Intrinsic checkpoints for lineage progression

A new T-cell receptor α-chain-like molecule has been identified in precursor T cells. This protein may be part of a receptor complex that induces T-cell maturation.

Development of the B and T lymphocytes that constitute the body's main means of recognizing foreign antigens proceeds via a discrete series of developmental stages. A key feature of this process is that proper rearrangement and expression of antigen-receptor genes are required for developmental progression [1]. The mechanism by which this process occurs in developing T cells has recently become clearer, as the molecular nature of an antigen-receptor-like complex expressed on precursor T cells has been defined [2].

The T-cell antigen receptor is composed of two immunoglobulin-like antigen-recognizing subunits — α and β chains for most T cells (hereafter called TCRα and TCRβ chains) — and a number of non-variable chains — the ζ and CD3 γ, δ and ϵ chains — that are required for cell-surface expression and signal transduction [3] (Fig. 1). Like the genes encoding B-cell immunoglobulin heavy μ and light κ chains, the genes encoding the TCRα and TCRβ chains are organized in multiple segments — V, D and J, for variable, diversity and joining — that are assembled by recombination during T-cell development. Distinct rearrangements occur in each lymphocyte, which consequently express an antigen receptor of effectively unique specificity [4].

Failure to produce functional rearrangements of T-cell receptor or immunoglobulin genes, either naturally because of the inherently error-prone V(D)J recombination process or as a result of various mutations in genetically altered mice, blocks the developmental progression of T or B cells, respectively [1,5]. For example, genetic inactivation of the TCRβ-chain locus [6,7] leads to T-cell development arresting at the early $CD4^-CD8^-$ 'double-negative' stage, defined by lack of expression of either CD4 or CD8 co-receptor chains. Restoration of TCRβ-chain expression with a rearranged transgene promotes maturation to the next stage of T cell development — the $CD4^+CD8^+$ 'double-positive' stage [6] (Fig. 2). These observations indicate that expression of a TCRβ chain by a developing T cell initiates a sensing event that acts as an intrinsic checkpoint for maturation. Expression of immunoglobulin heavy chain serves an analogous checkpoint function for B-cell development [8] (Fig. 2).

How does a precursor T cell sense productive TCRβ-gene rearrangement? Clues to a possible mechanism come from the observation that productive rearrangement of the TCRβ gene results in the expression of a TCRβ complex at the surface of precursor T cells [9]. Immunoprecipitation experiments show that the TCRβ chain in these cells covalently associates with a 33 kD glycoprotein, now called the pre-Tα chain [10]. The gene encoding this protein has recently been cloned [2]. From the gene's sequence, the pre-Tα chain is predicted to be a type 1 transmembrane protein with a cytoplasmic tail containing two potential protein kinase C phosphorylation sites. The extracellular domain contains a single immunoglobulin-like domain with conserved cysteine residues for intra-chain disulfide bonds and for dimerization with TCRβ. Like conventional TCRα chains, pre-Tα has charged residues within its putative transmembrane region [2]. These charged residues in the TCRα chain are required for proper assembly and cell-surface transport of conventional T-cell receptor complexes [3].

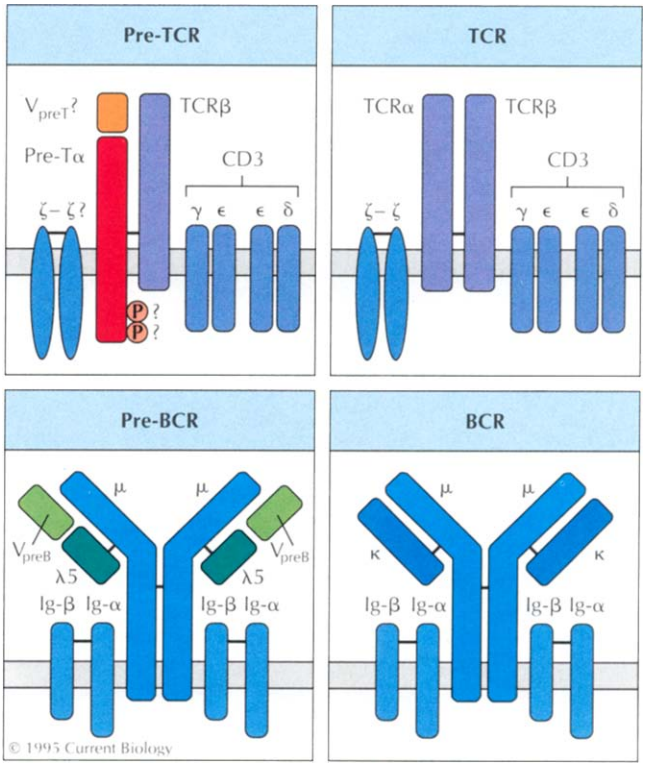

Fig. 1. Structural components of the T-cell antigen receptor (TCR) and B-cell antigen receptor (BCR) and of the related receptors expressed during T-cell or B-cell development (pre-TCR and pre-BCR, respectively). By analogy with the pre-BCR structure, the pre-TCR may contain an as yet unidentified component that contains a variable-type immunoglobulin domain to pair with the variable domain of the TCRβ chain. A ζ-ζ homodimer has not yet been found associated with the pre-TCR [10]; however, a possible weak association of the pre-TCR with ζ is indicated in the figure, because T-cell development is impaired in ζ-deficient mice [5].

