# IPEX Syndrome and IPEX-Related Disorders

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**Abstract**

Congenital immune disorders due to immune dysregulation usually present with multi-organ autoimmune manifestations, and recent genetic and molecular diagnosis techniques have deeply implemented the identification of new monogenic defects leading to an altered immune homeostasis. Regulatory T cells (Tregs) play an essential role in controlling immune response, and mutations affecting the transcription factor FOXP3 cause immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. Strikingly, similar clinical phenotypes resemble IPEX but due to distinct molecular defects have been identified and classified as IPEX-related disorders. These are associated to altered expression of a plethora of factors, either pivotal for Tregs biology or Tregs unrelated, and acting at different levels during immune response regulation. The clinical similarities and differences between these inborn errors of immunity, along with their molecular cause, diagnosis, and treatment options, will be discussed.

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**Keywords**

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Immune dysregulation · Autoimmunity · Tregs

## 10.1 Introduction

Primary immunodeficiency disorders (PIDs), also defined as inborn errors of immunity, are a heterogeneous group of diseases characterized by an impaired immune response and by an altered homeostasis. They include more than 400 diseases, caused by monogenic germline mutations affecting gene expression, and associated clinical features range from increased susceptibility to infections to immune dysregulation, resulting in autoimmunity, allergy, inflammation, and/or malignancy [1]. The overall incidence is around 1:10.000, and they are more prevalent in children. PIDs clinical and molecular characterization is currently boosting the identification of previously unknown immune regulatory mechanisms, in light of a parallel technological improvement in genetic analysis approaches. The next-generation DNA sequencing (NGS) technology has heavily contributed to new disease-associated genes discovery, having a profound impact on diagnosis and development of targeted therapies.

The emerging prevalence, among PIDs, of immune dysregulation signs as autoimmunity has shifted the attention toward immune tolerance. While central tolerance depends on thymic epithelial cells and self-antigens expression driving T cells selection, peripheral tolerance is maintained by regulatory T cells (Tregs). This chapter will discuss about immune dysregulation-associated diseases—IPEX syndrome and IPEX-related disorders—and how these distinct and partially overlapping manifestations can be seen, depending on the disease-causing gene, from a "Tregs point of view," in a wider context of immune response pathways and specific cellular mechanisms.

## 10.2 IPEX Syndrome

The immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome is a rare disorder causing life-threatening systemic autoimmunity due to immune dysregulation and is the first human disease whose characterization highlighted Tregs pivotal role in immune homeostasis. The IPEX hallmark features comprise severe early-onset enteropathy, chronic dermatitis, elevated IgE levels, and autoimmune endocrinopathies, such as early-onset insulin-dependent type 1 diabetes mellitus (T1D) and/or thyroiditis. Patients develop symptoms early in infancy, and most die prematurely. IPEX syndrome is caused by mutations in *FOXP3* gene, located on X chromosome, coding for a key transcription factor critical for the development and function of $CD4^+$ $CD25^+$ Tregs and able to regulate the expression of multiple genes involved in T cell response, as it will be discussed later.

The classical IPEX syndrome presents with a triad of diarrhea or enteropathy, endocrinopathy (most commonly T1D), and eczema (Table 10.1, Fig. 10.1).

**Table 10.1** IPEX-related disorders classification: genes, inheritance, and main manifestations are indicated (Table adapted from the IUIS classification 2017 [1, 6, 142, 143]) (All open access BY CC License 4.0)

| Disease | IUIS Classification table | Genetic defect | Inheritance | T cells | B cells | Other features | Immunoglobulins | Associated features |
|---|---|---|---|---|---|---|---|---|
| *CD25* deficiency | Table 4. Disease of immune dysregulation | IL-2RA | AR | Normal to decreased; no CD4+ CD25+ cells with impaired Tregs function | | | | Lymphoproliferation, autoimmunity and impaired T cell proliferation in vitro |
| STAT5b deficiency | Table2. Combined immunodeficiency with associated or syndromic features. | STAT5B | AR | Modestly decreased, reduced Tregs number and function | Normal | | Hypergammaglobulinemia, increased IgE | Growth-hormone insensitive dwarfism, dysmorphic features; eczema; lymphocytic interstitial pneumonitis; insulin like growth factor deficiency, prominent autoimmunity |
| CTLA-4 Haploinsufficiency | Table 4. Disease of immune dysregulation | CTLA4 | AD | Reduced T cell and impaired Tregs function | Reduced B cell | Impaired Tregs function | | Auto immune cytopenias, enteropathy, interstitial lung disease, extra lymphoid, lymphocytic tissue infiltration and recurrent infections |
| LRBA deficiency | Table 4. Disease of immune dysregulation | LRBA | AR | Normal or decreased CD4 + T cells and dysregulated T cells | Decreased or normal B cell | | Reduced IgG and IgA | Inflammatory bowel disease, autoimmune cytopenias, enteropathy, interstitial lung disease, extra-lymphoid, lymphocytic infiltration, lymphoproliferation, autoimmune and recurrent infections |

(continued)

**Table 10.1** (continued)

| Disease | IUIS Classification table | Genetic defect | Inheritance | T cells | B cells | Other features | Immunoglobulins | Associated features |
|---|---|---|---|---|---|---|---|---|
| STAT1 GOF | Table 6. Defects in intrinsic and innate immunity | STAT1 | AD | T cells are affected mainly associated with impaired development of Th17 cells | | B cells and monocytes may also be affected | | CMC, various fungal, bacterial and viral (HSV) infections, autoimmunity (thyroiditis, diabetes, cytopenias), enteropathy |
| STAT3 GOF | Table 4. Disease of immune dysregulation | STAT3 GOF | AD | Decreased, decreased Tregs with impaired function | Decreased | Enhanced STAT3 signalling, leading to increased Th17 cell differentiation | | Lymphoproliferation, solid organ auto immunity, recurrent infections |
| CD122 deficiency | Table 4. Disease of immune dysregulation | IL-2RB | AR | Increased memory CD8+ T cells, decreased Tregs | Increased memory B cells | Diminished IL-2RB expression, dysregulation signalling in response to IL-2 and IL-15, increased immature NK cells | | Lymphoproliferation, lymphadenopathy, hepatosplenomegaly, autoimmune haemolytic anaemia, dermatitis, enteropathy, hypergammaglobulinemia, recurrent viral (EBV, CMV) infections |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| DOCK8 deficiency | Table 1. Immunodeficiencies affecting cellular and humoral immunity | DOCK8 | AR | Reduced naïve CD8+ T cells, increased exhausted CD8+ TEM cells, reduced MAIT, NKT cells, increased γδ T cells; poor proliferation: few Tregs with poor function | Increased total B cells, reduced memory B cells and poor peripheral B cell tolerance | Low NK cells with poor function, eosinophilia | Low IgM, normal/high IgG and IgA, very high IgE, poor antibody responses | Recurrent infections, cutaneous viral, fungal and staphylococcal infections, severe atopy/allergic disease, cancer diathesis |
| Activated p110δ syndrome (APDS) | Table 3. Predominantly antibody deficiencies | PIK3CD GOF (APDS1) | AD | | Reduced memory B cells, increased transitional B cells, decreased pro B cells | | Normal of increased IgM, low IgA and IgG | Severe bacterial infections; EBV ± CMV viremia, lymphadenopathy/ splenomegaly, autoimmunity, lymphoproliferation, lymphoma |
| | Table 3. Predominantly antibody deficiencies | PIK3R1 (APDS2) | AD | | Reduced memory B cells, increased transitional B cells | | | Severe bacterial infections, lymphadenopathy/ splenomegaly, lymphoproliferation, lymphoma; developmental delay |

(continued)

**Table 10.1** (continued)

| Disease | IUIS Classification table | Genetic defect | Inheritance | T cells | B cells | Other features | Immunoglobulins | Associated features |
|---|---|---|---|---|---|---|---|---|
| Immunodeficiency with multiple intestinal atresias | Table 2. Combined immunodeficiencies with associated or syndromic features | TTC7A | AR | Variable; but may have low or absent TRECs on new born screening. Low T cells; may have SCID phenotype at birth | Normal or decreased | May have a SCID Phenotype at birth | Markedly low IgA, IgG, and IgM | Bacterial (sepsis), fungal, viral infections; multiple intestinal atresias, often with intrauterine polyhydramnios and early demise |
| Tricho-hepato-enteric syndrome | Table 2. Combined Immunodeficiencies with associated or syndromic features | TTC37 SKIV2L | AR | Impaired INFγ production | Variable decrease in numbers of switched memory B cells | | Hypogammaglobulinemia with possible low antibody responses (anti pneumococcal) | Respiratory infections; recurrent bacterial and viral infections IUGR; facial dysmorphic features, wooly hair; early onset intractable diarrhea, liver cirrhosis; platelet abnormalities and growth restriction |

