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Immunodeficiency and Immune Dysregulation Associated with Proximal Defects of T Cell Receptor Signaling

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Abstract

Engagement of the T cell receptor (TCR)/CD3 complex triggers a cascade of events that result in T lymphocyte activation and promote positive and negative selection of thymocytes, T lymphocyte migration and effector functions, development and activation of regulatory T cells. Gene mutations that abrogate early TCR signaling are associated with profound abnormalities of T lymphocyte development and function both in humans and in mice, causing susceptibility to severe infections since early in life. In recent years, a growing number of genetic defects have been discovered that reduce, but do not completely abrogate proximal TCR signaling. These defects result in complex phenotypic manifestations that are not limited to immunodeficiency, but also include immune dysregulation. The identification of these conditions may also prompt development of novel therapeutic strategies for autoimmune disorders.

Introduction

Integrity of T cell receptor (TCR) signaling plays a critical role during positive and negative selection of T cells in the thymus, and for effector and regulatory functions of peripheral T lymphocytes [1,2]. Upon engagement of the TCR by the peptide/Major Histocompatibility Complex (MHC), the Src kinase LCK is activated and phosphorylates the CD3 immunoreceptor tyrosine-based activation motifs (ITAMs). The Zeta-associated protein of 70 kDa (ZAP70) binds to the phosphorylated ITAMs and is activated by LCK. Zap70 phosphorylation enables ZAP70 kinase activity, resulting in phosphorylation of the adaptor molecules SLP-76 and Linker of Activated T cells (LAT). The interleukin-2 inducible tyrosine kinase (ITK) interacts with phosphorylated SLP-76 and LAT, and undergoes autophosphorylation. This promotes ITL-dependent phosphorylation of phospholipase C-γ1 (PLC-γ1), allowing hydrolysis of phosphatidylinositol (4,5) diphosphate (PIP_2) to inositol (1,4,5) trisphosphate (IP_3) and diacylglycerol (DAG), release of Ca^{2+} from endoplasmic

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reticulum stores, Ca^{2+} influx, activation of Erk, reorganization of the actin cytoskeleton and activation of transcriptional program.

Genetic defects that affect components of the TCR signaling machinery cause immunodeficiency. However, there is growing evidence that mutations that perturb strength of TCR signaling may also cause autoimmunity. Here, we will review genetic disorders of TCR signaling associated with immune dysregulation, with a special focus on defects that have been reported in patients.

ZAP70 defects affect thymocyte selection and alter mechanisms of immune homeostasis

The first evidence that the ZAP70 tyrosine kinase plays a critical role in T cell development and function was provided in the '90s by observations that patients with ZAP70 mutations lack circulating CD8+ lymphocytes, whereas CD4+ cells are present but fail to proliferate in response to mitogens and antigens [3-5]. Soon thereafter, it was demonstrated that *Zap70*$^{-/-}$ mice have a complete block at double positive (DP) stage of T cell development in the thymus, and consequently lack circulating CD4+ and CD8+ single positive (SP) cells [6]. These studies were followed by a series of seminal observations in mice demonstrating that hypomorphic mutations of the *Zap70* gene that alter the strength of TCR signaling may cause immunodeficiency and immune dysregulation. In particular, *Zap70*^{skg} mice, harboring the W163C amino acid substitution in the SH2 domain of Zap70 that is involved in recognition of CD3ζ ITAMs, have attenuated TCR signaling. As a result of this, thymocytes that bind self-antigens with high affinity and that would normally be negatively selected are positively selected instead, and when exported to the periphery mediate autoimmune arthritis [7,8]. Furthermore, *Zap70*$^{mrd/mrt}$ mice carrying compound heterozygous mutations in the *Zap*70 gene resulting in decreased TCR signaling, have decreased number of naïve and an increased proportion of activated CD4+ lymphocytes in the periphery, preserved generation of CD8+ cells, and features of immune dysregulation, as shown by hypergammaglobulinemia, increased serum IgE and autoantibody production [9]. This peculiar immunological phenotype was the result of defective negative selection and impaired generation of regulatory T (Treg) cells in the thymus. Overall, these data are consistent with the notion that distinct TCR signaling thresholds can promote apoptosis or survival of self-reactive thymocytes, and affect negative selection of SP CD4+ cells.

