LEF-1 (*40*), and Egr-1 family members (*41*)], the phenotype of $CD3\text{-}\zeta/\eta^{-/-}$ DP cells suggests that survival, proliferation, and differentiation of pre-TCR selected cells mobilize com-

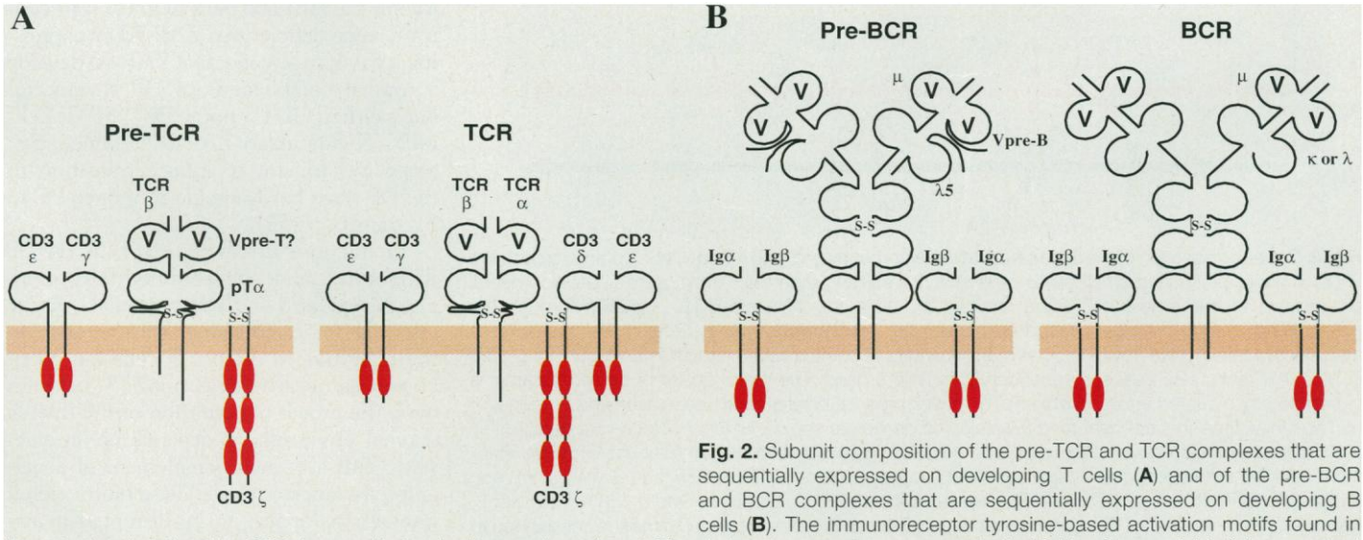

Fig. 2. Subunit composition of the pre-TCR and TCR complexes that are sequentially expressed on developing T cells (**A**) and of the pre-BCR and BCR complexes that are sequentially expressed on developing B cells (**B**). The immunoreceptor tyrosine-based activation motifs found in the cytoplasmic tails of the CD3, Igα, and Igβ subunits are shown in red. The circles show sequence segments that fold as for a variable (V) or constant immunoglobulin domain or are predicted to do so. S–S: disulphide bond.

plex gene cassettes. Despite being part of the pre-TCR complex (*35*), the CD3-δ subunit appears dispensable for pre-TCR function, because CD3-δ–deficient mice have normal numbers of DP cells (*42*).