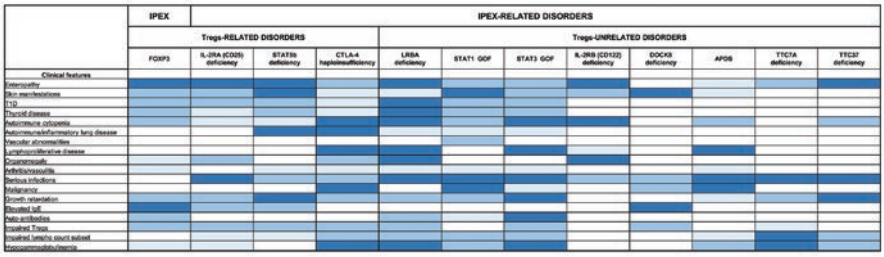


**Fig 10.1** IPEX syndrome and IPEX-related disorders clinical features. (Modified from Ciullini Mannurita *et al.*, 2017)

Histopathological features of endoscopic biopsies done in IPEX patients included a heterogeneous array of findings, as pale fragile mucosa of upper gastrointestinal tract, duodenal villous atrophy, with varying signs of inflammation, ulceration in addition to severe colitis. Furthermore, such cases are associated with inflammation of the lamina propria with lymphocytic, macrophagic, eosinophilic, and plasmocyte infiltration in the duodenum, while patients with lower gastrointestinal tract involvement show predominant inflammatory cell infiltrates with lymphocytes and eosinophils [2]. Additionally, IPEX patients can present with T1D very soon after birth or, sometimes, congenital T1D can occur. Studies have demonstrated that early-onset insulin-requiring diabetes mellitus can sometimes be the only feature of IPEX in infants, especially in the absence of other known genetic causes [3]. Nonetheless, patients can also present with other endocrine disorders, most commonly thyroid disease and less commonly adrenal disease or adrenal insufficiency. The endocrine features of the disease are most entirely due to autoimmunity, with the presence of thyroid, glutamic acid decarboxylase (GAD), and islet cells autoantibodies.

Skin manifestations, and specifically dermatitis, represent a strong arm of the classical IPEX triad. Eczema is the most common presentation in IPEX; however, several reports have revealed that, in addition to eczematous dermatitis, other dermatological manifestations occurring in IPEX patients include erythroderma, psoriasiform dermatitis, alopecia, and bullous pemphigoid, with the latter two most prevalent in older individuals [4]. These manifestations are usually associated with the presence of autoantibodies versus different skin antigens.

In addition to diarrhea/enteropathy, multiple endocrinopathies, and dermatological diseases, patients with classical IPEX phenotype can present with acute, chronic, or recurrent autoimmune cytopenias, lymphoproliferation, and autoimmune hepatitis [5].

Autoimmune cytopenias frequently associated with IPEX syndrome are anemia, neutropenia, and thrombocytopenia. Interestingly, autoimmunity can present at varying degrees of systemic and/or organ-specific autoimmunity, which can include arthralgia/arthritis as well as renal and neurological manifestations, with the latter two mostly associated with vasculitis. Renal disease can present with different

patterns, and the most common manifestations described include glomerulonephritis, interstitial nephritis, hypertension, persistent proteinuria, or hematuria. Renal disease is usually either secondary to autoimmunity or secondary to side effects of immunosuppressive therapy [5]. Other serious autoimmune manifestations include pulmonary disease, which is associated with asthma, lymphadenopathy, and interstitial lung disease. Interestingly, many reports have described the different cardiovascular diseases commonly associated with IPEX and IPEX-like disorders: pericarditis, pericardial effusions, aneurysms, atrial flutter, and dilated aortic root. Furthermore, other IPEX-associated clinical features included neurological manifestations such seizures, developmental delay, and ventricular disease [6]. Most importantly, as IPEX and IPEX-like diseases are classified as primary immunodeficiency diseases, it is essential to mention that they can also present with recurrent severe infections, usually complicated by sepsis, such as meningitis, peritonitis, and pneumonia, with the varying causative organisms including *Staphylococcus aureus spp., Cytomegalovirus, and Candida spp.* Other serious infections associated with PIDs are due to *Pneumocystis jirovecii* infection and usually are due to the immunosuppressive therapy rather than to the primary disease [4–6].

## 10.3 IPEX-Related Disorders

Significantly, there is an emerging group of children presenting various clinical features typical of IPEX syndrome not associated with *FOXP3* mutations and whose clinical phenotype has been defined as "IPEX-like." Our recent study shows as, among a cohort of 173 patients, almost half of them have no *FOXP3* gene mutations, but they do display gene variants in other genes [6]. The IPEX-like clinical presentation can include other manifestations which can differ from the classical IPEX syndrome as patients usually present with a more severe systemic autoimmunity. Despite this, the common feature between these two clinical syndromes is enteropathy, usually evident as watery diarrhea [6]. Moreover, the severe persistent diarrhea usually leads to failure to thrive; although most commonly attributed to enteropathy, it can also be due to food allergy and malabsorption. Autoimmune enteropathy is a common pivotal feature of many inherited primary immune deficiencies, as IPEX and IPEX-like, although the severity and the clinical course might vary among patients with the same genotype. In this regard, the role of microbial diversity and composition is still unclear. The intestine functions as a major gateway for external environment and contains an extensive network of secondary lymphoid organs, and it is home to several lymphocytes including intestine-specific subpopulations. It has become evident that individual commensal species influence the makeup of T lymphocytes subsets, modulating a range of effector function with a general impact on immunity that reaches well beyond the intestinal lamina propria (Fig. 10.1).

Recently, the International Union of Immunological Society (IUIS) has updated PIDs classification, classifying IPEX-like disorders under different categories despite having similar clinical manifestations as IPEX syndrome. This is due to the

fact that these diseases are associated with other main clinical manifestations, which are considered primary features. Nevertheless, this doesn't deny that these disorders can commonly present with an IPEX-like picture, whose main manifestations are shown in Table 10.1 [6, 7].

Indeed, rapid development of next-generation DNA sequencing deeply impacted the discovery of new genetic aberrations affecting factors not previously associated to immune response regulatory mechanisms. In a cost-effective and time-efficient manner, sequencing of targeted gene panels, as well as whole exomes or whole genomes, allows efficient analysis of cohorts of patients whose disease is suspected to have a monogenic cause. Currently, IPEX-like disorders are defined as presenting with a similar phenotype to IPEX syndrome and are due to mutations in *CD25* (*IL-2RA*), *CD122* (*IL-2RB*), *STAT1*, *STAT3*, *STAT5B*, *PI3KCD*, *PI3KR1*, *CTLA4*, *LRBA*, *DOCK8*, *TTC7A,* and *TTC37* genes (Fig. 10.1). While these disorders are defined according to clinical manifestation, a parallel nomenclature according to the role played by impaired factors in these monogenic and partially overlapping disease can be applied by classifying regulatory mechanisms and their consequent dysfunction in light of their role (direct or indirect) in Tregs homeostasis.

## 10.4 Tregs or Not Tregs: When the Deficiency is Defining You

An efficient adaptive immune response is pivotal for survival, as well as self-reactivity control in the host, mediated by regulatory T cells (Tregs). Tregs are specialized T cells required for peripheral immune tolerance and immune response regulation in both human and mice. They are able to suppress many effector T cell (Teff) functions, such as proliferation, pro-inflammatory cytokines, and growth factors production [8]. The discovery of *FOXP3* gene deficiency as cause of IPEX has been followed by extensive Tregs biology dissection along with the characterization of IPEX-related disorders. As most patients with IPEX-like features have wild-type FOXP3 molecules, several biochemical studies indicated that FOXP3 may cooperate with multiple partners in a dynamic assembled supermolecular complex to modulate gene transcription and regulatory T cell function [9, 10]. Thus, it became evident that other genetic factors may be responsible for the immune dysregulation observed in IPEX-like patients.

