Review

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Fine-tuning T cell receptor signaling to control T cell development

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T cell development from immature CD4$^+$CD8$^+$ double-positive (DP) thymocytes to the mature CD4 or CD8 single-positive (SP) stage requires proper T cell receptor (TCR) signaling. The current working model of thymocyte development is that the strength of the TCR-mediated signal – from little-or-none, through intermediate, to strong – received by the immature cells determines whether they will undergo death by neglect, positive selection, or negative selection, respectively. In recent years, several developmentally regulated, stage-specifically expressed proteins and miRNAs have been found that act like fine-tuners for signal transduction and propagation downstream of the TCR. This allows them to govern thymocyte positive selection. Here, we summarize recent findings on these molecules and suggest new concepts of TCR positive-selection signaling.

Emerging fine-tuning molecules in T cell development

During the process of negative selection in central immunological tolerance, immature T cells (i.e., thymocytes) that respond strongly to self MHC peptide (MHCp) through their TCRs are effectively eliminated from the pool of conventional mature T cells. Negative selection avoids generalized T cell aggression towards healthy tissue, while preserving a naïve T cell pool available to generate T cell responses against foreign MHCp when summoned to fight infection or malignant cell transformation [1]. Fulfilling the latter task is only ensured via the survival during thymocyte development, of immature T cells that recognize self MHCp with low-to-moderate strength, in a process termed positive selection. Positive selection generally precedes negative selection in the thymus, signaling survival of the selected thymocytes and enabling these cells to distinguish foreign antigen in the context of their own self-MHC (i.e., MHC restriction). Remarkably, these two contrasting developmental outcomes, as well as the activation and differentiation process of mature T cells in response to foreign antigens, are all managed by a TCR signaling machinery of qualitatively similar composition [2]. The essential difference is in the interpretation of signals of different strength leading to antagonistic gene expression and metabolic changes that predispose to survival and differentiation, or that trigger cell death. How these diverse roles are achieved by the TCR in different cellular contexts, and indeed how these contexts affect TCR signaling, remains unclear.

The TCR signaling machine is dynamically assembled from at least three membrane-resident modules that are physically segregated at steady-state but which coalesce upon binding of TCR to MHCp with adequate affinity [3–5]. The TCR recognition module (TCRαβ-CD3γεδεζ2) governs MHCp recognition and the association of the protein tyrosine kinase (PTK) zeta-chain-associated protein kinase of 70 kDa (ZAP70) with the intracellular immunoreceptor-tyrosine-based activation motifs (ITAMs) of CD3 molecules. These are phosphorylated by the Src family PTK lymphocyte-specific protein tyrosine kinase (Lck). The Src kinase module is in charge of regulating the activity of the PTKs Lck and Fyn, and ensures the TCR activation threshold. Finally, the diversification/regulatory module rapidly forms highly cooperative multiprotein complexes that fine tune the amplitude and duration of signal propagation along several pathways. The ZAP70, Lck, and Fyn PTKs and the membrane-associated linker of activated T cells/SH2 domain containing leukocyte protein of 76 kDa (LAT/SLP67) scaffold complex together govern this critical signaling step. These signals trigger the activation of multiple biochemical pathways and the increase in cytosolic free Ca^{2+} concentration (Figure 1).

Part of the problem in understanding TCR-dependent signaling in thymocyte development is that many of the biochemical and imaging studies of TCR signaling have been performed with mature T cells or tumor lines, whereas thymocyte development has mostly been studied at the genetic level. Moreover, those signaling experiments performed on thymocytes have mainly used antibody cross-linking, rather than a natural ligand, to stimulate the cells. Thus, our understanding of signaling processes in thymocyte development suffers from the entanglement of interpretations based on these different methodologies. In the past 30 years the main players in the signaling cascades have been identified, but only relatively recently have we started to identify molecules with more subtle roles that can distinguish signal strengths to give different responses