Analyses of mice deficient in cytoplasmic PTKs of the Src and Syk families have revealed their critical and redundant role in early T cell development. $Lck^{-/-}$ mice have a partial inhibition of T cell development (*43*) reminiscent of that observed in CD3-$\zeta/\eta^{-/-}$ mice, and the deletion of Fyn had no measurable effect on T cell development (*44*). However, combined disruption of Fyn and Lck totally prevented development beyond the $CD44^{-/low}CD25^{+}$ stage (*45*). Mice deficient in Syk showed no major abnormalities in αβ T cell development (*46*), whereas both human patients and mice deficient in ZAP-70 had salient defects that become manifest only at the DP to SP transition (*47*). Mice lacking both Syk and ZAP-70 are completely blocked in the transition from the $CD44^{-/low}CD25^{+}$ stage (*48*). A few complexes consisting of calnexin and the CD3-γ, -δ, and -ε subunits are expressed at the surface of TN thymocytes. It is unlikely, however, that such partial complexes have a normal signaling function, because mice lacking most CD3 subunits (CD3-$\varepsilon^{\Delta 5/\Delta 5}$ + CD3-$\zeta/\eta^{-/-}$ genotype) or with a combined disruption of Lck + Fyn or ZAP-70 + Syk produce immature T cells that can reach the $CD44^{-/low}CD25^{+}$ stage and faithfully initiate TCRβ rearrangement (*38*, *45*, *48*). Thus, the CD3 subunits and the Lck + Fyn and ZAP-70 + Syk PTK pairs become mandatory only when the pre-TCR is expected to operate. Accordingly, it is tempting to arrange mutations belonging to class 6 (Fig. 1) in the following linear (but oversimplistic) cascade: V(D)J recombination machinery → TCRβ polypeptides → TCRβ-pTα/CD3 assembly → Lck-Fyn activation → ZAP70-Syk activation → phenotypic changes associated with transition beyond the $CD44^{-/low}CD25^{+}$ stage. These interactions have not yet been validated by the establishment of interaction suppressors or enhancers. It is not yet clear whether the pre-TCR needs to engage with a ligand to exert its signaling function. However, the presence of the Vβ domain within the TCRβ polypeptide or expression of the class I and class II molecules encoded by the major histocompatibility complex (MHC) is dispensable for proper pre-TCR function (*49*).

TCR. After productive TCRα gene rearrangements, DP cells are rescued from programmed cell death and induced to differentiate into SP cells only if their αβ TCRs bind with low affinity to self–peptide-MHC complexes expressed on thymic stromal cells. Such a phenotypic shift, often denoted as TCRαβ selection, is ablated by a host of genetic defects (classes 10, 10a, and 10b; Fig. 1) affecting (i) the assembly and proper cellular display of peptide-MHC complexes [from mutations in β_2-microglobulin (*50*), the MHC class I heavy chain (*51*), subunits of the TAP peptide transporter (*52*), transcription factors regulating MHC class II chain genes (*4*), and H-2M (*53*)]; (ii) the synthesis of TCRα chains [mutations in the TCRα locus (*29*, *30*) and TCRα gene enhancer (*54*)]; (iii) the transducing CD3 subunits and intracellular effectors associated with the αβ TCR (below); and (iv) the coreceptors CD4 and CD8 (*55*). The CD3-ζ/η (*37*), Lck (*43*), Vav (*56*), or CD45 (*57*) gene defects each correspond to "leaky" mutations of the pre-TCR sensor. Because of this characteristic, it has been possible to score their effect on the αβ TCR sensor and confirm that the pre-TCR and TCR share many functional components. Unexpectedly, CD3-δ is required for TCRαβ selection but is dispensable for the development of functional γδ T cells (*42*). Thus, the pre-TCR and γδ TCR contrast notably with the αβ TCR in that they appear to be able to tolerate loss of CD3-δ. This may reflect a lower signaling threshold for pre-TCR selection than for TCRαβ selection (*35*).

In humans deficient in ZAP-70, $CD4^{+}$, but not $CD8^{+}$, SP cells are found in the thymic medulla and periphery (*47*). In contrast, mice deficient in ZAP-70 or expressing a catalytically inactive ZAP-70 develop a normal complement of DP thymocytes but neither $CD4^{+}$ nor $CD8^{+}$ SP T cells (*47*). Therefore, in ZAP-$70^{-/-}$ mice, Syk alone is sufficient to induce transition to the DP stage but is unable to induce DP to SP transition (*58*).