Tregs constitutively express CD25, the subunit of the trimetric receptor for interleukin-2 (IL-2). The IL-2 plays a critical role in the maintenance of Tregs in vivo. The signaling cascade activated upon IL-2 binding to its receptor activates the signal transducer and activator of transcription 5b (STAT5b), which translocates to the nucleus and binds to a highly conserved STAT-binding site located within the first intron of the *FOXP3* gene, thus enhancing its transcription [8]. Patients with *STAT5b* or *CD25* (*IL-2RA*) mutations have been reported and showed decreased number and function of Tregs and a clinical phenotype similar to IPEX. The *IL-2R* transcription is upregulated by the complex formed by FOXP3 and the nuclear factor of activated T cells (NFAT), which binds to *IL-2* and *CD25* promoters, respectively suppressing and enhancing their transcription [8]. Moreover, the cytotoxic T-lymphocyte

antigen 4 (CTLA-4) protein is a key regulator of immune response involved in maintenance of peripheral tolerance by regulatory T cells. Its loss is causing fatal autoimmunity in mice, and it has been identified as a cause of a dysregulation syndrome with autosomal dominant inheritance characterized by hypogammaglobulinemia, recurrent infections, and autoimmune manifestations. In human, the *CTLA4* haploinsufficiency causes a dysregulation of FOXP3$^+$ Tregs, with cell hyperactivation and effector T lymphocyte infiltrates [8].

However, diseases of immune regulation are not exclusively caused by an altered regulatory T cells function. Other factors involved in alternative and/or overlapping mechanisms are found to be responsible for IPEX-like clinical phenotype. For instance, patients with IPEX-like phenotype due to monoallelic *STAT1* mutations have been described. They show a broad spectrum of clinical symptoms including a variety of infectious, in particular fungal infections, and autoimmune features, as well as carcinomas and aneurysms associated with a poor outcome [11]. In addition, germline gain-of-function (GOF) mutations in *STAT3* have also been associated with autoimmune disease, with involvement of the endocrine glands, skin, and hematologic compartment. The transcription factor STAT3 is involved in the regulation of STAT1 and STAT5, and *STAT3* GOF mutations cause a lack of STAT1 and STAT5 phosphorylation, resulting in Tregs dysfunction [8]. Lastly, patients with clinical phenotype resembling IPEX syndrome with mutation in the gene encoding the LPS-responsive beige-like anchor (LRBA) protein have been reported [12]. These patients are characterized by low expression of regulatory T cells markers such as FOXP3, CD25, and CTLA-4. All these factors, along with the phosphatidylinositol 3-kinase (PI3K) subunits, dedicator of cytokinesis 8 (DOCK8), and the tetratricopeptide repeat domain proteins (TTC7A and TTC37) recently identified, contribute along with Tregs to immune homeostasis, whose perturbation leads to the dysregulation-observed patients with IPEX-like clinical features.

In light of these evidences, single gene defects causative of IPEX syndrome or IPEX-like disorders can be classified as Tregs-related disorders when displaying a direct effect on regulatory T cell function, as occurs with mutations affecting *FOXP3*, *CD25*, *STAT5B,* and *CTLA4* gene expression; on the other hand, Tregs-unrelated disorders can be considered those having an indirect effect on immune homeostasis and Tregs biology, as observed for *CD122* (*IL-2RB*), *STAT1*, *STAT3*, *PI3KCD*, *PI3KR1*, *LRBA*, *DOCK8*, *TTC7A,* and *TTC37* genes defects. The altered molecular mechanisms, the clinical consequences of inefficient immune response, and diagnosis and therapy for these diseases will be discussed.

## 10.5 Tregs-Related Disorders

### 10.5.1 FOXP3 Deficiency

IPEX syndrome was first described by Powell et al. in 1982 in a family of 19 males. They clinically characterized eight male patients' cohort displaying variable clinical symptoms, as diarrhea, polyendocrinopathy, severe enteropathy,

T1D, and dermatitis. Most of the patients died in infancy, and in light of normal B cell function, T cell numbers, polymorphonuclear leukocytes chemotaxis, and complement system, they speculated a T cell defect of X-linked recessive inheritance [13]. In 2000, Chatila and collaborators identified the *JM2* genetic locus in patients suffering from early-onset T1D, chronic diarrhea, and food allergic reactions and displaying a skewed Th2 cells phenotype [14]. The *JM2* gene, which encodes a candidate transcription factor containing a forkhead homology domain, was later called *FOXP3* when Bennett et al. and Wildin et al. discovered additional mutations in seven IPEX patients [15, 16]. Interestingly, the X-linked *scurfy (sf)* mutation spontaneously arose in a mouse strain at the Oak Ridge National Laboratory in 1949 [17], and it is considered the IPEX mouse model. Scurfy male mice present with scaly and ruffled skin, reddened eyes, lymphadenopathy, and splenomegaly and undergo premature death. They also display dermis lymphohistiocytic infiltrates, anemia, elevated serum IgG and IgM, and positive direct Coomb's test, suggestive of an immune dysfunction/hyperactivity [18]. Molecular investigations conducted by Brunkow et al. showed a 2 bp insertion within the *sf* gene coding region, responsible for a frameshift leading to a truncated protein. By functional complementation of the *sf* mutation in transgenic mice, harboring gene copy number ranging between 3 and 70, they observed a complete rescue of scurfy defect and high expression of *scurfy* gene in thymus and spleen. They also observed smaller lymph nodes in transgenic animals due to a decrease in total T cell number [19].

The *FOXP3* gene is the human homolog of mouse gene *Foxp3*. This new evidence confirmed IPEX syndrome as human equivalent of the *scurfy* mouse phenotype [18]. It is located on the short arm of X chromosome (Xp11.23) and contains a 5′-untranslated region (exon-1) followed by 11 translated exons, encoding a protein of 431 amino acids; the mouse protein contains 429 amino acids and shares 86.5% amino acid sequence identity with human FOXP3. The gene is mainly expressed in lymphoid tissues (thymus, spleen, and lymph nodes) and, in particular, by $CD4^+$ $CD25^+$ Tregs, which play a pivotal role in peripheral tolerance to self and non-self-antigens by controlling reactive T cells [20]. IPEX patients harbor *FOXP3* mutations distributed throughout the gene, even if most of them are found within functional domains, as the repressor N-terminal domain, the leucine zipper, and the C-terminal forkhead domain [6].

FOXP3 is a member of the forkhead box (FOX) protein superfamily of transcriptional regulators, which play a role in cell proliferation, differentiation, survival, and apoptosis during embryonic development and homeostasis of adult tissues [21]. The FOXP3 protein displays multiple structural domains: a proline-rich domain at the N-terminus, interacting with factors involved in transcription regulation; central zinc finger and leucine zipper domains, required for oligomer formation; and at C-terminus, a conserved forkhead/winged helix domain (FKH) required for DNA binding and nuclear localization [22]. In humans, *FOXP3* transcripts alternative splicing leads to the expression of four isoforms: a full-length protein (FOXP3) and shortened isoforms, as a result of exclusion of either exon 2 (FOXP3Δ2), exon 7 (FOXP7), or both exons 2 and 7 (FOXP3Δ2Δ7) [23–25].

FOXP3 full-length and the FOXP3Δ2 isoform are the most abundantly expressed, the latter displaying mostly nuclear localization due to loss of nuclear export signal located within exon 2 [26].

FOXP3 is a master regulator of Treg lineage commitment [27], and studies conducted on murine T cell hybridomas provided insights into Foxp3-mediated transcriptional regulation, showing as its targets are those associated with the TCR signalling pathway, such as *Il2ra*, *Tnfrsf18* (GITR), *Nrp1,* and *Ccr4* [28]. Intriguingly, FOXP3 is able to interact with about 700 genes, and it acts as both activator or repressor to regulate Treg cell development, function, and homeostasis [28, 29]. Moreover, FOXP3 forms large protein complexes of 400–800 kDa and associates with more than 360 proteins [9, 10]. Many FOXP3 interactors are transcription factors, such as RUNX1, Eos, Helios, IRF4, RORγ, RORα, HIF1α, STAT3, TCF1, EZH2 [10], and, as already mentioned, NFAT [30].

Interestingly, genetic deletion of these transcription factors in mice does not induce a phenotype as severe as the one observed in *scurfy* mouse, whereas conditional deletion of posttranslational modifiers in Tregs, modulating FOXP3 transcriptional activity, results in more severe autoimmunity [31]. Strikingly, FOXP3 can be modulated by phosphorylation, O-GlcNAcylation, acetylation, ubiquitination, and methylation [32], and transcription regulation in Tregs is also dependent on its ability to shape chromatin remodelling at target gene loci. In particular, FOXP3 repression of IL-2 and IFNg genes upon TCR engagement is mediated by histone H3 deacetylation, while expression of GITR, TNFRSF18, CD25, and CTLA-4 occurs through histone acetylation [33].

