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INSIGHTS

IL2RB maintains immune harmony

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How the IL-2 receptor β-chain specifically shapes immunity has remained enigmatic. In this issue of *JEM*, Zhang et al. (https://doi.org/10.1084/jem.20182304) and Fernandez et al. (https://doi.org/10.1084/jem.20182015) independently report the first observations of autosomal recessive mutations in *IL2RB*, revealing a requirement for *IL2RB* in immunity and peripheral immune tolerance.

Human primary immunodeficiencies have provided key insights to immune function that often also raise new, unexpected questions. In a landmark paper published in 1993, Warren Leonard and colleagues identified mutations in *IL2RG*, encoding the IL-2 receptor γ-subunit, as a cause of X-linked severe combined immunodeficiency (X-SCID) associated with defective T and natural killer (NK) cell development (Noguchi et al., 1993). Biochemical studies had previously determined that the IL-2 receptor consists of a heterotrimeric receptor complex comprised of a low-affinity α-chain (CD25), a β-chain (IL-2Rβ/CD122), and the γ-chain (CD132). While providing a molecular explanation for X-SCID (popularly known as "bubble boy" syndrome), the association of *IL2RG* mutations with X-SCID was initially puzzling to immunologists, as IL-2 is not required for the development of T cells in the thymus. We now know that the IL-2 receptor γ-chain is shared between multiple cytokine receptors, including those for IL-4, IL-7, IL-9, IL-15, and IL-21 (Rochman et al., 2009). Consequently, SCID is also associated with autosomal recessive mutations in *IL7RA*, which encodes a polypeptide forming the IL-7 receptor together with the common γ-chain, thereby explaining the defective T cell development observed in X-SCID. However, important questions still remain with respect to the role of the shared IL-2 and IL-15 receptor in human immunity. This receptor is expressed on T cells and NK cells and is comprised of the common γ-chain and IL-2Rβ. One missing piece to this puzzle is the immunological phenotypes and clinical characteristics of individuals with mutations in *IL2RB*, who have hitherto not been described.

In the current issue of *JEM*, Zhang et al. and Fernandez et al. describe a total of five kindreds with autosomal recessive mutations in *IL2RB*—seven affected live-born children with immunodeficiency and autoimmune disease, and three perinatally affected fatalities. Clinical hallmarks of the disease included enteropathy, skin abnormalities, autoimmune hemolytic anemia, and hypergammaglobulinemia, in addition to susceptibility to respiratory and herpesvirus infections. As such, human *IL2RB* deficiency shares several features of immune dysregulation with *Il2rb* knock-out mice, including autoimmune hemolytic anemia, hypergammaglobulinemia, elevated autoantibodies, lymphadenopathy, and splenomegaly (Suzuki et al., 1995). In contrast to *Il2rb* knock-out mice, however, human patients also displayed enteropathy and skin abnormalities. The severe, early-onset autoimmune manifestations of human *IL2RB* deficiency bear resemblance to the immunodysregulation, polyendocrinopathy and enteropathy X-linked (IPEX) syndrome caused by mutations in *FOXP3* (Ziegler, 2006). However, a distinctive feature of patients with *IL2RB* mutations is their susceptibility to herpesviruses, especially CMV and EBV. The combination of early-onset autoimmunity and severe CMV infections has also been reported in three patients with different *IL2RA* mutations (Sharfe et al., 1997; Caudy et al., 2007; Goudy et al., 2013). Notably, an additional patient lacking *IL2RB* transcripts and IL-2Rβ expression was previously described

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by Gaspar and colleagues (Gilmour et al., 2001). This infant presented with severe viral and fungal infections; however, no autoimmune manifestations were reported. Nonetheless, overall, autosomal recessive mutations in *IL2RA* and *IL2RB* are characterized by severe autoimmunity and susceptibility to viral infections.