Interactions involving αβ TCR on DP thymocytes and self–peptide-MHC complexes expressed on thymic stromal cells are essential for establishing self-tolerance in the repertoire of mature T cells (an event known as negative selection) (*31*). Moreover, the proper organization of the thymic stromal environment depends on interactions with a normal complement of developing thymocytes (*59*). These bidirectional interactions appear to be disrupted in patients with Omenn's syndrome, a clinical condition characterized by the presence of a

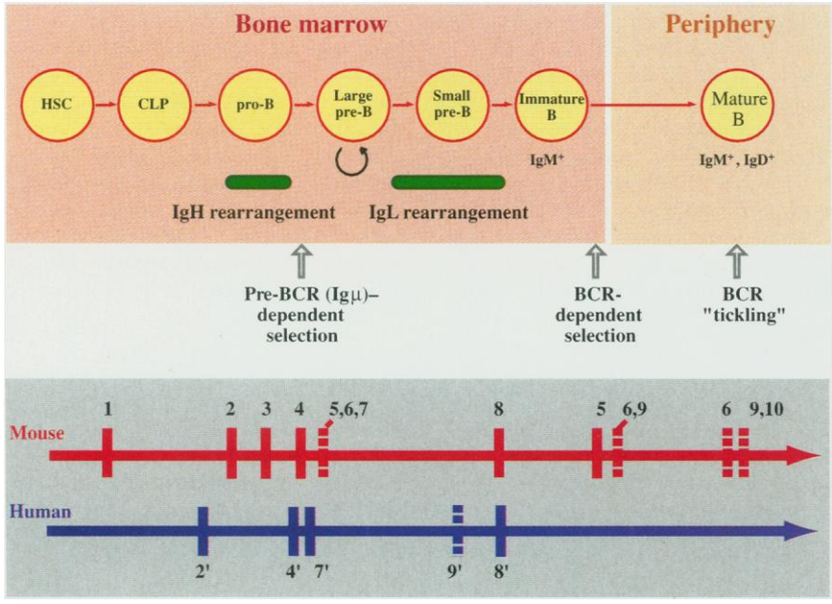

Fig. 3. Genetic dissection of the B lymphocyte developmental pathway. (**Top**) Upon commitment to the B cell lineage, pro-B and small pre-B cells start to rearrange their IgH chain genes. Developmental progression to the large pre-B stage requires the assembly of a pre-BCR. Signaling through the pre-BCR induces IgL chain gene rearrangements. After the expression of a BCR, a few immature B cells are selected to emigrate from the bone marrow and continue to mature into IgM^{+} and IgD^{+} long-lived B cells that recirculate between secondary lymphoid organs. The major phase of cell proliferation is highlighted by a curved arrow. (**Bottom**) The developmental blocks observed in mice (red time line) or humans (blue time line) deficient for a few selected genes are shown. Stringent blocks are depicted as continuous bars and leaky mutations as dashed bars. The bars interrupting the mouse B cell development time line correspond to mutations in the following: 1, *Ikaros*; 2, E2A, EBF, and Sox-4; 3, Igβ and Pax5; 4, RAG-1, RAG-2, SCID (DNA-PK catalytic subunit), Ku80, and μ; 5, Syk; 6, $Igβ^{\Delta c/\Delta c}$; 7, λ5; 8, Kappa; 9, Btk; and 10, Lyn. The bars interrupting the human B cell development time line correspond to mutations in the following: 2′, autosomal recessive agammaglobulinemia; 4′, SCID RAG-1 and RAG-2, μ, and other SCID; 7′, λ5; 8′, Ig 107; and 9′, Btk.

very small number of T cell clones that exhibit overt signs of activation and predominantly infiltrate the skin and the gut (60). Omenn's syndrome was found in one patient with a leaky RAG-2 deficiency (18), whereas in other patients its molecular basis is still unknown. Given the extreme disorganization of the thymic microenvironment found in these patients, negative selection may have been inoperative, and a few residual T cell clones may have expanded in the periphery because of their autoreactive character.