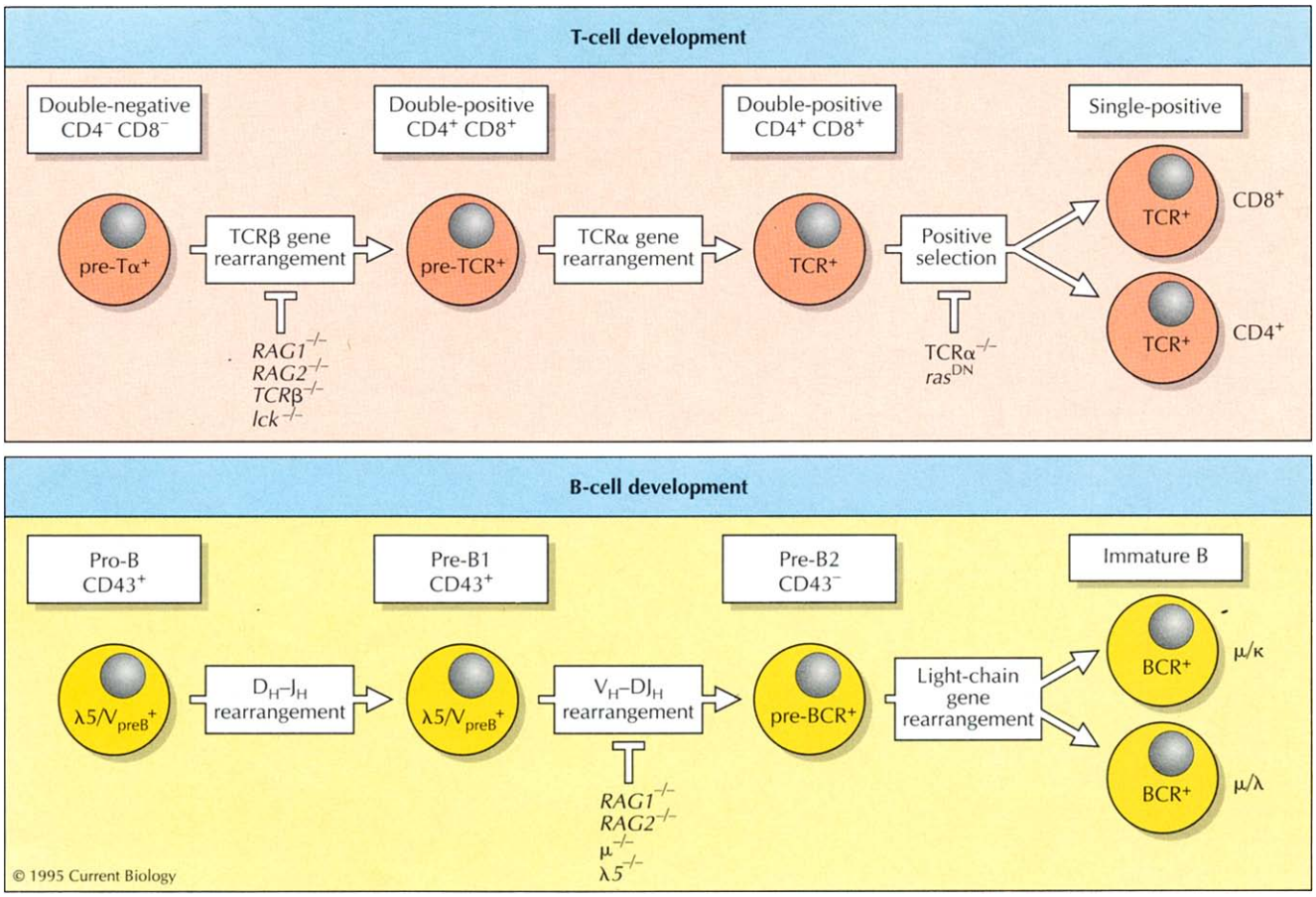

Fig. 2. Developing T cells and Bcells progress through a series of discrete stages. Early T-cell precursors (double-negative thymocytes) are characterized by lack of expression of CD4 and CD8. TCRβ chain expression and Lck function are required for progression to the next stage, $CD4^+CD8^+$ double-positive thymocytes, which initially express the pre-TCR. These cells stop rearranging TCRβ loci and start rearranging TCRα loci. Once a TCRα chain is made, the cells undergo positive and negative selection based on the specificity of the T-cell receptors they express. Positive selection based on reactivity for major histocompatibility complex (MHC) molecules leads to progression to the $CD4^+$ or $CD8^+$ single-positive stage, after which the T cells are fully mature and leave the thymus. B cells follow an analogous developmental pathway, with the exception that positive selection based on antigen specificity may not be required. Expression of the pre-BCR is required for progression from the pre-B1 to the pre-B2 stage. Functional light-chain gene expression is required to complete development and allow exit of B cells from the bone marrow. Stages at which development is arrested in specified genetically defective mice are shown (ras^{DN} refers to expression of a gene encoding a dominant-negative form of Ras).

Thus, the structure of the newly described pre-Tα chain supports the hypothesis that it combines with the TCRβ chain to form an antigen-receptor-like complex, hereafter called the pre-TCR (Fig. 1). The pre-TCR complex is postulated to induce developmental progression to the $CD4^+CD8^+$ double-positive stage (Fig. 2). Subsequent rearrangement and expression of a functional TCRα-chain gene allows the formation of a conventional T-cell receptor αβ heterodimer. The resulting precusor T cell further develops to the mature T-cell state if the ligand-binding specificity of its T-cell receptor is appropriate to allow positive, but not negative, selection (see [11,12] for review).

One striking feature of the pre-TCR is its similarity to antigen-receptor-like molecules expressed by developing precursor B cells. During B-cell development, precursor cells transit through a characteristic series of developmental stages (Fig. 2). The gene for the immunoglobulin μ heavy chain rearranges first during B-cell development [13,14]. Once a functional μ heavy chain is produced, it forms a complex (the pre-BCR) with two immunoglobulin light-chain-like proteins, called λ5 and V_{preB1} [15,16]. Like pre-Tα, λ5 