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Human *IL2RA* null mutation mediates immunodeficiency with lymphoproliferation and autoimmunity

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Abstract Cell-surface CD25 expression is critical for maintaining immune function and homeostasis. As in few reported cases, CD25 deficiency manifests with severe autoimmune enteritis and viral infections. To dissect the underlying immunological mechanisms driving these symptoms, we analyzed the regulatory and effector T cell functions in a CD25 deficient patient harboring a novel *IL2RA* mutation. Pronounced lymphoproliferation, mainly of the $CD8^+$ T cells, was detected together with an increase in T cell activation markers and elevated serum cytokines. However, Ag-specific responses were impaired *in vivo* and *in vitro*. Activated $CD8^+STAT5^+$ T cells with lytic potential infiltrated the skin, even though $FOXP3^+$ Tregs were present and maintained a higher capacity to respond to IL-2 compared to other T-cell subsets.

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Thus, the complex pathogenesis of CD25 deficiency provides invaluable insight into the role of IL2/IL-2RA-dependent regulation in autoimmunity and inflammatory diseases.

1. Introduction

The interleukin-2 (IL-2) receptor is formed by the α (IL-2RA, CD25) [1], β (IL-2RB, CD122) [2] and γ_{common} (IL-2RG, CD132) [3] subunits, and plays a vital role in maintaining the immune system. Among the IL-2 receptors, CD25 is a unique subunit that exclusively binds IL-2, while CD132 binds the common γ_c family cytokines (IL-4, IL-7, IL-9, IL-15 and IL-21), and the CD122 subunit binds IL-15. CD25 is constitutively expressed at high levels by $CD4^+CD25^+FOXP3^+$ regulatory T cells (Tregs), and enables them to be the first responders to IL-2 during an immune response [4] and promotes the transcription of FOXP3 by amplifying IL-2 signaling in a STAT5-dependent fashion [5]. Interestingly, single nucleotide polymorphism (SNP) studies of the *IL2RA* gene have been associated with several forms of autoimmunity [6–10] demonstrating that IL-2 signaling via CD25 is an important axis in regulating tolerance. CD25 is also critical for effector T cell expansion in response to IL-2 immediately after antigenic stimulation. Although both $CD4^+$ and $CD8^+$ T cells up regulate CD25 and IL-2RB upon activation, $CD8^+$ T cells are more susceptible to IL-2 stimulation, probably due to their higher level of IL-2RB expression both in mice [11] and humans [12,13].

The immunological consequence resulting from the loss of CD25 has been ill-defined in man. Roifman's group was the first to describe a CD25 deficient patient who suffered from chronic infections and severe autoimmunity [14] resembling Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome, caused by mutations in *FOXP3* gene [15]. This IPEX-like patient possessed a translation frameshift mutation in the *IL2RA* gene ablating its expression. Similarly, a second report described a patient with a different frameshift mutation in the *IL2RA* gene leading to a CD25 null phenotype with comparable clinical manifestations [16].

Here we describe the immunological findings of a patient carrying an *IL2RA* mutation not previously reported, selectively abrogating CD25 cell surface expression. Our results show, for the first time in human, the complex immunopathology associated with CD25 deficiency, and reveal a distinct pathogenetic mechanism of immune dysregulation.

2. Material and methods

2.1. *IL2RA* molecular analysis

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's recommendations. PCR for each of the 8 exons of the human *IL2RA* gene (including exon/intron boundaries) was performed using PCR techniques as previously reported [17] and sequence conservation analysis of mutations was performed using PolyPhen, SIFT and SNPs3D tools.

2.2. Flow cytometry

PBMCs were isolated using Lymphoprep (Axis-shield) density gradient centrifugation. Surface Ab staining was performed for 30 min on ice in the absence of light using a 2% bovine serum albumin PBS mixture. Cells were washed and fixed with either 2% paraformaldehyde (Pierce) for later acquisition or with FOXP3 perm/fix buffer (eBioscience) to be further stained for FOXP3 or Ki67 The following Abs (all antibodies purchased from BD Biosciences unless otherwise noted): CD4 (SK3), CD8 (SK1), CD25 (2A3; M-A251), CD45RA (HI100), CD49d (L25), CD62L (SK11), CD69 (FN50), CD122 (MIKB2), CD132 (TUGh4), Ki67 (B56), FOXP3 (eBioscience PCH101), HLA-DR (L243), FASL (NOK-1), and HELIOS (22F6) (Biolegend).

2.3. T cell line generation and stimulation

Healthy donor cell lines were generated by stimulating 1×10^6 PBMCs with PHA 1 μg/ml (Sigma) in X-Vivo media (Biowhitaker) containing 5% human serum (Biowhitaker), 1% penicillin and streptomycin (Lonza), IL-2 (40 U/ml, Proleukin (Novartis)). On days 9, 14 and 20 the cells were washed and plated in the presence of IL-2 (100 U/ml), IL-7 (10 ng/ml), and IL-15 (10 ng/ml). For the CD25 deficient patient, $CD4^+$ T cells were enriched using $CD4^+$ T cell negative selection beads (Miltenyi) and cultured with IL-2 (100 U/ml), IL-15 (10 ng/ml), IL-7 (10 ng/ml). Cells were washed and restimulated with the same conditions on days 7, 11, and 20. On day 24, cells were washed and stimulated in 24 well plates (Corning) containing plate bound anti-CD3 (10 μg/ml) (BD Pharmingen) and anti-CD28 (1 μg/ml) (BD Pharmingen) in the presence or absence of IL-2 (100 U/ml) and IL-15 (10 ng/ml) for 6 h.

2.4. Measurement of sCD25

Levels of sCD25 were evaluated using a commercially available ELISA kit (BD Pharmingen). To measure sCD25 from activated cells, PBMCs (1×10^5) were stimulated for 72 h in complete RPMI (Biowhitaker) with plate-bound anti-CD3 (OKT3) (10 μg/ml) and soluble anti-CD28 (2 μg/ml) in the presence or absence of IL-2 (1000U/ml), TPA (Sigma)/Ionomycin (Sigma), or left unstimulated.

2.5. Phospho flow cytometry

To determine the phosphorylation (p) status of STAT3 and STAT5 after cytokine stimulation, a barcode technique was