This hypothesis has been largely confirmed by observations in patients. Katamura et al. described a patient who presented with infiltrative cutaneous erythematous lesions and accumulation of activated $CD4^+$ $CD25^+$ $CD45R0^+$ DR^+ cells and eosinophils in the perivascular area of the skin [10]. Turul et al. reported two other patients with homozygous *ZAP70* missense mutations, whose clinical phenotype was characterized by immune dysregulation, with wheezing, generalized erythroderma, lymphadenopathy, eosinophilia and elevated serum IgE [11].

Recent studies have provided mechanistic insights on the immune dysregulation that may associate with ZAP70 deficiency in humans. In particular, it has been demonstrated that CD4+ cells from ZAP70-deficient patients have an altered TCR repertoire [12]. Moreover, these cells express reduced levels of *CTLA4*, *IL10*, and *TGFB* transcripts, and are less sensitive to FAS-mediated apoptosis [12], which represent signatures of autoimmunity.

Furthermore, it has been shown that impaired TCR signaling in ZAP70-deficient patients does not affect uniquely T cell function, but has also important consequences on lymphostromal cross-talk in the thymus and on organization and maturation of thymic epithelial cells (TECs) and dendritic cells. We have recently shown that the thymus of two patients with ZAP70 deficiency was characterized by smaller medullary area, and a reduced number of AIRE+ medullary TECs (mTECs) [13*]. Hassall corpuscles expressed minimal amounts of involucrin, a marker of terminally differentiated mTECs. Furthermore, the number of FOXP3+ cells and of thymic dendritic cells was also reduced as compared to what observed in control thymus [13*]. Therefore, immune dysregulation of ZAP70 deficiency is the consequence of multiple abnormalities that affect cell differentiation and selection in the thymus, as well as function of peripheral CD4+ cells. Importantly, several other genetically-determined defects that affect TCR signaling have been recently identified in patients with immunodeficiency and immune dysregulation.

LCK deficiency

Hauck et al. have described a child who presented with recurrent respiratory infections, nodular skin lesions, arthritis, vasculitis, and autoimmune thrombocytopenia. Genetic studies revealed a homozygous missense mutation of the *LCK* gene, causing defective expression of a mutant protein that was devoid of kinase activity [14**]. CD4+ T cell lymphopenia (including reduced absolute count of Treg cells), oligoclonal T cell repertoire, increased proportion of “exhausted” effector memory CD8+ T (T_{EMRA}) cells were present. A proximal defect of intracellular signaling in response to TCR/CD3 stimulation was observed, consistent with the genetic defect. Furthermore, activation-induced cell death (a late T cell response requiring intact TCR signaling) was markedly reduced. It is likely that abnormal TCR signaling in developing thymocytes may have caused altered positive and negative selection, with emergence of self-reactive T cells undergoing peripheral expansion. Furthermore, the low number of Treg cells, and the defect of activation-induced cell death, may have also contributed to induce and perpetuate clinical manifestations of immune dysregulation.