IPEX syndrome classical hallmarks include severe early-onset enteropathy, dermatitis, and type 1 diabetes mellitus, and, due to its severity, it is considered fatal if not treated with immunosuppressive therapy and/or hematopoietic stem cell transplantation (HSCT) [34]. Nevertheless, patients with later onset and mild phenotype have been described by our team and others (Fig. 10.1) [6, 35]. Thus, FOXP3 can be considered the master regulator of Treg cell lineage identity [27] as retrovirus-mediated *Foxp3* expression in mouse-naïve $CD4^+$ T cells results in lower proliferation and IL-2, IFNg, IL-4, and IL-10 expression [36], and IPEX patients characterization heavily contributed to dissect the immune tolerance mechanisms as well as the consequences of an altered Tregs function.

### 10.5.2 CD25 (IL-2Rα) Deficiency

The interleukin-2 is a cytokine primarily produced by activated T cells, which plays a pivotal role in maintaining the immune system. The IL-2 receptor (IL-2R) is composed by three subunits: α (IL-2RA, CD25), β (IL-2RB, CD122), and γ common (IL-2RG, CD132), the latter shared by other five cytokine receptors (IL-4R, IL-7R, IL-9R, IL-15R, and IL-21R). Two functional receptors for IL-2 are known: one is a heterodimeric complex formed by the β and γ chains, which binds IL-15 and IL-2 with intermediate affinity, and it is constitutively expressed on resting $CD8^+$ T cells and NK cells; the other is a trimeric membrane-spanning complex composed of the

α, β, and γ subunits, and it has a higher affinity for IL-2 than the former [37]. The IL-2-mediated signalling starts with formation of a quaternary IL-2-IL-2R complex, whose signal transduction is mediated by receptor-associated tyrosine kinases JAK1 and JAK3, associated with IL-2RB and IL-2RG, respectively. The phosphorylated tyrosines on IL-2RB subunit act as docking sites for signalling molecules, as the adaptor protein Shc, STAT5a, and STAT5b, thus leading to both STAT5 and MAPK pathway activation [38].

CD25 (IL-2Rα) is constitutively expressed at high levels by Treg cells—making them the first responders to IL-2 during immune response—and promotes *FOXP3* transcription by amplifying IL-2 signalling via STAT5b activation pathway [39, 40]. High expression of CD25 is considered as a Tregs marker [41], and this protein is essential for Tregs development and function [20]. Moreover, IL-2-mediated signalling controls antigen-specific peripheral T cell clonal deletion [42], displaying a dual role in lymphocyte homeostasis.

The human *CD25* gene is located on chromosome 10p15.1, and CD25 deficit, due to homozygous mutations, is described as associated with severe bacterial, viral, and fungal infections. Affected patients may also present with adenovirus gastroenteritis, chronic diarrhea, failure to thrive, lymphadenopathy, hepatosplenomegaly, autoimmunity, and dermatitis. Additionally, they have impaired Treg function and T cell proliferation in vitro (Table 10.1, Fig. 10.1) [1, 43–47]. Interestingly, the clinical phenotype due to a deficit of CD25 and STAT5b is partially overlapping, confirming as IPEX-related disease can be considered as "different shades" of a common and profound immune defect.

### 10.5.3 STAT5b Deficiency

The signal transducer and activator of transcription (STAT) pathway is a key signalling cascade able to mediate cell response to extracellular stimuli and regulate cell proliferation, differentiation, activation, and survival during immune and inflammatory responses. STATs are transcription factors located into the cytoplasm, activated by interferons, cytokines, and growth factors through Janus kinases (JAK)-mediated phosphorylation. Seven members have been identified within the STAT protein family: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6. They display a common structure, consisting of a Src homology 2 (SH2) domain required for STATs homo- or heterodimerization; a coiled-coil domain, pivotal for nuclear localization; a DNA-binding domain, essential for transcription of target genes; and a transactivation domain required for coactivators recruitment [48]. STAT-mediated transcription regulation starts with extracellular stimuli, which interact with their surface-specific receptors. The JAK kinases (JAK1, JAK2, JAK3, and TYK2), constitutively associated with type I and type II receptors cytoplasmic tail, undergo transphosphorylation generating docking sites for cytoplasmic STATs binding. Thus, STATs phosphorylation on tyrosine residues and dimerization occurs, followed by nuclear translocation and binding to specific DNA sequences to activate or suppress gene transcription [48].

The JAK/STAT pathway shapes the fate of immune cells and, in particular, of helper T cells: STAT1 and STAT4 are key factors for Th1 cells, required for intracellular pathogens clearance, while Th2 cells—active in host defense against parasites—require STAT6 for signal transduction; STAT3 mediates RORγt expression, a Th17-specific transcription factor, thus contributing to immune response against extracellular bacterial and fungal infections; STAT5, instead, binds *FOXP3* promoter, shaping Tregs pool and host immune tolerance [48]. The plethora of factors involved at each step of these pathways showing as a tight orchestration—in terms of timing and specific molecules involved—of cellular response is required, resulting in specific transcriptional landscape able to shape immune cells' fate. Moreover, the discovery of *STAT1*, *STAT3,* and *STAT5b* germline mutations, leading to immune dysregulation and to an IPEX-like clinical profile as consequence of a GOF for the formers and a LOF for the latter, heavily contributed to dissect the exact role of STAT-mediated signalling during immune response. While STAT5b deficiency is considered as Tregs-related disease, thus discussed in this section, STAT1 and STAT3 GOF will be described in the following section, along with the Tregs-unrelated disorders.

STAT5b mediates signal transduction in response to IL-2, IL-4, IL-7, IL-9, IL-13, IL-15, IL-21, growth hormone (GH), erythropoietin, thrombopoietin, and granulocyte colony-stimulating factor (G-CSF) [49]. Upon activation, STAT5a and/or STAT5b mostly form homo- or heterodimers and translocate to the nucleus, where they act as a transcriptional activator for *FOXP3*, *CD25*, *Bcl-2,* and insulin-like growth factor-I (*IGF-1)* target genes [50]. The *STAT5B* gene is located on chromosome 17q11.2, approximately 12 kb apart from *STAT5A* [51]; the encoded proteins are highly homologous, sharing more than 90% sequence similarity, and in humans they do not have redundant function [50].

STAT5b deficiency is a rare autosomal recessive disease, first described in 2003 and presenting with failure to thrive, chronic diarrhea, eczema, and recurrent pulmonary infections. Taking into account that FOXP3 and CD25 expression is pivotal for Tregs differentiation and maintenance [20, 52] and that impaired IL-2R signalling pathway also affects effector $CD4^+$ and $CD8^+$ T cells activation in response to IL-2, it is not surprising that altered STAT5b-mediated signal transduction leads to immune dysregulation. Patients affected by STAT5b deficiency present with hypergammaglobulinemia and T cells lymphopenia, in particular a Tregs defect, and the loss of immune homeostasis drives autoimmune manifestations [53]. Moreover, the GH-induced IGF-I expression promotes skeletal development and fat metabolism, and missed regulation through STAT5b results in growth delay [49]. Additionally, STAT5b deficit results in reduced STAT5 phosphorylation in response to GH or IFNg, whereas increased phosphorylation of STAT1 and STAT3 is observed (Fig. 10.1) [52, 54].

Clinical and molecular characterization of mutations affecting *STAT5B* expression—as well as *STAT1* and *STAT3* genes, as it will be described later—within an "IPEX-like scenario" strongly contributed to elucidate the role of these factors and the connections required for a proper immune response.