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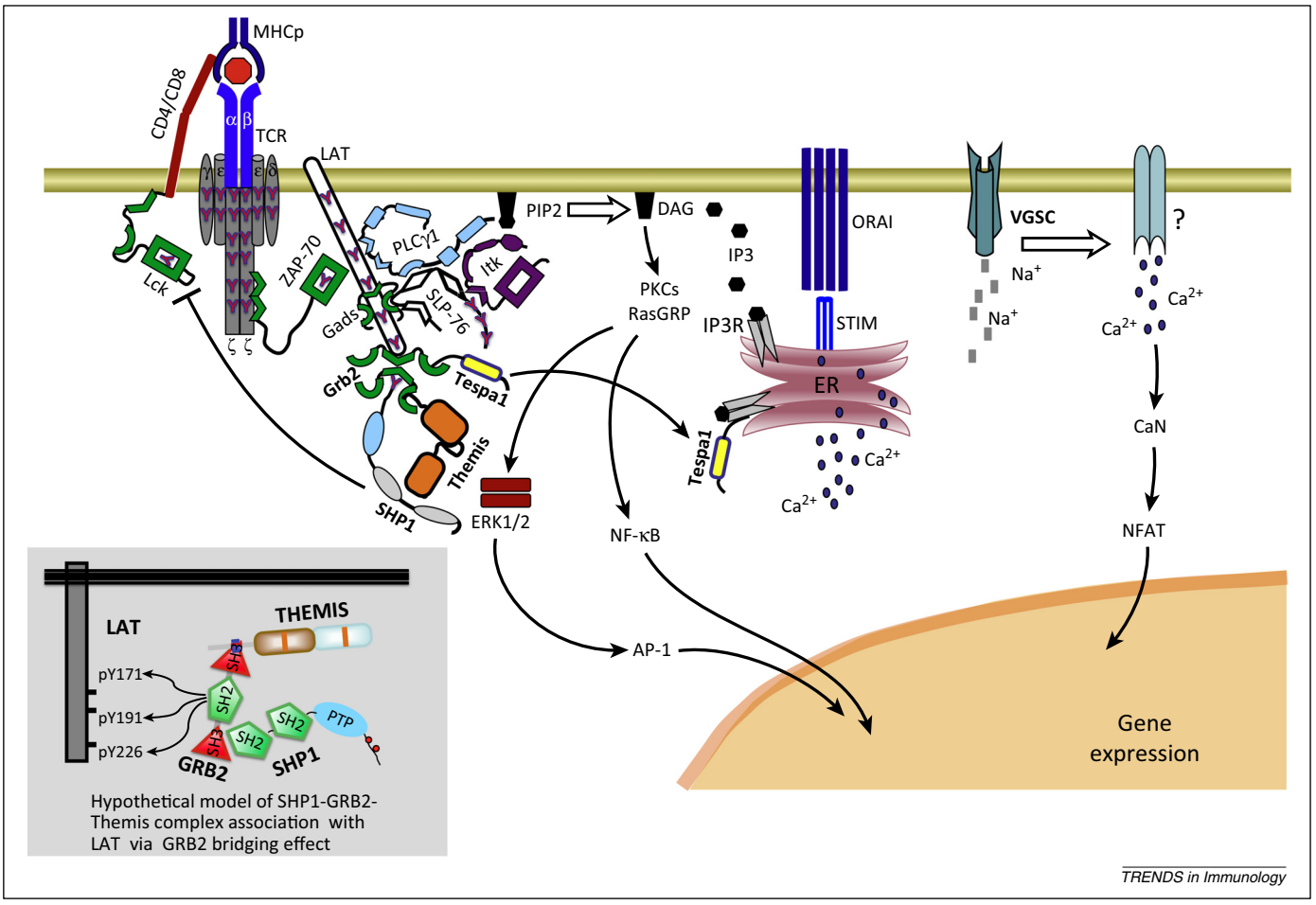