Mutations affecting the life-span of naïve lymphocytes. It was thought that after emigration to the periphery, mature T cells circulate through the blood and peripheral lymphoid organs and wait for their antigen in a quiescent stage. However, elegant experiments relying on mice deficient in MHC molecules (51, 61) and ablation of the genes coding for transcription factors LKLF and Ets-1 (39, 62) have shown that maintenance of a pool of "resting" immunocompetent T cells requires continual stimulation of their TCR by the same MHC alleles involved in intrathymic TCRαβ selection. These signals, which may mobilize the LKLF and Ets-1 factors, are subtle in that they do not result in the induction of activation markers and entry into the cell cycle. The B cell antigen receptor (BCR) also plays an essential role in the persistence of mature peripheral B cells, as shown with Cre-loxP–mediated inducible gene targeting (63).

Genetic Disorders of B Lymphocyte Development

The proper development of B and T cells relies on analogous molecular sensors, the stepwise assembly of which depends on the outcome of V(D)J recombination reactions (Figs. 1 and 3). In the case of B cells, sequential assembly of the pre-BCR and BCR (Fig. 2B) depends on productive rearrangements at the Ig heavy (IgH) and IgL chain loci, respectively. A fascinating filtering process, based on the specificity of the BCR antigen-binding unit, has evolved to select only those Ig^+ B cells that will be most useful in mounting responses to antigens in the periphery (64). Some rare patients show a blockade at an early stage of B cell differentiation, before the onset of IgH rearrangements. This phenotype resembles that found in mice deficient in the transcription factors E2A, EBF, Pax-5, and Sox-4 but is not due to a defect in the corresponding human genes (65). The pre-BCR is critical during early B cell development, as shown by the creation of mice deficient in either a component of the pre-BCR complex [the membrane form of the Igμ heavy chain (66) or the λ5 subunit of the surrogate light chain (67)] or one of its proximal cytoplasmic effectors [for example, Syk (46)]. In humans, an almost complete block in the transition between the pro-B and pre-B cell stage was documented in a child with mutations on both alleles of a gene that codes for the human equivalent of the mouse λ5 surrogate light chain (68). For unknown reasons, the defect in that λ5-like gene resulted in a more severe phenotype than that seen in mice deficient in λ5 (34).

The pre-BCR triggers selective maturation of IgH^+ pre-B cells through its Igα- and Igβ-transducing subunits. Igβ-deficient mice are completely blocked in B cell development at a stage corresponding to the $CD44^{-/low}CD25^+$ stage of T cell development (69). V_H to D_HJ_H rearrangements were found to be markedly reduced in Igβ-deficient mice, whereas D_H to J_H rearrangements proceeded normally. Thus, although the CD3 components are dispensable for the onset of V_β to $D_\beta J_\beta$ recombination (38), Igβ appears to initiate V_H to D_HJ_H recombination independently of and before its function as a component of the pre-BCR. A mouse mutant that lacked most of the Igα cytoplasmic tail ($Ig\alpha^{\Delta C}$) exhibited only a limited impairment in early B cell development but was blocked in the establishment of the peripheral B cell pool (70). The few B cells found in the periphery of $Ig\alpha^{\Delta C/\Delta C}$ mice were capable of responding to T-independent, but not T-dependent, antigens. These features resemble the Xid mouse that has mutations in the Btk, a member of the TEC cytoplasmic PTK family (71). Inactivation of the gene encoding the Btk in humans corresponds to the clinical condition known as X-linked agammaglobulinemia (XLA) and results in a phenotype much more severe than that in mice (5, 71). Within human kindreds, the very same Btk mutation was associated either with the absence of mature B cells and agammaglobulinemia or with the presence of mature B cells and hypogammaglobulinemia (5). Thus, other factors (for instance, modifier genes) (72) may account for the phenotypic variability observed in human XLA.

Redundancy and Adaptive Response to Certain Mutations

Genetic redundancy has often been invoked as an explanation when a null mutation in a gene results in no detectable phenotypic abnormalities or less severe abnormalities than expected. In some cases, functional redundancy constitutes a sound explanation (73), but an alternative possibility is that the gene performs a nonredundant function that can be unraveled only under challenging experimental or environmental conditions (74).