### 10.5.4 CTLA-4 Haploinsufficiency

Cytotoxic lymphocyte antigen 4 (CTLA-4, CD152) is an inhibitory receptor constitutively expressed by Treg cells and plays a key role in their suppressive function during immune response through inhibition of proliferation and cell cycle progression of antigen-stimulated T cells [55–57]. Additionally, CTLA-4 is also expressed by activated T cells and, upon binding of CD80 (B7-1) and CD86 (B7-2) molecules on APCs, competes with CD28 receptor for opposite regulatory functions [55, 58] as both CTLA-4 and CD28 share 30% protein identity [59]. Furthermore, CTLA-4 binds to CD80 and CD86 ligands with greater avidity [60, 61]. In fact, a CTLA-4 homodimer is able to bind two CD80 molecules, leading to a latticelike structure in the immunological synapse [62, 63], and in this configuration, the CD28-mediated co-stimulation and proteins assembly may be impaired. Moreover, upon CD80/CD86 binding, CTLA-4 is able to remove the ligands from cell surface via trans-endocytosis, with consequent T cells proliferation inhibition [64]. Being CTLA-4 a key factor in immune activation checkpoint, its localization is tightly regulated. In T cells, CTLA-4 is continuously internalized via endocytosis, and it localizes in intracellular compartments; however, after internalization, either it can be reexpressed on plasma membrane (recycling), or it can be targeted to lysosomes for degradation [65]. Despite the exact nature and signalling pathways related to CTLA-4-mediated immune response inhibition are still under debate, an effect on T cells motility—related to the limited contact between T cells and APCs—has also been proposed [66, 67].

The *CTLA4* gene is located on chromosome 2q33.2 in human and on chromosome 1 in mouse and harbors four exons: exon 1 encodes the signal peptide; exon 2, the dimerization and ligand-binding domains; exon 3, the transmembrane region; and exon 4, the cytoplasmic tail [68, 69]. Differential splicing of CTLA-4 transcript has been described, leading to the expression of different isoforms: a full-length transmembrane form, a soluble CTLA-4 form lacking exon 3, and a transcript encoding only for exons 1 and 4 are detected in humans, while mice also express a ligand-independent CTLA-4 isoform, lacking exon 2 [70]. CTLA-4 expression is regulated by NFAT and, in Tregs, also by FOXP3 [30, 71]. Regarding *CTLA4* mutations, more than 50 heterozygous gene variants have been identified in exons 1, 2, and 3: most of them are missense mutations, followed by insertions or deletions and several nonsense mutations [72].

CTLA-4 deficit has a profound impact on both mouse and human immune system. *Ctla4* knockout mice die prematurely within 1 month after birth from multiorgan inflammation [73, 74], while heterozygous mice appeared to be normal [56]. In humans, CTLA-4 haploinsufficiency leads to impaired Tregs function and loss of suppression, with hyperactivated immune response. Patients display immune dysregulation associated to a variable disease phenotype, not correlated with CTLA-4 protein expression, and the main clinical presentations include hypogammaglobulinemia, lymphoproliferation, cytopenia, and gastrointestinal complications (Table 10.1, Fig. 10.1) [75, 76]. Interestingly, both asymptomatic and symptomatic mutation carriers have been described as carrying lower CTLA-4 protein levels than

healthy controls, and further studies have confirmed the clinical variability observed in these cohorts, supporting the loss of correlation between genotype, phenotype, and penetrance [77, 78]. Additionally, SNPs on the human CTLA4 gene have been associated to increased susceptibility to autoimmune manifestations [79]. CTLA-4 reveals to be a key negative regulator of immune response, and its altered expression has a profound impact on immune defense.

## 10.6 Tregs-Unrelated Disorders

In addition to immune defects due to an inefficient Tregs-mediated regulation of response, genetic studies have evidenced other monogenic diseases leading to immune dysregulation and affecting molecular pathways indirectly related to Tregs, and their molecular and clinical characterization provides, once more, an excellent example of how immune response is based on overlapping and tightly orchestrated mechanisms.

### 10.6.1 LRBA Deficiency

Lipopolysaccharide-responsive and beige-like anchor (LRBA) is a cytosolic protein which interacts with CTLA-4 in recycling endosomes, favoring its expression on T cell surface [65]. The *LRBA* gene is located on human chromosome 4q31.3 and contains 57 exons, encoding a 2851 amino acids cytosolic protein expressed in different cell types, such as hematopoietic, neural, gastrointestinal, and endocrine cells [80]. Additionally, increased *LRBA* expression has been detected in several cancers [81]. LRBA harbors different functional domains, required for biological processes regulation. In particular, the PH-like domain and the BEACH domain, which precede the highly conserved WD40 domain at C-terminus, have been implicated in CTLA-4 regulation [82]. In B cells and macrophages, LRBA expression is mediated by LPS stimulation, and it has been shown that the protein localizes in trans-Golgi and endocytic vesicles, lysosomes, endoplasmic reticulum, and plasma membrane [83].

LRBA loss of function (LOF) due to biallelic mutations leads to immune dysregulation, whose manifestations are similar to those observed in presence of CTLA-4 haploinsufficiency, and the main clinical features observed are autoimmunity—as autoimmune cytopenia—enteropathy, lymphoproliferation, and humoral immunodeficiency [84]. In patients displaying LRBA deficit, a lower intracellular and cell surface CTLA-4 expression is detected in Tregs, which seems to be LRBA dose-dependent, whereas normal *CTLA4* mRNA levels found suggest a posttranslational regulation exerted by LRBA [82]. Strikingly, LRBA mutations identified are located throughout the protein, making a possible genotype/phenotype correlation unlikely [85]. Patients harboring biallelic LRBA mutations display a Treg cells defect (decreased numbers and FOXP3 and CD25 expression, reduced suppression activity), autoimmunity, and reduced number and function of B cells, unable to proliferate upon activation (Table 10.1, Fig. 10.1) [12]. If the regulatory T cells

defect is consistent with the role played by LRBA in CTLA-4 recycling, and consequent negative regulation of immune response, the humoral deficiency and the B cell compartment impairment seem to be associated with increased apoptosis and altered B cells autophagy in response to starvation [12].

Interestingly, *Lrba*-deficient mice display normal B and T cell development, as well as B cells proliferation, class switch recombination, and survival. In spite of reduced *Ctla4* expression in Tregs and activated T cells, which recapitulates what observed in *LRBA*-deficient patients, *Lrba*$^{-/-}$ mice do not show any clear sign of autoimmunity, neither any sign of abnormalities or pathologies, especially in the gastrointestinal tract [86, 87]. A similar discrepancy between human disease and mouse model, unable to recapitulate the clinical phenotype, is also observed for CTLA-4 haploinsufficiency [56], and the hypothesis of compensatory mechanisms occurring in mice and not in human has been made.

The recent identification of patients harboring LRBA mutations and the role played by this protein in multiple processes regulating immunological homeostasis—alone or in combination with CTLA-4—lead to many open questions about additional regulatory mechanisms which may occur in immune cells, and thus justify the highly variable clinical phenotype associated to its loss of function.

### 10.6.2 STAT1 Gain of Function

STAT1 plays an important role in antiviral and antimycobacterial defense by transducing signals from type I IFNs (IFNα/IFNβ) and IFNγ. During viral infection, IFNα and IFNβ activate JAK1/TYK2 kinases, leading to STAT1/STAT2 heterodimer formation, which is able to bind p48—a member of the IFN regulatory factor (IRF) family—and to form the IFN-stimulated gene factor 3 (ISGF3) complex. ISGF3 thus translocates to the nucleus and, upon binding to type I IFN-stimulated response element (ISRE), activates gene expression of viral replication-blocking enzymes, MHC class I and CD69 [48]. On the other hand, during mycobacterial infections, T cells and NK cells produce IFNγ, which through JAK1/2 induces STAT1 activation and homodimerization; thus, STAT1 homodimers act as IFNγ activation factor (GAF) and, into the nucleus, activate IFNγ-responsive genes such as *IRF8*, *GATA2,* and those coding for the NADPH oxidase components in macrophages [48].

The *STAT1* human gene is located on chromosome 2q32.2, and heterozygous GOF mutations, detected in the DNA-binding domain or coiled-coil domain, have been identified in patients presenting with IPEX-like clinical features, associated or not with chronic mucocutaneous candidiasis (CMC) [88–90]. In particular, patients harboring heterozygous STAT1-activating mutations present with various fungal, bacterial, and viral infections, especially herpesviruses infections, as well as autoimmunity (thyroiditis, diabetes, cytopenias), enteropathy, and vasculitis. Moreover, recurrent respiratory infections, lung and liver granulomas, bronchiectasis, and cerebral aneurysms can also be observed. Strikingly, most patients can have

different features, even if harboring the same mutation (Table 10.1, Fig. 10.1) [1, 11, 91]. The clinical phenotype associated to STAT1 GOF is related to an enhanced STAT1 activation, as observed in monocytes and $CD4^+$ T cells stimulated, respectively, with IFNα/IFNγ and IFNα/IL-27 [92], and recent evidence suggests that increased STAT1 protein expression, rather than a delayed dephosphorylation, may be responsible for its hyperactivation [93–95]. Moreover, increased STAT1 signalling exerts a negative effect on STAT3-dependent cytokine production, thus impairing IL-17-mediated immune response and favoring *Candida* infections predisposition [89, 90].