has a domain that is homologous to immunoglobulin constant regions. In contrast to pre-Tα, λ5 does not have transmembrane or cytoplasmic domains [17]. This difference is to be expected, however, as the TCRα chain is a transmembrane protein, whereas immunoglobulin light chains are localized outside of the membrane (Fig. 1). A more important apparent difference between the T-cell and B-cell results is the presence in the pre-BCR complex of the other non-covalently associated polypeptide, V_{preB1} [18] — so far no analogous component of the pre-TCR has been discovered.

The similarity of λ5 to an immunoglobulin constant domain and of V_{preB1} to an immunoglobulin variable domain suggested that these two polypeptides bind to an immunoglobulin heavy chain in a manner analogous to a normal light chain, and act as surrogate light chains [17]

(Fig. 1). Just as the pre-Tα–TCRβ complex associates with CD3 components, the V_{preB}–λ5–μ complex associates with the accessory chains Ig-α and Ig-β [19]. In mature B cells, Ig-α and Ig-β facilitate the transport of immunoglobulin heavy and light chains to the cell surface, and mediate signal transduction events initiating from the B-cell antigen receptor [20]. In light of the structural similarities between the pre-BCR and pre-TCR, it seems likely that a V_{preB}-like molecule is associated with the pre-Tα–TCRβ complex.

The precise roles of the pre-TCR and the pre-BCR are not known, although gene 'knock-out' experiments have clearly shown that they are required for proper lymphoid development. For example, B-cell and T-cell development ceases at characteristic stages if the immunoglobulin heavy-chain and TCRβ genes, respectively, are not successfully rearranged [21,22] (Fig. 2). Similarly, the λ5 gene is critical in B-cell development, as knocking-out this gene results in mice with greatly reduced production of B cells [23]. One possibility is that the newly identified pre-Tα chain assists in receptor assembly and expression at the cell surface. Indeed, the λ5 chain of the pre-BCR is required for μ-chain expression at the cell surface [16]. A second possible function for the pre-TCR and the pre-BCR is recognition of a stromal-cell or epithelial-cell ligand in the supporting environment. Lymphoid development occurs in a specific thymic microenvironment in the case of T cells, and in the bone marrow in the case of B cells, and is dependent on cell–cell contact within these microenvironments. The pre-TCR could mediate interactions with specific components within the thymic microenvironment.