Macrophage Stimulating 1 (MST1) deficiency

The MST1 protein, also known as serine threonine kinase 4 (STK4), is a component of the Hippo signalling pathway, which regulates apoptosis and organ size. In the immune system, MST1 is activated in response to TCR or chemokine receptor engagement. Studies in mice have shown that Mst1 induces phosphorylation of Foxo1/Foxo3 [15*], two transcription factors required for Treg development [16,17]. Moreover, Mst1 also inhibits Akt activation [15*] and prevents Akt-mediated destabilization of Foxo1/Foxo3 [18]. Consistent with these observations, both the number and the function of natural and inducible Treg cells are reduced in *Mst1*$^{-/-}$ mice, which are prone to autoimmune disease, with lymphocytic infiltrates in multiple organs, and presence of anti-nuclear and anti-dsDNA antibodies in serum [15*,19]. Importantly, bone marrow chimeric experiments have shown that autoimmune manifestations of *Mst1*$^{-/-}$ mice are suppressed by wild-type Tregs, indicating that they are not intrinsic to Mst1-deficient effector T cells, but rather reflect abnormalities of the Treg compartment. Indeed, it has been recently shown that Mst1 expression by Tregs is required for immunological synapse formation and contact-dependent suppressive

function [20*]. However, perturbed Treg function is not the only mechanism by which Mst1 deficiency may cause immune dysregulation. It has been shown that Mst1 regulates integrin-dependent thymocyte trafficking, and is required for efficient antigen-specific association of single positive (SP) cells with mTECs. Accordingly, both thymic egress, as well as positive and negative selection of *Mst1*$^{-/-}$ thymocytes are affected [19,21,22]. Furthermore, absence of Mst1 in mice is associated with progressive loss of naïve T cells, reduced IL-2 production, increased activation-induced cell death of Th1 cells, skewing to a Th2 profile, and depletion of peripheral lymphoid organs [23-26].

Two groups have recently reported several patients with autosomal recessive MST1 deficiency [27**,28**]. The clinical phenotype of this disease includes recurrent bacterial and viral infections (including warts and molluscum contagiosum), dermatitis, and autoimmune haemolytic anemia. Severe and progressive reduction of naïve T cells, increased proportion of $CD8^+$ T_{EMRA} cells, restricted T cell repertoire, impaired proliferation to mitogens, and increased activation-induced cell death of T lymphocytes have been reported in these patients [27**,28**]. The B cell compartment of affected patients was also abnormal, with increased proportion of transitional B cells and a reduced frequency of marginal zone and switched memory B cells. Despite B cell lymphopenia, hypergammaglobulinemia and elevated levels of thyroglobulin and red blood cells autoantibodies and of rheumatoid factor were detected [27**]. Overall, these data confirm the critical role played by MST1 in immune system development and homeostasis.

ITK deficiency

ITK is a member of the TEC family of tyrosine kinases, and promotes maximal PLC-γ1 activation and Ca^{2+} influx in response to TCR stimulation [2]. Loss of Itk in mice causes only modest changes in the number of CD4+ cells in the thymus and the periphery [29]. However, both positive and negative selection of thymocytes are impaired due to reduced TCR signaling strength [29,30]. Importantly, Itk-deficient mice are nearly devoid of conventional CD8+ T cells, and instead have a marked expansion of innate-like, activated (CD44hi CD122hi CD62L-) CD8+ cells expressing Eomes and rapidly producing IFN-γ [31,32]. Recent data suggest that this aberrant developmental program reflect impaired expression of IRF-4, which normally suppresses Eomes expression [33]. Moreover, invariant NKT (iNKT) development, survival and function are impaired in *Itk*$^{-/-}$ mice [34-36].

ITK plays also an important role in peripheral immune homeostasis. A twofold increase in the proportion of activated/memory peripheral CD4+ lymphocytes, and impaired activation-induced cell death and defective expression of Fas ligand upon T cell activation have been reported in *Itk*$^{-/-}$ mice [37]. Production of IL-4 is reduced, and of IFN-γ is increased, upon activation of Itk-deficient CD4+ cells [38]. In spite of this, *Itk*$^{-/-}$ mice have increased serum IgE, resulting from expansion of γδ NKT lymphocytes [39]. Finally, ITK plays a critical role in the balance between TCR- and cytokine-induced signaling, allowing generation of protective immune responses while avoiding autoimmunity [40**]. Loss of Itk in mice is associated with decreased (but not abolished) TCR signaling, reduced phosphorylation of mTOR targets, and increased expression of Foxp3 [40**,41]. Moreover, Itk negatively

regulates expansion of natural Treg cells in response to IL-2, but is indispensable for Treg-mediated suppression of naïve CD4+ cell-induced colitis in *Rag*$^{-/-}$ mice [42*].