### 10.6.3 STAT3 Gain of Function

STAT3 is activated by many proinflammatory and anti-inflammatory cytokines, as IL-6, IL-10, IL-17, IL-21, IL-22, IL-23, IL-27, IFNα/IFNβ, and IFNγ, and is able to regulate many processes, such as cell growth, apoptosis, organogenesis, inflammation, infection, and oncogenesis [48]. JAKs engagement results in STAT3 phosphorylation and consequent dimerization, translocation to the nucleus, and activation of target genes transcription. Phosphorylated STAT3 forms mainly homodimers, but also heterodimers with STAT1, in response to IL-6-mediated activation, and STAT5, upon M-CSF stimulation, thus controlling different transcriptional programs [96].

The *STAT3* gene is located on chromosome 17q21.2, and GOF mutations, detected in the DNA binding, SH2, transactivation, or coiled-coil domain, lead to immune dysregulation associated with multiple clinical manifestations. Patients usually present with lymphoproliferation, organ-specific or solid organ autoimmunity, and recurrent infections and failure to thrive. Despite the main presentation, they can present with a clinical phenotype similar to IPEX as diabetes and enteropathy have also been observed; moreover, increased Th17 cells differentiation, secondary to enhanced STAT3 signalling, is also observed along with low Tregs number and function (Table 10.1, Fig. 10.1) [1, 6, 97–101]. Activating *STAT3* mutations are found throughout all the protein, and most of them are missense [102]. Strikingly, it has been showed as mutated STAT3 protein is not constitutively phosphorylated compared to wild-type, even if a delayed dephosphorylation has been reported, and that both resting and stimulated cells expressing *STAT3* GOF variant show higher transcriptional activity [97]. Moreover, some patients with *STAT3* GOF mutation show a reduced number of Treg cells, and decreased CD25 expression, maybe as consequence of SOCS3 upregulation [97].

### 10.6.4 CD122 (IL-2RB) Deficiency

The human CD122 (IL2RB) protein, whose coding gene is located on chromosome 22q12.3, beyond being a IL-2R subunit is also a subunit of IL-15 receptor (IL-15R), and homozygous recessive deficiency has been described [103, 104]. The main clinical features are gastroenteritis, dermatitis, severe diarrhea, and infections;

additionally, all patients who survived the neonatal period also had recurrent infections, as well as autoimmune disease (mainly autoimmune hemolytic anemia), leading to early death in most cases [103]. Moreover, lymphoproliferation, lymphadenopathy, hepatosplenomegaly, and elevated IgG levels have also been associated with CD122 deficiency (Table 10.1, Fig. 10.1) [1].

Mutation analysis revealed three mutations, located in the extracellular protein domain and leading to different outcomes: (a) the L77P missense mutation results in CD122 sequestration in the endoplasmic reticulum, with consequent abrogation of its surface expression and impaired IL-2 signalling in T cells, despite normal NK cells responsiveness to IL-2 and cytotoxic activity; (b) the patient harboring the S40L mutation shows decreased IL-2-mediated response in spite of CD122 surface expression, while (c) the Q96 stop-gain mutation is causative of a more severe phenotype due to complete absence of CD122 surface expression and IL-2 signalling [103]. Fernandez et al. also identified a CD122 mutation leading to reduced CD122 expression and altered downstream signalling, observing reduced Tregs frequency, lymphocyte populations skewing toward memory T cells, and tissues lymphocytic infiltration. Moreover, they detected increased numbers of less differentiated NK cells, as well as memory $CD8^+$ T cells and memory B cells, associated with increased IgG levels [104].

### 10.6.5 DOCK8 Deficiency

Dedicator of cytokinesis 8 (DOCK8) is a member of the DOCK180 superfamily of atypical guanine exchange factors (GEFs) involved in actin cytoskeleton regulation. DOCK proteins activate CDC42 and RAC, members of the Ras homolog gene family (Rho) of small guanine triphosphate binding proteins (GTPases), required for actin polymerization and cytoskeletal rearrangement regulation. DOCK8 is predominantly expressed in hematopoietic cells and plays a key role in both humoral and cellular immune responses [105]. In particular, DOCK8 has been shown to regulate cell differentiation, adhesion, and survival by activating CDC42, and through actin cytoskeleton remodelling, it allows spatial redistribution of signalling molecules at immunological synapses, as observed in T, B, and NK cells [106]. Additionally, DOCK8 is also important for lymphocyte subsets differentiation and survival: by acting on STAT3 nuclear translocation, it indirectly drives Th17 differentiation [107], and its absence leads to a memory $CD4^+$ T cells polarization toward a Th2 cytokine phenotype [108]. Furthermore, DOCK8 acts on memory B cells activation and antibody-mediated response [109, 110].

DOCK8 deficiency is a combined immunodeficiency first reported in 2009, when biallelic mutations in *DOCK8* gene were discovered in patients suffering from autosomal recessive hyper-IgE syndrome (AR-HIES) [111, 112]. So far, mutations identified are mainly large deletions even though splicing, frameshift, or nonsense mutations are also detected [113]. A deficit of DOCK8 leads to severe and recurrent mucocutaneous bacterial infections, as well as viral and fungal infections, eczema, and food allergies. Patients affected may display lymphopenia, normal/elevated IgG

and IgA levels, hyper-IgE, and decreased serum IgM; an impaired antigen-specific antibody responses may also be detected, and high incidence of malignancy is reported (Table 10.1, Fig. 10.1) [114].

### 10.6.6 Activated Phosphoinositide 3-Kinase D Syndrome (APDS): PIK3CD Gain of Function and PIK3R1 Loss of Function

Activated phosphoinositide 3-kinase δ syndrome (APDS) is a combined immunodeficiency disorder due to GOF mutations in the *PIK3CD*, encoding for the p110δ catalytic subunit of phosphatidylinositol 3-kinases (APDS1), to LOF mutations in *PIK3R1*, encoding for the p85α regulatory subunit of the kinase (APDS2) or due to LOF mutations in phosphatase and tensin homolog (PTEN) coding gene (APDS-L) [115].

The phosphatidylinositol 3-kinases (PI3Ks) are a family of heterodimeric lipid kinases, whose signalling pathway is involved in many cellular processes, such as growth, metabolism, differentiation, proliferation, and motility [115]. Within the PI3K protein family, class IA PI3Ks play a pivotal role in mammals' immune system, and they form heterodimers comprised of a catalytic subunit (p110α, p110β, or p110δ) and an Src homology 2 (SH2)-containing regulatory subunit [p85α, p55α, p50α, p85β, or p55γ]. The catalytic isoform p110δ is mainly expressed by immune cells, whereas p110α, p110β, and the regulatory isoforms p85α and p85β display ubiquitous expression and broad tissue distribution [115]. The p110δ protein, associated with the p85α regulatory subunit, forms PI3Kδ, which is mainly expressed in hematopoietic cells, and its activation is triggered by T and B cell antigen receptors (TCR and BCR), Toll-like receptors (TLRs), costimulatory molecules, and cytokine receptors in T, B, and myeloid cells. Active PI3Kδ catalyzes the addition of a phosphate group to the membrane phospholipid phosphatidylinositol (4,5)-bisphosphate ($PI(4,5)P_2$) to generate phosphoinositide-3,4,5-trisphosphate ($PIP_3$), which acts as a docking site to pleckstrin homology (PH) domain-containing intracellular signalling proteins. Signalling termination is mediated by PTEN, which dephosphorylates $PIP_3$ to $PIP_2$, or by SH2 domain-containing inositol 5-phosphatase (SHIP), which dephosphorylates $PIP_3$ to phosphatidylinositol (3,4)-bisphosphate ($PI(3,4)P_2$) [116]. Among the many PH domain-containing proteins interacting with $PIP_3$, the most characterized is the serine/threonine kinase AKT and the mTOR/FOXO1 signalling, which leads to glucose uptake and glycolysis, shifting cell metabolism toward growth, proliferation, and differentiation.