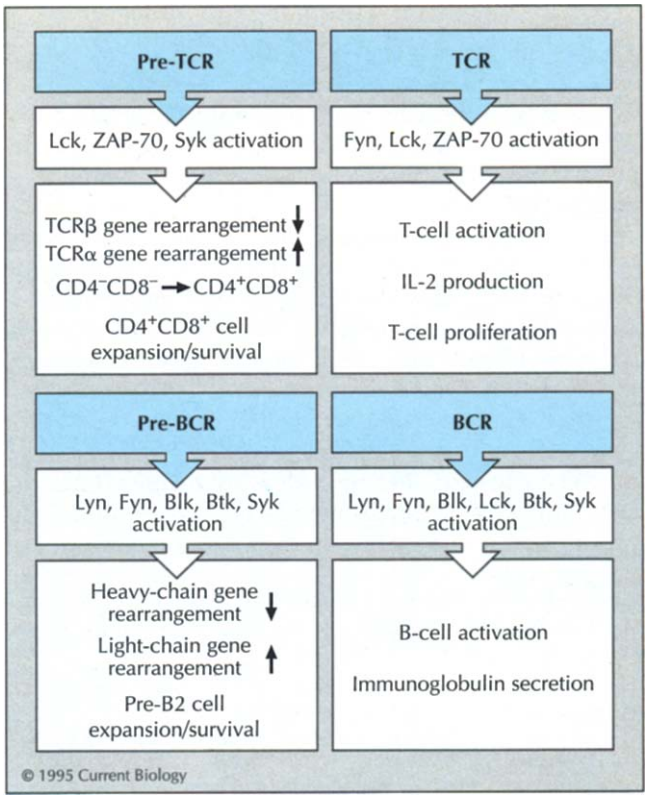

Fig. 3. Distinct biological phenomena regulated by the antigen-receptor and related complexes expressed by lymphocytes. Some of the same protein-tyrosine kinases associated with, and potentially activating, the T-cell receptor (TCR) or B-cell receptor (BCR) can also associate with the pre-TCR or pre-BCR. Stimulation of the receptors of mature T and B cells, however, induces functional activation of the cells, whereas expression of precursor receptors induces differentiation events.

As expression of the pre-TCR or the pre-BCR is required for lymphocyte developmental progression, it has been hypothesized that these molecules initiate signaling events that indicate their presence. The signal transduction mechanisms used by the pre-TCR and pre-BCR are therefore of considerable interest. Cross-linking pre-BCRs results in a number of signaling events, including protein-tyrosine phosphorylation and calcium elevation [24]. These experiments demonstrate a potential signaling ability of the pre-BCR. Hints as to what intracellular components mediate pre-TCR signaling have come from the generation of mice in which the protein-tyrosine kinase Lck is inactivated, either by expression of a dominant-negative allele or by knocking-out the *lck* gene [25,26]. In either case, T-cell development is specifically abrogated at the $CD4^-CD8^-$ to $CD4^+CD8^+$ transition, the same point at which it is blocked in the absence of TCRβ-chain production. Moreover, expression of an activated allele of *lck* can drive thymocytes through this transition, even in the absence of TCRβ chain [27].

Thus, Lck function is critical to promote the transition between these T-cell stages or to drive the expansion and survival of emerging $CD4^+CD8^+$ cells. In contrast, Ras function is not required at this stage, but is required later for positive selection [28]. A model for T-cell development emerges in which TCRβ chains assemble with pre-Tα chains and the non-polymorphic CD3 chains to form a receptor complex in progenitor T cells. This receptor complex functions as a signal-transducing unit, using Lck to transduce signals into the cell that effect transition to the $CD4^+CD8^+$ stage. In this regard, it is interesting that the pre-Tα cytoplasmic domain contains a proline-rich region that may bind the Src homology 3 (SH3) domain of Lck [2,29].

Signal transduction by the T-cell receptor in mature T cells requires Lck as well as another cytoplasmic tyrosine kinase, ZAP-70 [30]. Interestingly, humans with defective ZAP-70 have been found among patients with T-cell immunodeficiency [31]. In these patients, mature T cells are largely unresponsive to stimulation via the T-cell receptor. However, these patients show only a partial defect in T-cell development, perhaps because thymocytes express moderate levels of the highly related tyrosine kinase Syk, whereas mature T cells express much less Syk [32]. Another immunodeficiency, X-linked agammaglobulinemia, involves loss-of-function mutations of a third type of intracellular tyrosine kinase, Btk. In this disease, there is a strong block in B-cell development [33]. The role of Btk in B-cell development is not well understood,

but some evidence suggests that it plays a role in B-cell receptor and possibly pre-BCR signaling [34–36].

The identification and characterization of the structural components of the pre-BCR and pre-TCR provides insight into the mechanisms regulating lymphocyte development. The structure of these precursor lymphocyte receptors is analogous to those of the antigen receptors of mature B and T cells. However, the biological outcome of signaling through these different receptors is distinct (Fig. 3). The challenge for the future is to dissect the signal transduction events induced by these receptors and to determine the mechanisms that relay proper and distinct developmental information to the progenitor lymphocytes. The cloning of the gene encoding pre-Tα [2] will facilitate molecular analysis of the pre-TCR, and may provide a means to identify how T-cell precursors transit through checkpoints in their development.

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