Adaptive mechanisms can also be set in motion to compensate for a missing gene product and to buffer the severity of the mutation. Such compensatory mechanisms occur in multigene families, the members of which display overlapping expression patterns [for example, Src family kinases (75) and BCL-2 family members (76)]. Because of the epigenetic mechanisms that shape the repertoire of antigen receptors expressed on mature B and T cells, lymphoid development is particularly prone to compensatory mechanisms. For instance, the density and output of the αβ TCR–CD3 complexes expressed by DP thymocytes contribute to setting the threshold for TCRαβ selection. However, it is possible to artificially reset TCR selection windows by engineering mutations that either reduce (56, 77, 78) or increase (79) TCR-transmitted signals. When these mutations were bred into mice capable of generating a complete repertoire of αβ TCRs, T cell development was unabated, and selection of αβ TCRs with lower or higher affinity for self-MHC was thought to compensate for those TCR-CD3 complexes with a boosted or impeded signaling capacity, respectively. Consistent with this view, the effect of most of these mutations was revealed when they were bred into mice in which αβ TCR variability was neutralized by expressing matched pairs of TCRα and TCRβ transgenes that had been calibrated in a normal thymus. Similar conclusions can be readily applied to B cell development (80) and may have practical relevance. For instance, peripheral blood lymphocytes have been considered as primary targets for gene therapy of inherited immune disorders. It has been suggested that transduction of a gene encoding CD3-ε into the peripheral blood lymphocytes of an immunodeficient patient lacking CD3-ε chains should rescue normal surface expression of TCR-CD3 complexes (81). However, for such mutant T cells that have already undergone a phase of internal calibration in primary lymphoid organs, gene therapy trials aiming at correcting defects in T cell recognition or activation events may result in the production of cells reactive with self-MHC products (78).

Selective events at play during B and T cell development can also favor the survival and expansion of rare precursor cells with spontaneous somatic reversion of an inherited mutation. For instance, study of a SCID-X1 patient showed that a reverse mu-

tation in the gene encoding γc restored an oligoclonal repertoire of αβ T cells that were γc^+ and lacked the Cys → Arg substitution at position 115 (*82*). NK cells were undetectable, whereas all other cell lineages, including B cells and myeloid cells, exhibited the γc gene mutation. Considering that the mother was a carrier for this mutation and that there was no evidence for mosaicism in the patient, a reverse mutation occurring beyond the NK-T branching point (Fig. 1) and conferring selective advantage on T cell precursors is likely to account for this phenotype. This suggests that T cell precursors are endowed with high proliferative capacity before TCR gene rearrangements and supports the feasibility of gene transfer as a treatment of SCID-X1 (*83*).

Conclusions

In the mouse, the analysis of the in vivo consequences of constitutive gene inactivation has been most rewarding. However, this methodology has clear limitations. For instance, when a gene defect results in embryonic lethality or prevents the development of a given lineage, any late potential impacts will be missed. This problem might be, at least in part, solved by the use of conditional gene inactivation techniques (*63*). Other limitations stem from the fact that experimental gene inactivation studies are usually restricted to known genes and that the choice of the gene to be inactivated often depends on preconceived ideas. Alternatively, genetic analysis of natural models of immune disorders has been, and still is, useful for basic studies, medical diagnosis, and therapeutic perspectives. Its limitations are nevertheless obvious, because most models are human and thus subject to a number of restrictive constraints (ethical constraints, rarity of patients, and complexity of genetic background). To identify novel gene products involved in the regulatory pathways governing the immune system, one might therefore need to develop saturation mutagenesis screens or insertional mutagenesis approaches (*84*). By targeting immune systems in zebrafish or mice, these methodologies are likely to provide new models of immunodeficiencies and consequently new insights into the immune system developmental pathways.

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Homeostasis and Self-Tolerance in the Immune System: Turning Lymphocytes off

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The immune system responds in a regulated fashion to microbes and eliminates them, but it does not respond to self-antigens. Several regulatory mechanisms function to terminate responses to foreign antigens, returning the immune system to a basal state after the antigen has been cleared, and to maintain unresponsiveness, or tolerance, to self-antigens. Here, recent advances in understanding of the molecular bases and physiologic roles of the mechanisms of immune homeostasis are examined.