In B cells, PI3Kδ activation is mediated by BCR cross-linking, TLRs, chemokines (CXCR5), and cytokines (IL-4, IL-21, BAFF), and signalling through AKT leads to protein synthesis and cell growth. Additionally, this also results in FOXO1 phosphorylation and removal from the nucleus as FOXO1 is a transcription factor which regulates *RAG* genes, *IKAROS*, *CD62L*, and *AICDA* expression. In T cells, PI3Kδ activation is mainly mediated by TCR, ICOS, and IL-2R engagement and leads to *IL7RA* and *CD62L* downregulation, resulting in T cells mobility from

lymph nodes to circulation. Furthermore, PI3Kδ promotes T cell activation and effector phenotypes [115, 117].

In 2013 and 2014, heterozygous GOF mutations in the *PI3KCD* gene—encoding the p110δ protein—were identified, the most recurrent being the E1021K mutation, and affected patients were presenting with recurrent respiratory infections and progressive airway damage, e.g., bronchiectasis, lymphopenia, hyper-IgM, hypogammaglobulinemia, and impaired vaccine responses [118–121]. Additionally, patients can present with autoimmune features and diseases including cytopenias; thyroid, kidney, and liver disease; and enteropathy. Examination findings include lymphadenopathy and hepatosplenomegaly [122]. Significantly, hyperactivation of PI3K signalling has also been associated with malignant transformation, most commonly EBV-derived lymphomas [120–122]. The main laboratory findings in most patients with APDS include hypogammaglobulinemia and low $CD4^+$ T cell counts (Table 10.1, Fig. 10.1) [122].

Additionally, heterozygous LOF mutations in *PIK3R1* were reported as associated with a similar clinical phenotype, named APDS2 (Table 10.1, Fig. 10.1). These were heterozygous point mutations at splice donor site, resulting in skipping of exon 11 (coding exon 10), encoding amino acids 434 to 475 of p85a. This mutation affects all three proteins encoded by *PIK3R1* (p85α, p55α, and p50α regulatory subunits), thus leading to impaired p110δ inhibition [123, 124]. Recently, a novel heterozygous missense *PIK3R1* mutation, N564K, has been identified; it is predicted to influence binding to p110δ and is associated with APDS2 clinical features [125].

## 10.7 The Tetratricopeptide Repeat Domain Proteins

The tetratricopeptide repeat domain 7A (TTC7A) and tetratricopeptide repeat domain 37 (TTC37) are two factors associated with immune dysregulation, whose mutated genes we identified in a large cohort of IPEX-like patients, as they showed an overlapping clinical presentation [6].

### 10.7.1 TTC7A Deficiency

The tetratricopeptide repeat domain 7A (TTC7A) is a factor involved in multiple process regulating cell polarization, adhesion, and proliferation. The *TTC7A* gene is located on chromosome 2p21 and contains 20 exons, encoding an 858 amino acids protein, localized within the cytoplasm [126]. The encoded protein is expressed in many tissues and organs during development, such as brain, bone marrow, testis, pancreas, ovaries, liver, and blood, and is supposed to have some redundancy with its paralog, tetratricopeptide repeat domain 7B (TTC7B), as they share 49.47% sequence identity [126]. In thymus, TTC7A is expressed in thymic epithelial cells and, at a lower extent, is also detected in thymocytes [127]; in the gastrointestinal tract,

instead, TTC7A is strongly expressed in duodenum, ileum, and colon enterocytes, and this expression pattern is lost in patients harboring mutations predicted to reduce protein expression [128]. In gastric epithelial cells, TTC7A is proposed to regulate PI-4P synthesis and cells survival, cell polarity, apoptosis, cell adhesion, cytoskeletal homeostasis, cell motility, and barrier function, the latter resulting in bacteria translocation into lamina propria and inflammatory response triggering [126].

TTC7 proteins contain nine TPR domains, structurally conserved motifs which seem to be involved in multiprotein interactions [126]. Avitzur et al. described as the specific TTC7A partner phosphatidylinositol 4-kinase IIIa (PI4KIIIa), expressed by enterocytes and immune cells, is able to catalyze the production of phosphatidylinositol 4-phosphate (PI-4P) at plasma membrane and to regulate cell survival and polarity [128, 129]. Moreover, co-expression of TTC7A with the plasma membrane protein EFR3B is proposed to relocate TTC7A from cytosol to cell surface, where it acts as a scaffold leading to TTC7A/PI4KIIIa/EFR3 complex formation [126]. Additionally, TTC7A acts on cytoskeleton regulators within the RhoA/Rho-associated kinase (ROCK) pathway, thus influencing cell shape, polarization, and motility [130], although the exact regulatory mechanism is still not fully understood.

In human, biallelic mutations in the *TTC7A* gene are causative of hereditary multiple intestinal atresia (MIA), a rare cause of intestinal obstruction often associated with a profound combined immunodeficiency (MIA-CID) [127, 128, 130–133]. The immunological manifestations include severe hypogammaglobulinemia and lymphopenia, increased susceptibility to bacterial and opportunistic infections, and higher risk of graft-versus-host disease [134]. Furthermore, extraintestinal manifestations as integumentary hyperplasia and reduced hepatic function have been reported in TTC7A-deficient patients (Table 10.1, Fig. 10.1) [126].

Significantly, three spontaneous mouse models harboring Ttc7 mutations are known: a] the *Ttc7*$^{fsn}$ (flaky skin) mouse, presenting with papulosquamous skin disease and multisystem defects, including anemia, testicular degeneration, imbalance of CD4/CD8 T cells, and apoptotic cecal intestinal epithelial cells; b] the *Hea* mouse model, presenting with hematological anomalies as severe anemia, abundant circulating erythroblasts, thymic atrophy, defective thymocytes differentiation, and increased number of apoptotic and necrotic cells; and c] the *Ttc7*$^{fsn\text{-}Jic}$ mouse, which displays low body weight, skin and hematological abnormalities, reduced white pulp in the spleen, and inflammatory infiltrates in the liver [134]. In spite of a loss of unique mouse model able to fully recapitulate the MIA-CID phenotype observed in patients, the important role played by TTC7A in functional regulation of both epithelial cells and the hematopoietic system becomes more evident [135].

### 10.7.2 TTC37 Deficiency

The RNA exosome is an evolutionarily conserved ribonuclease complex required for processing and degradation of different RNAs within the cell. It degrades RNA in 3′ to 5′ direction and consists of a barrel-like catalytic core and accessory

proteins, which recruit RNA substrates. The RNA exosome functional specificity depends on its cofactors as the multiprotein superkiller (SKI) complex, involved in cytosolic exosome-mediated RNA surveillance through regulation of normal mRNA and decay of nonfunctional mRNA, and which includes—among its proteins—the tetratricopeptide repeat domain 37 (TTC37) [136].

The human TTC37 protein contains 20 predicted TRP motifs, involved in protein-protein interaction, and its coding gene is located on chromosome 5q15 [136]. Mutations in the TTC37 encoding gene are associated with trichohepatoenteric syndrome (THES1), a rare autosomal recessive disorder presenting with growth restriction, severe infantile diarrhea, trichorrhexis nodosa-like hair morphology, hepatopathy, facial dysmorphism, and immunodeficiency [137–139]. The immunological features are represented by recurrent infections, hypogammaglobulinemia, and low vaccination response (Table 10.1, Fig. 10.1) [139, 140]. So far, no clear genotype/phenotype correlation has been made, most likely due to the broad spectrum of mutations identified. Interestingly, both TTC7A and TTC37 proteins harbor TPR domains, and little is known about protein function, in particular about the role of mutations in both of these TPR-domain-containing proteins resulting in immune system and gut dysfunction [126], even though recent data on Drosophila *ski3* mutant, ortholog of TTC37, propose a role in mitochondrial function and thus open new directions for future investigations [141].

## 10.8 Diagnosis of IPEX Syndrome and IPEX-Related Disorders

Diagnosis of IPEX and IPEX-related disorders mostly depends on strong suspicion based on clinical presentation. Usually, patients will have normal immunoglobulin levels, apart from increased IgE and, sometimes, increased IgA. Hypogammaglobulinemia, if present, is mostly associated with IPEX-like diseases, in particular with CTLA-4 haploinsufficiency, STAT3 GOF, and LRBA deficiency. Low immunoglobulin levels may also be seen if the patient has wasting syndrome. Lymphocyte counts are usually normal in IPEX, as well as lymphocyte proliferation in response to mitogens in vitro, and immunization responses, including those to protein antigens. However, lymphocyte impairment can be found in other diseases (i.e., DOCK8, CTLA-4 haploinsufficiency, CD25 deficiency, etc.). The $CD4^+$ $CD25^+$ $FOXP3^+$ Treg cells are usually present in IPEX, unless *FOXP3* mutations prevent or limit normal gene expression. Tregs levels are variable in IPEX-related disorders. Bacchetta et al. reported autoantibodies specific to IPEX, mainly anti-harmonin antibodies and anti-villin antibodies; however, their role in IPEX pathogenesis and diagnosis is yet to be revealed [4–6]. However, a first screening on Treg cells is recommended. Lack of CD25 expression by flow cytometry should highly raise the suspicion of *IL2Rα* mutation and address genetic testing appropriately to confirm the disease.