Several patients with autosomal recessive ITK deficiency have been reported in recent years [43,44*,45,46]. Their clinical phenotype includes both infections (especially due to herpesviruses) and immune dysregulation, with autoimmune cytopenias, lymphadenopathy, hepatosplenomegaly and lymphoproliferative disease, especially affecting the lungs. Laboratory data have revealed naïve CD4^{+} cell lymphopenia, increased proportion of activated T cells, defective proliferation to CD3 stimulation, deficiency of NKT cells, and progressive hypogammaglobulinemia.

Conclusions

Characterization of naturally-occurring or gene-targeted animal models, and of patients with defects in proximal components of TCR signaling has revealed an unanticipated complexity of phenotypes associated with these disorders. While impaired development and function of T cells cause the immunodeficiency associated with these disorders, immune dysregulation is also frequently observed and may be accounted for by multiple, non mutually exclusive mechanisms, including: a) abnormalities of positive and negative selection in the thymus as the result of reduced TCR signaling strength; b) effects of impaired thymopoiesis on maturation of thymic epithelial cells, affecting thymocyte cell fate; c) abnormalities of Treg development and/or function; d) defects of T cell survival and apoptosis; e) restriction in the number and diversity of T cells, and homeostatic T cell proliferation in the periphery; f) skewing in the distribution of different types of T helper (T_H) lymphocytes; g) inability to control infections, resulting in dysregulated activation of immune responses.

Importantly, individual mutations within the same gene may have a different effect on TCR signaling strength, causing distinct clinical and immunological phenotypes. Besides defects in *ZAP70*, *LCK*, *MST1*, and *ITK* genes described above, other examples of altered proximal T cell signaling associated with immune dysregulation have been described in mice, but not yet in humans. In particular, mice with a missense mutation in a critical tyrosine residue of SLP-76 have impaired intrathymic deletion of self-reactive CD4 single positive (SP) cells, whereas generation of Treg cells is largely preserved [47]. On the other hand, introduction of the LAT Y136F mutation in mice is associated with decreased phosphorylation of ERK1/2 and PLC-γ1, but increased phosphorylation of CD3ζ and Zap-70 [48], resulting in increased survival of self-reactive thymocytes, severe autoimmunity and lymphoproliferation [49].

Immune dysregulation has been frequently observed also in patients with defects of distal components of TCR signaling, such as those with mutations in ORAI1 and STIM1 that control store-operated Ca^{2+} entry [50,51*], or in patients with DOCK8 deficiency, in which reduced number and function of Treg cells and defective peripheral B cell tolerance have been recently reported [52*].

Widespread availability of potent tools to investigate the human genome (whole exome and whole genome sequencing) and to analyze function at the single cell level (RNA sequencing

on single sorted cells) is expected to lead to further advances of the field in the near future, permitting identification of novel genetic defects of TCR signaling, and of distinct clinical and immunological phenotypes associated with novel mutations in known genes.

Finally, inhibition of TCR signaling represents an attractive strategy to treat autoimmunity. For example, following demonstration that concurrent deletion of *Itk* or administration of Itk inhibitors in *Ctla4*$^{-/-}$ reduce lethality and inhibit accumulation of T cell infiltrates in peripheral organs [53*], several ITK inhibitors are under development [54*]. The study of patients with inborn errors of immunity may help identify thresholds of TCR signaling that may temper immune activation without affecting immune regulatory mechanisms, and thus guide towards development of novel pharmacological agents with defined immunosuppressive activity.

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* of special interest

** of outstanding interest

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Highlights

- We review recent discoveries of proximal defects of T cell receptor (TCR) signaling
- Altered TCR signaling may cause immunodeficiency with immune dysregulation
- The study of defects of TCR signaling may help develop novel immunosuppressive drugs