The immune system has a remarkable capacity to maintain a state of equilibrium even as it responds to a diverse array of microbes and despite its constant exposure to self-antigens. Immunologists have focused largely on defining the stimuli that induce the growth, differentiation, and effector functions of lymphocytes, and over the last two decades, the essential features of lymphocyte activation and immune responses have been defined in considerable detail. Less is known about the mechanisms that terminate immune responses. These mechanisms are important in two main situations. First, after a productive response to a foreign antigen, the immune system is returned to a state of rest, so that the numbers and functional status of lymphocytes are reset at roughly the preimmunization level. This process is called homeostasis, and it allows the immune system to respond effectively to a new antigenic challenge. The size and content of the preimmune lymphocyte repertoire are also closely regulated, as new emigrants from the generative lymphoid organs compete for "space" with resident cells. The mechanisms responsible for maintaining homeostasis before antigen exposure have been the subject of other reviews (*1*) and are not discussed here. Second, lymphocytes with receptors capable of recognizing self-antigens are generated constantly, yet normal individuals maintain a state of unresponsiveness to their own antigens, called self-tolerance. In this article, we review the principal control mechanisms for maintaining homeostasis after active immune responses to foreign antigens and for preventing or aborting responses to self-antigens. Our emphasis is on T lymphocytes, because many of the recent advances have come from studies of T cells, but it is likely that the general principles are applicable to all lymphocytes. Elucidating the nature of these homeostatic mechanisms may lead to better strategies for suppressing harmful immune responses and for augmenting and sustaining beneficial responses to microbial vaccines and tumors.

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Signals for Lymphocyte Maintenance, Growth, and Differentiation

Phenotypically and functionally naïve lymphocytes may survive for long periods, up to several months in mice, in the absence of overt antigen exposure (*2*). The basal survival of naïve B cells requires the expression of antigen receptors (*3*), and that of T cells requires the presence of major histocompatibility complex molecules, which are essential components of the ligands that the T cells recognize (*4*). Thus, the maintenance of the pool of naïve lymphocytes, before immunization, is dependent on antigen receptor–mediated signals (Fig. 1). These signals might be generated by spontaneous aggregation of antigen receptors or by the engagement of these receptors by extracellular ligands. The requisite ligand or ligands have not been identified; they may include environmental antigens or even self-antigens that are recognized with low affinities by mature lymphocytes.

For an immune response to occur, lymphocytes must be exposed to two types of stimulus. The first signal, an antigen, ensures the specificity of the response. "Second signals" are elicited by microbes, either directly or by the initial innate immune response, which identifies the antigen as a potential pathogen (*5*, *6*). Second signals for T lymphocytes include costimulators and cytokines that promote clonal expansion of the specific T cells and their differentiation into effector and memory cells (Fig. 1). The best defined costimulators for T cells are the two members of the B7 family, B7-1 (CD80) and B7-2 (CD86), which are induced on antigen-presenting cells (APCs) by microbes and by cytokines produced during innate immune reactions (*7*). The CD28 receptor on T cells recognizes B7 molecules and delivers activating signals; a second receptor for B7, called CTLA-4, functions to terminate T cell responses and is discussed later. The engagement of CD28 by B7 results in the expression in T cells of antiapoptotic proteins of the Bcl family, notably Bcl-x_L (*8*), and the production of cytokines, such as interleukin-2 (IL-2) (*9*). Thus, costimulation promotes the survival of T cells that encounter an antigen, allowing autocrine cytokines to initiate clonal expansion and differentiation. A second system of costimulation may be the CD40 molecule on antigen-presenting cells, which interacts with its ligand on T cells (*10*). Neither the biochemical consequences of CD40L engagement in T cells nor the ability of CD40-CD40L interactions to replace B7-CD28 signals has been established. The best defined second signal for B cells is a breakdown product of complement activation, called C3d, which engages its receptor, CD21, on B cells and functions in concert with an antigen to trigger B lymphocyte proliferation and differentiation (*5*, *11*). Microbes may directly activate the complement cascade by the alternative pathway, generating C3d, or an initial immunoglobulin M antibody re-