T-cell signaling and immunopathologies

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The understanding of the factors responsible for T-cell signaling has developed enormously over the past two decades. Our initial discovery of the CD4- and CD8-p56lck complexes and their ability to phosphorylate the T-cell receptor (TCR) complex led to the discovery of a second protein-tyrosine kinase ZAP-70 and an array of adaptor proteins such as LAT and adhesion and degranulation-promoting adaptor protein (ADAP) that are responsible for integrating TCR signaling events. These pathways are in turn modified by phosphatases such as LYP (PTPN22), E3 ligases of the ubiquitin pathway such as Cbl-b and Itch, and co-receptor modules such as signaling lymphocyte activation molecule (SLAM). TCR signals then determine the function of T-cells subsets such as regulatory T-cells (Tregs) and follicular helper T-cells (Tfh) in their regulation of immune function and autoimmunity. This volume of *Seminars in Immunopathology* provides an up-to-date review of the current status of mutations in the TCR signaling cascade and T-cell subsets that account for immunodeficiencies and autoimmune pathologies.

During the past two decades, the field of T-cell signaling has developed in leaps and bounds. Our initial discovery of the CD4- and CD8-p56lck complexes identified the initiators of the TCR tyrosine phosphorylation cascade in T-cells (i.e., referred to as the “TCR signaling paradigm”) [1–3]. p56lck phosphorylates the antigen-receptor complex [2, 4] in a cascade that leads to the recruitment of another kinase termed ZAP-70 to immunoreceptor tyrosine-based activation motifs (ITAMs; YxxL x6–8 YxxL) on the CD3 and zeta chains [5]. These observations combined with

availability of antiphosphotyrosine-specific antibodies led to the identification of an array of p56lck, p59fyn, and ZAP-70 targets that have served as the mainstay of research on T-cell activation over the past two decades. While p56lck can phosphorylate ITAMs, related src kinase p59fyn preferentially phosphorylates ADAP [6, 7], and ZAP-70 targets linker for activation of T-cells (LAT) and 76-kDa src homology 2 domain-containing leukocyte phosphoprotein (SLP-76) [8, 9]. From this work evolved the identification of so-called immune cell specific “adaptor proteins” or “molecular scaffolds” that possess discrete molecular domains or binding sites for the generation of complexes [10–12]. These complexes of proteins integrate TcR signals for downstream effector functions such as cytokine production [11]. For example, LAT has multiple sites for binding to phospholipase C γ 1 (PLC γ 1), GADS, and growth factor receptor-bound protein 2 [13, 14], while SLP-76 constitutively binds to GADS and has binding sites for Vav1, IL2-inducible T-cell kinase, PLC γ 1, 14-3-3, and an SH2 domain that binds to ADAP and hematopoietic progenitor kinase 1 [8, 10]. At the same time, key protein-tyrosine phosphatases such as CD45, SHP-1, and LYP have been uncovered that amplify or dampen the function of these molecules by dephosphorylating proteins [15]. Over-arching on this scheme is the ubiquitination pathway, a mechanism that controls the expression of signaling proteins [16, 17]. Ubiquitination is controlled by an array of E1-E3 ligases that tag proteins for degradation by proteasomes. In addition, co-receptors such as CD28 can amplify the immune response [18, 19]. In this context, SLAM uses a unique recognition mode of tyrosine phosphorylation to induce co-signals [20, 21]. These components in turn can regulate the development and function of T-cell subsets. Of particular note are TRegs and their crucial role in regulating autoimmunity [22]. Given these develop-

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ments in T-cell signaling, it is perhaps not surprising that various mutations have been uncovered that are causally linked to autoimmunity and immunodeficiency disorders [23]. The present issue of *Seminars in Immunopathology* reviews our current knowledge of signaling events and mutations in the TCR signaling cascade whose dysregulation results in autoimmunity and immunodeficiency.

The first article by Ohkura and Sakaguchi review their seminal work on the identification and function of CD4⁺CD25⁺ Tregs in maintaining self-tolerance and immune homeostasis. In addition to describing their central role in regulating autoimmunity, they outline the importance of TCR signaling for suppressor function, and in particular, how TCR signaling alters Foxp3-dependent transcriptional regulation and recruitment to the specific genomic loci.

This article is then followed by Alan Fischer and colleagues who review their key work on ZAP70 mutations in patients with severe T-cell immunodeficiencies and the use of ZAP-70 expression as diagnostic marker in prognosis of chronic lymphocytic leukemia (B-CLL). They also review recent data from mouse models implicating the inactivation of ZAP-70 SH2 domains in collagen-induced arthritic models of autoimmunity. Mallisen and colleagues then outline their model of “LAT signaling pathology” whereby LAT knock-in develop lymphoproliferative disorders that are accompanied by the loss of TRegs. These studies underscore the possibility of opposite outcomes (i.e., immunodeficiency versus autoimmunity) with different mutations in a single kinase such as ZAP-70 or within the same signaling cascade (i.e., ZAP-70-LAT pathway).

Stanford, Bottini, and Mustelin et al. then review their seminal discovery of the R620W mutation in LYP (encoded by the *PTPN22* gene), a negative regulator of TCR signaling in a group of autoimmune disorders including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), Graves disease, Addison's disease, vitiligo, myasthenia gravis, and systemic sclerosis. They outline how this mutation may act in the TCR signaling pathway to elicit this effect and review studies on other autoimmune disorders that derive from the loss of SHP-1 or a gain-of-function mutation in the transmembrane phosphatase CD45.