In their studies, the teams of Lenardo and Hsieh characterized patients with several different *IL2RB* mutations. Fernandez et al. (2019) identified a nine nucleotide in-frame *IL2RB* deletion resulting in the loss of three amino acids (p.Pro222_Gln225del) that disrupts the extracellular, highly conserved WSXWS motif. This motif is common to type I cytokine receptors and serves to stabilize interactions between the subunits and conduce ligand-binding conformational changes for receptor activation. As assessed by flow cytometry on patient lymphocytes, the *IL2RB* p.Pro222_Gln225del mutation drastically reduced IL-2Rβ surface expression. Despite this, cells still retained some capacity to respond to IL-2 or IL-15, exhibiting induction of low levels of STAT5 phosphorylation upon

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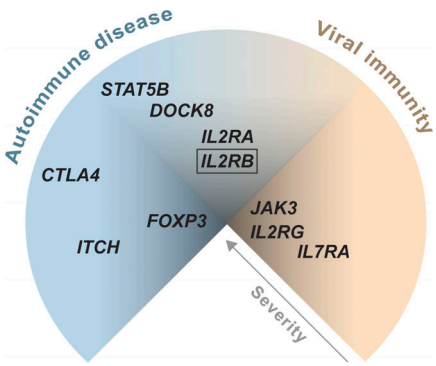

An illustrative representation of genetic deficiencies comparable to the *IL2RB* deficiencies reported by Zhang et al. (2019) and Fernandez et al. (2019). Genes are clustered by affiliation with presentation of autoimmune disease (left, blue) or defective viral immunity (right, orange), with increasing severity depicted by proximity to the center. IPEX syndrome (caused by mutations in *FOXP3*) is a hallmark autoimmune disorder, occurring through a lack of regulatory T cells. In this issue, it is reported that patients with *IL2RB* mutations presented with an IPEX-like syndrome and viral susceptibility. Similarly, patients with deficiencies in *IL2RA*, *DOCK8*, *CTLA4*, and *ITCH* have been described with IPEX-like disease. IL-2Rβ forms a signaling complex with the common γ-chain, and deficiencies in *IL2RG* cause X-SCID, which imparts extreme susceptibility to viral infection. SCID is also associated with deficiencies in *JAK3* (which transmits signals from the γ-chain) as well as *IL7RA* mutations. Finally, STAT5 transduces signals downstream of IL-2Rβ (among other receptors), with deficiencies in *STAT5B* involving autoimmune presentation and some increased susceptibility to viral infection. Human deficiencies in *STAT5A* have yet to be identified.

stimulation. Similarly, two kindreds with an *IL2RB* p.Leu77Pro mutation displayed severely reduced IL-2Rβ surface expression (Zhang et al., 2019). Microscopy revealed that the IL-2Rβ p.Leu77Pro mutant was intracellularly sequestered due to misfolding. Recapitulating patient IL-2 receptor signaling through transfection of HEK-293T cells with constructs encoding IL-2Rβ variants, the common γ-chain, and the signaling JAK3 and STAT5 components, Zhang et al. (2019) demonstrated that the p.Leu77Pro mutant retained some signaling capacity. In contrast, from a fourth kindred, surface expression of the IL-2Rβ p.Ser40Leu mutant was only partially reduced, but this mutant could not induce STAT5 phosphorylation in reconstituted HEK-293T cells. The p.Ser40Leu mutation is located at a ligand-binding interface, and was therefore predicted to interfere with receptor activation. Thus, four of the kindreds carried homozygous *IL2RB* missense mutations with at least the *IL2RB* p.Leu77Pro and p.Pro222_Gln225del representing hypomorphic mutations retaining some residual activity. Notably, the fifth kindred carried nonsense *IL2RB* p.Gln96* and consisted of two fetuses and a prematurely born neonate who died of respiratory failure shortly after delivery. All affected individuals in this family displayed intra-uterine growth retardation and reduced fetal movement. All three were also noted to have skin-floating membranes in the amniotic fluid, mirroring the autoimmune skin desquamation in utero observed in prenatal IPEX patients (Louie et al., 2017). Conceivably, nonsense *IL2RB* mutations might generally result in prenatal lethality.

The severe clinical manifestations of *IL2RB* deficiency raise important questions with respect to the pathophysiological basis of the disease. The infant-onset autoimmune manifestations are shared with IPEX syndrome, in which an absence of regulatory T cells breaks peripheral immune tolerance through uncontrolled autoreactive T cells. Fittingly, the frequency of $CD4^+CD25^+FoxP3^+$ regulatory T cells were clearly diminished in the two *IL2RB*-deficient patients examined. Relative to *Il2rb*-deficient mice, autoimmune manifestations in *IL2RB*-deficient patients appear more severe. These differences could in part be explained by the use of syngeneic animal models housed in pathogen-free environments, but could also represent distinctions between human and mouse fetal immune development (Mold and McCune, 2012). In humans, $CD4^+CD25^+FoxP3^+$ regulatory T cells constitute a large fraction of T cells during the second trimester of fetal development where they can control responses to maternal alloantigens (Mold et al., 2008). Thus, mutations in *FOXP3*, *IL2RA*, and *IL2RB* appear to highlight a requirement for regulatory T cells in suppressing fetal immune responses that otherwise could lead to autoimmunity.