Patients without *FOXP3* mutation and clinical picture resembling IPEX can be screened according to the recent IUIS update for inborn errors of immunity, based on basic clinical, laboratory, and immune phenotyping features (Table 10.1). Thus,

patients can then be referred for further laboratory or genetic testing, if required. The IUIS classified IPEX in the "IV. Diseases of immune dysregulation: B. Syndromes of autoimmunity and others, with regulatory T cell defects." CTLA-4 haploinsufficiency, CD25 deficiency, STAT3 GOF and LRBA deficiency are included in the same classification. Other disorders with immune dysregulation as only part of the clinical picture were classified according to their main clinical features and immunological phenotype (e.g., DOCK8 deficiency, STAT5b deficiency, STAT1 GOF, TTC37, and TTC7A deficiency) [142].

The definitive diagnosis of IPEX and IPEX-like conditions is made through genetic and molecular analysis to identify possible causative mutations leading to these disorders. Methods applied are whole-exome sequencing (WES) or Sanger sequencing in cases of positive family history of IPEX or IPEX-like diseases.

In spite of IPEX being an X-linked disease, the study by Lin et al. showing as somatic and germline *FOXP3* mutations can occur concomitantly [144]. They reported a mosaicism of somatic and germline mutations in the mother of an affected patient; however, only one of the two reported mutations was vertically transmitted to her children, including a daughter and an affected son, as both daughter and son had the same de novo point mutation, with the healthy sister being a disease carrier [144]. Moreover, CTLA-4 haploinsufficiency often shows an incomplete clinical penetrance not fully understood. This makes diagnosis very challenging and further proves that all family members should be screened for mutations.

It would be appropriate to investigate patients presenting with an IPEX clinical picture for specific diseases according to a certain strategy based on their main presenting features and basic laboratory investigations findings [6]. Thereafter, if attainable, single gene sequencing can be attempted. Yet giving the wide clinical overlap of these conditions, with the recent advances in genetics, WES remains the most suitable and recommended method for definitive diagnosis [6].

## 10.9 Management and Treatment

Patients with suspected IPEX syndrome or IPEX-like manifestation must be monitored, followed up, and cared for by a multidisciplinary team of physicians, specialists, nurses, and nutritionists. Furthermore, this interdisciplinary patient management approach requires supportive therapy, immunosuppressive therapy, and—in most cases—hematopoietic stem cell transplantation (HSCT) as it is the current definitive treatment.

Most patients will present with failure to thrive, eczema, and skin manifestations, mostly consistent with atopic dermatitis. Hence, a combination of dermatological team follow-up, extensive nutritional guidance, and support is required, especially prior to confirming the diagnosis. The most used therapeutic approach includes induction of remission with steroids in a tapering dose, usually in combination of immunosuppressive medications, although patients sometimes tend to respond to a single immunosuppressive agent, and remission is usually maintained through a combination of at least two immunosuppressive medications. The most commonly

used therapeutic agent is rapamycin (Sirolimus), at a dose of 0.15 mg/kg/day, that is adjusted to maintain a serum Sirolimus level of 12–18 ng/mL [2, 5]. Rapamycin or Sirolimus Rapamycin has been proved to be extremely beneficial in resolving or improving IPEX-related autoimmune manifestations, and it was mostly used as a monotherapy [34]; it acts by inhibiting the mTOR pathway and it selectively inhibits effector T cell proliferation, while sparing rapamycin-resistant regulatory T cells, hence increasing Tregs number and function. Other immunosuppressive treatments used include calcineurin inhibitors, such as cyclosporine and tacrolimus, in addition to azathioprine and mycophenolate mofetil, methotrexate, or monoclonal antibodies as anti-TNF alpha antibodies, anti-CD20 mAbs, CTLA-4 infusion proteins, and more [34]. Barzaghi et al. [34] showed that a few patients in their cohort, harboring *FOXP3* mutations, have improved spontaneously without requiring immunosuppressive therapy or any other intervention. While one patient had improved under supportive therapy, only without requiring immunosuppressive medications, another patient with the same mutation remained asymptomatic throughout the period of the study [34]. However, this review pointed out that most patients will require immunosuppression with concomitant use of steroids. Calcineurin inhibitors as cyclosporine had some benefit in some patients (40%), and their conditions resolved with significant improvement of their nutritional status, autoimmune manifestations, and recurrent infections [34].

Apart from classical immunosuppression, other treatments have been described. These include targeted therapies, such as monoclonal antibodies directed against specific molecules that directly cause disease: CTLA4-Ig, anti-IL-6 monoclonal antibodies (tocilizumab), rituximab, and IL-2. The CTLA-4-Ig is a fusion protein composed of the Fc region of IgG, fused to the extracellular domain of CTLA-4. It is used mainly in patients with CTLA-4 haploinsufficiency and acts by controlling auto-inflammation, with improvement seen specifically in patients with interstitial lung disease; the CTLA-4-Ig is also used to treat patients with LRBA deficiency, who benefit from the addition of hydroxychloroquine which inhibits lysosomal degradation. Moreover, tocilizumab inhibits IL-6-related Th17 cells differentiation, that is mostly observed in patients with STAT3 GOF disease, while increasing the number of circulating Tregs [8].

Rituximab is an anti-CD20 monoclonal antibody used for $CD20^+$ B lymphocytes depletion in patients with autoimmune conditions. However, in IEI, it is mostly used to treat patients with evident autoimmune cytopenia and granulomatous lymphocytic interstitial lung disease (GLILD). Also, it has been used in patients with *FOXP3*, *CTLA4*, *CD25*, *LRBA,* and *STAT3* GOF mutations. Nevertheless, rituximab is associated with complications related to B cell depletion, such as increased risk of infections, which might exacerbate the severity of certain patients' conditions, especially in those presenting with increased susceptibility to infection.

Other targeted therapies include IL-2 cytokine administration, which enhances and maintains the function of $FOXP3^+$ Tregs by increasing CD25 expression. However, even when administered in low doses, it can worsen patients' conditions, such as thrombocytopenia, in addition to its toxic side effects [8]. Some studies are focused on enhancing native IL-2 with concurrent administration of engineered

autologous Tregs in order to avoid effector T cells activation caused by IL-2 cytokine infusion [8].

Nevertheless, patients who received either immunosuppression or HSCT showed similar outcomes, but those who underwent HSCT benefited more, mainly with longer disease-free survival [34]. A recent survey on HSCT outcome in patients with primary immune regulatory disorders indicated that transplantation was the most definitive treatment, especially in patients with regulatory T cells defects including IPEX and IPEX-like clinical phenotypes, with good overall 5-year survival rate regardless of the donor type, match, or conditioning regimen received prior to HSCT. Strikingly, HSCT was more successful in patients with inactive or quiescent disease, mainly after immunosuppressive therapy [145].

Lastly, gene therapy represents a solid therapeutic option, especially for patients with monogenic diseases affecting Tregs function, since the mutation effects can be readily accessible in immune cells. Many gene therapy approaches have been studied and tested in patients. The most studied approaches include ex vivo HSCs gene correction, where the patient's own stem cells are manipulated to correct the genetic mutation through a lentiviral vector and are then transferred back to the patient at a specific genome site in order to prevent oncogenic gene activation. Also, conventional $CD4^+$ T cells from IPEX patients can be reprogrammed to express wild-type FOXP3, and hence to suppress the disease. Other approaches include patient's HSCs gene editing through clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 ribonucleoproteins: this technique is based on a chemically modified guide RNA and homology-directed DNA repair, and it has been used in T cells from a patient with a nonsense mutation in *CD25* gene, with acceptable CD25 expression and function [8]. Despite all the efforts and scientific advances made so far, more studies are required for this technique to be fully implemented in clinical practice, and HSCT remains the most favorable curative treatment for patients presenting with IPEX and IPEX-like clinical phenotype.

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