A third theme then involves descriptions of the exciting connection between ubiquitin E3 ligases and autoimmunity. E3 ligases regulate a key step in the ubiquitination pathway whereby ubiquitin is coupled to proteins for degradation. In an exciting area, mutations in E3 ligases such as autoimmune regulator (AIRE), tumor necrosis factor receptor-associated factor 6 (TRAF6), Casitas B cell lymphoma b (Cbl-b), gene related to anergy in lymphocytes (GRAIL), Itch, and Roquin recently have been linked to autoimmunity [16, 24, 25]. Paolino and Penninger discuss their pioneering work on the link between immune tolerance and the E3 ligase Cbl-b, while Yun Cai Cai, the first to identify

Cbl-b as a E3 ubiquitin ligase, focuses on another E3 ligase termed *Itch* whose deficiency is linked to enhanced immune responses, abnormal T helper cell differentiation, and failed T-cell anergy. As in the case of LAT, *Itch* regulates the development of Foxp3-positive Tregs with a specific connection to TGF- β -induced Foxp3 expression.

A fourth theme of the volume outlines the interesting connection between co-receptors and autoimmunity. Co-receptors such as CD28 have long been implicated in the amplification of T-cell responses [19, 26, 27]. Terhorst et al. review their seminal work in uncovering the first connection between the co-receptor SLAM and X-linked lymphoproliferative disease (XLP; also known as Purtilo's syndrome and Duncan's disease) and susceptibility to SLE. XLP disease is an inherited immunodeficiency triggered by Epstein–Barr virus infection. SLAM family currently includes nine members named SLAM, CD48, CD229, 2B4, CD84, NTB-A, CRACC, BLAME, and CD2F-10 that are differentially expressed on immune cells, and regulate T-cell development and function and modulate lytic activity and cytokine production [20, 21]. SLAM family receptors become tyrosine phosphorylated for the recruitment of an adaptor known as SAP (SLAM-associated protein) and the Src kinase Fyn. As outlined by Terhorst et al., SLAM signaling involves an unusual phosphotyrosine recognition event that influences the outcome of interactions between T-cells and antigen-presenting cells (APCs) as well as between NK cells and their targets.

In the fifth theme of the volume, Wang et al. review our current understanding of a different category of immune cell regulators, namely, the integrins. These proteins that include lymphocyte function-associated antigen 1 (LFA-1) mediate T-cell adhesion required for movement of T-cells to sites of inflammation within lymph nodes and mediate binding to APCs [28]. Immunodeficiency has been observed with leukocyte adhesion deficiency disorder patients who are unable to clear pathogens and present with recurrent life-threatening infections and impaired wound healing. Altered LFA-1, a fucosylation defect in the ligand (i.e., SLeX) for selectins and mutations in signaling protein Kindlin 3, accounts for these disorders. They also review our understanding of the components that comprise the TCR “inside-out” pathway for adhesion, possible future targets of immunodeficiency disorders.

The final section of the volume then involves further information on subsets of T-cells in autoimmunity. Lintermann and colleagues describe recent insights on the function of the T follicular helper (Tfh) T-cell and the consequences of Tfh dysregulation for immunodeficiency and autoimmune diseases. Tfh constitute a distinct CD4⁺ T helper lineage that is critical for humoral immunity. They also outline the role of the novel E3 ligase Roquin and other factors in Tfh function. Lastly, Johnson and Jirik explore the contributions of CD4

and CD8 T cell populations to disease initiation and progression of multiple sclerosis (MS). While CD8⁺ T lymphocytes are more abundant in MS lesions than CD4⁺ T lymphocytes, too little attention has been paid to the involvement of this subset in disease. They highlight several novel murine systems for investigating CD8⁺ involvement in autoimmune de-myelination.

Overall, the volume includes articles from leading players who have made key links between TCR signaling events with the development of immune pathologies. Genome-wide screening of patients with single-site polymorphisms has generated additional connections between other signaling proteins and autoimmune disorders such as type 1 diabetes [29]. These include the negative co-receptor CTLA-4 whose ablation in mice results in a severe tissue infiltration with death by 3–4 weeks [18, 30–32]. CD28 and CTLA-4 are structurally homologous members of the immunoglobulin (Ig) gene superfamily. CTLA-4 binds to CD80/86 with a 20–100-fold higher affinity than CD28. The autoimmune phenotype may in part be related to effects on motility and T-cell-APC dwell times [33], 2,3-dioxygenase indoleamine (IDO) production [32, 34, 35], regulation of TReg function [32], and other mechanisms [36]. One surprising aspect of these screens is the absence of mutations in some of the key regulators of TCR signaling. For example, no mutations in p56lck and ZAP-70 have been found in type 1 diabetes. This may underscore the curious correlation between mutations in certain proteins and certain autoimmune diseases. For example, while mutations in the PTPN22 gene have defined one group of autoimmune disorders including RA and SLE, no mutations are apparent in others such as MS. As in the case of PTPN22, commonality amongst certain autoimmune diseases may only become apparent with the definition of mutations in signaling proteins that regulate the different disease states. This regulatory effect could occur at the level of a specific T-cell function, or in the development of subsets of T-cells such as TRegs that influence signaling thresholds in the immune response. Future studies will almost certainly lead to the identification of new TCR signaling proteins and mutations responsible for immune pathologies.

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