Figure 1. Proteins affecting signaling during thymocyte positive selection. During thymocyte development, upon TCR interaction with self-MHCp complex, a cascade of signaling events is triggered. This process includes a series of tyrosine phosphorylations mediated by protein tyrosine kinases (e.g. Lck and ZAP-70), sequential signaling complex formation mediated by scaffold proteins (e.g., LAT and SLP-76), and more downstream diversified signaling pathways (e.g., ERK and NFAT). Over the past 30 years, many molecules important for thymocyte positive selection were discovered, characterized, and put in the context of the signaling network shown here. Recently, some newly identified molecules were found to be specifically involved in thymocyte positive selection (i.e., Themis, Tespa1, and VGSC). These each serve different functions in signaling in positive selection, acting with other molecules that may have a more general role in T cell signal transduction and negative selection. **Insert**. Model for GRB2-Themis-SHP1 complex association with LAT. Upon TCR stimulation during thymocyte positive selection, the scaffold protein LAT is tyrosine phosphorylated at multiple sites, which serve as the docking sites for many SH2-domain-containing molecules. Among these LAT-binding molecules, the adaptor protein GRB2 has a central SH2 domain flanked by two SH3 domains. Recent studies showed that Themis binds the C-terminal SH3 domain of GRB2 via its proline-rich region, whereas SHP1 binds the SH2 domain of GRB2 via its C-terminal phosphotyrosines. Thus GRB2 brings Themis and SHP1 together, and Themis is required for activity of SHP1. Abbreviations: DAG, diacylglycerol; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; Gads, GRB2-related adapter protein 2; GRB2, Growth factor receptor-bound protein 2; IP3, inositol 1,4,5-trisphosphate; IP3R, IP3 receptor; Itk, interleukin-2-inducible T cell kinase; LAT, linker of activated T cells; Lck, lymphocyte-specific protein tyrosine kinase; MHCp, MHC peptide; NF-κB, nuclear factor-κB; NFAT, nuclear factor of activated T cells; PIP2, Phosphatidylinositol 4,5-bisphosphate; PKC, protein kinase C; PLC, phospholipase C; PTP, protein tyrosine phosphatase; SH2, Src homology region 2 domain; SH3, Src homology region 3 domain; SHP1, Src homology region 2 domain-containing phosphatase-1; SLP-76, SH2 domain containing leukocyte protein of 76kDa; TCR, T cell receptor; Tespa1, thymocyte-expressed, positive selection–associated-1; Themis, thymocyte-expressed molecule involved in selection; VGSC, voltage-gated sodium channel; ZAP-70, zeta-chain-associated protein kinase of 70 kDa.

for positive versus negative selection. Several genes involved in controlling proximal TCR signaling in thymocyte selection processes are found to be differentially expressed during thymocyte development, and Ca^{2+} signaling during thymocyte development is differentially 'wired' compared to that in mature T cells [6]. The idea is emerging that developmentally regulated, stage-specific expression of molecules that finely tune the TCR signal transduction and propagation processes govern positive selection. Here, we review these recent findings and suggest new concepts in TCR positive selection signaling.

Specialized signaling regulation for thymocyte positive selection

Thymocytes are more sensitive than mature T cells to low-affinity ligands [7,8]. This has biological consequences, because it allows weakly binding self-MHCps to readily trigger thymocyte positive selection without incurring the undesirable activation of mature T cells in the periphery. This relatively weak stimulation of thymocytes does not lead to overt activation that would induce negative selection or lineage deviation into one of the agonist-selected T cell subsets. Agonist-selected cells include the TCR$\alpha\beta^+$ CD8$\alpha\alpha$-expressing intraepithelial lymphocytes, Natural killer (NK)T cells, and thymus-derived T regulatory cells; all of which require interaction with higher-affinity ligands than are required for positive selection of conventional naïve $CD4^+$ or $CD8^+$ T cells (agonist selection is comprehensively reviewed in [9]). Because of this intertwined complexity and subtlety, it is reasonable to expect that specialized signaling regulators exist in thymocyte-positive selection to help translate signals into different cell fates [2].