With respect to immunodeficiency, NK cells play an important role in the control of herpesviruses, such as CMV and EBV. While *Il2rb* knock-out mice have diminished NK cell numbers (Suzuki et al., 1997), patients with *IL2RB* p.Ser40Leu, p.Leu77Pro, and p.Pro222_Gln225del mutations surprisingly all displayed increased peripheral blood NK cell numbers and frequencies. This difference between humans and mice could be due to the fact that the patients carried hypomorphic *IL2RB* mutations that supported low levels of IL-2Rβ expression and signaling in NK cells—more so than that observed in T cells. In vivo, such signaling was likely promoted by the highly elevated levels of serum IL-2 and IL-15. Despite preservation of NK cell numbers, *IL2RB*-deficient patients exhibited a more prominent immature NK cell phenotype, with elevated frequencies of $CD56^{bright}$ cells and negligible expression of the differentiation marker CD57. In one examined patient, $CD56^{bright}$ NK cells expressed unusually elevated levels of the cytotoxic granule constituent proteins perforin and granzyme B, possibly in response to the chronic cytokine exposure. Functionally, NK cells in the *IL2RB*-deficient patients were capable of degranulation and target cell killing, which was enhanced by IL-2 or IL-15 priming. In contrast, NK cells appeared to display a selective resistance to IFN-γ production through IL-2 or IL-15 stimulation, while responding normally to IL-12 and IL-18. These findings suggest *IL2RB* deficiency differentially affects NK cell functions.

The activating NKG2C receptor has been associated with protective NK cell responses to CMV, driving expansions of $NKG2C^+$ NK cells that display a so-called adaptive phenotype. A large population of $NKG2C^+$ NK cells was observed in one of the patients with the *IL2RB* p.Leu77Pro mutation, but these NK cells lacked other hallmarks of adaptive NK cells (Tesi et al., 2016). Thus, susceptibility to CMV in the *IL2RB*-deficient patients may be attributed to defects in NK cell differentiation toward adaptive NK cells, but the relative contribution of differentiated $CD8^+$ T cells and NK cells to control of these viruses in the patients is difficult to

		IL-2Rβ	
		Surface expression	pSTAT5 response
IL2RB mutation	WT	+++	+++
	p.Gln96*	–	–
	p.Ser40Leu	++	–
	p.Leu77Pro	+	+
	p.Pro222_Gln225del	+	+

A summary of the relative expression and functional capacity of the *IL2RB* mutations described by Zhang et al. (2019) and Fernandez et al. (2019) in this issue of *JEM*, compared with WT IL-2Rβ (+++, high; ++, moderate; +, low; –, negligible).

discern as these lymphocytes share several molecular pathways promoting differentiation (Tesi et al., 2016). Furthermore, other immune cells mediating antiviral immunity, including γδ T cells and mature dendritic cells, express IL-2Rβ. Although not examined in the papers published in this issue, these cell types may also be affected by the *IL2RB* mutations, potentially influencing the course of CMV disease in these patients.

Importantly, two *IL2RB*-deficient patients were successfully treated with allogeneic hematopoietic stem cell transplantation. Zhang et al. (2019) also discuss the potential use of engineered IL-2 proteins as a means of stimulating lymphocytes in patients with hypomorphic *IL2RB* mutations, but the efficacy of such therapeutic approaches remains to be tested. Using this strategy may also present challenges given the patients already expressed elevated serum IL-2 and IL-15.

In summary, mutations in *IL2RB*, *FOXP3*, and *IL2RA* share clinical features of severe immune dysregulation, reflecting an important role of regulatory T cells in maintaining immune tolerance. Contrasting *FOXP3* deficiency, however, mutations in *IL2RB* and *IL2RA* predispose to severe herpesvirus disease, highlighting the requirement for intact signaling through the IL-2/IL-15 receptor for effective immunity to complex viral infections. An outstanding question is the relative contributions of IL-2 versus IL-15 to immune tolerance and antiviral immunity. With time, the study of new primary immunodeficiency patients promise to provide answers to this and other intriguing questions.

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