

## R E V I E W

## Update in primary immunodeficiencies

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**Abstract.** Primary immunodeficiencies (PIDs) are inherited disorders classically characterized by increased susceptibility to infections. Nevertheless, in the last two decades, genomic analysis (such as NGS) coupled with biochemical and cellular studies led to a more accurate definition for a growing number of novel genetic disorders associated with PIDs. This revealed new aspects of the immune system and its function and regulation within these diseases. In particular, it has been clarified that the clinical features of PIDs are much broader than originally thought and extend beyond an increased susceptibility to infections. More specifically, immune dysregulation is very often described in novel characterized PIDs and can lead to multiple autoimmune diseases, lymphoproliferation and malignancies. If not promptly diagnosed, these could negatively impact patient's prognosis. The aim of this review is to increase the awareness of recently discovered PIDs, characterized predominantly by immune dysregulation phenotypes. Findings highlighted in this review suggest screening for immunodeficiency in patients with lymphoproliferation or early onset/multiple autoimmune diseases. Prompt diagnosis would potentially allow most successful treatment and clinical outcome for patients with PIDs. ([www.actabiomedica.it](http://www.actabiomedica.it))

**Key words:** IPEX-like, ALPS-like, APDS1-2, CTLA-4 haploinsufficiency, LRBA deficiency, RAG1-2, immune dysregulation, autoimmunity

### Introduction

Since at least a decade, in light of a growing body of knowledge on primary immunodeficiencies (PIDs), experts suggest that the 10 warning signs promoted as a screening tool for PIDs diagnosis by the Jeffrey Modell Foundation are unable to identify more than 30% of PID patients (1). These 10 warning signs do not take into account that many PIDs are sporadically

accompanied by infections, while their early symptoms may be predominantly autoimmune diseases, autoinflammation and malignancies (2).

As shown by different studies aimed at determining the incidence of autoimmunity and inflammation in these patients, primary immunodeficiencies must now be considered conditions characterized by a broad dysregulation of the immune system, rather than a simple deficiency. A cornerstone study published in

2017 by Fisher et al. from the French national PID registry (on a cohort of 2183 patients) reported that 26.2% of PID patients developed one or more autoimmune or inflammatory complications with the risk of onset spread throughout the patient's lifetime. All types of PIDs were associated with a tenfolds higher risk of autoimmune and/or inflammatory complications than the general population in particular for patients with T-cells deficiency and common variable immunodeficiency (CVID). Autoimmune diseases mostly reported were cytopenias and gastrointestinal disorders. Moreover, these patients showed an overall survival time significantly shorter compared to PID patients without autoimmune/inflammatory manifestations (3).

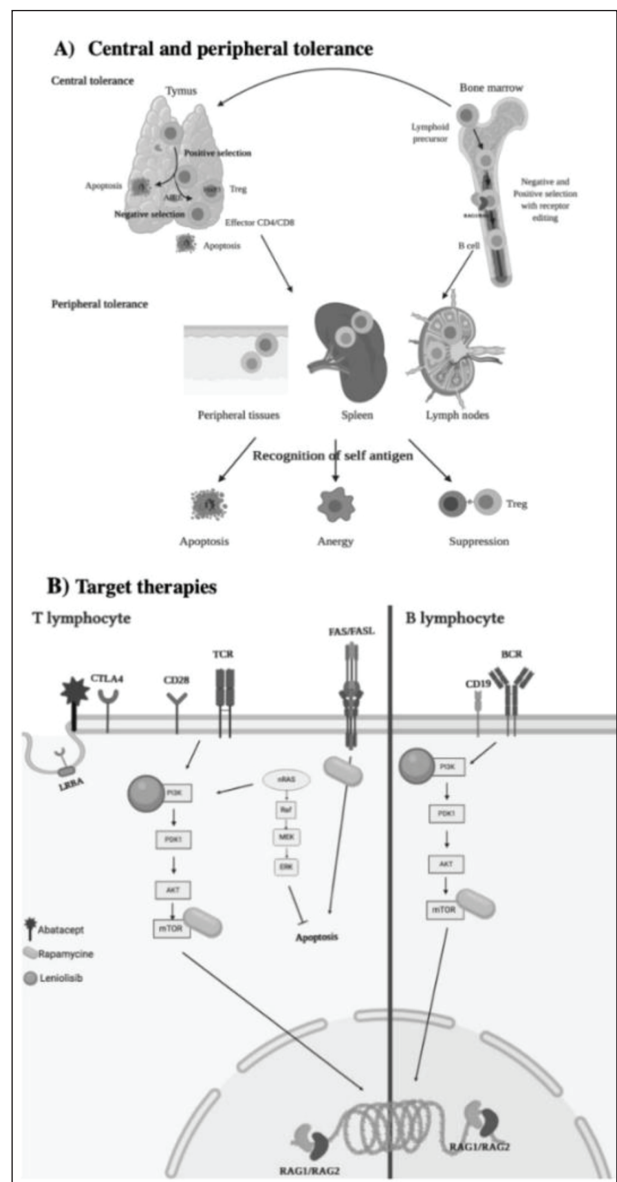
Recently, a more accurate characterization of dys-regulated immunophenotypes in known PIDs, as well as in PIDs more recently discovered, led to the identification of new disorders associated with immune dysregulation such as multiple autoimmunity, lymphoproliferation and malignancies (4).

An integrated approach based on genomics analysis, biochemical, cellular and molecular characterization for each PIDs phenotypes is a more effective approach in discriminating the pathways involved in isolated autoimmunity, immunodeficiency as compared to healthy individuals. These novel insights on the underlying mechanisms of immune dysregulation, including loss-of-function and gain-of-function genetic defects, allowed tailored therapeutic strategies targeting specific cell functions beyond a general immunosuppressive strategy (5).

### Clinical and laboratory features of selected, newly described PIDs associated with immune dysregulation

The impairment of different pathways and mechanisms underlying PIDs can trigger autoimmunity and inflammation that interfere with regulation of immune homeostasis and induction of tolerance. Understanding the pathogenesis of PIDs allows a better categorization of these conditions although clinical or pathogenetic overlap between categories is often reported.

The induction of tolerance starts during several



**Figure 1.** A) Central tolerance develops during early maturation of T and B lymphocytes in the Thymus and bone marrow respectively. Peripheral tolerance leads to avoidance of autoimmunity through apoptosis, anergy or suppression of autoreactive cells. B) The identification of dysregulated pathways or mutated receptors in certain immunodeficiencies has allowed the development of specific targeted therapies for the treatment of PIDs

early stages of lymphocytes development (Fig 1-a).

Alterations of lymphocyte receptors constitution or selection may underlie PIDs associated with immune dysregulation due to the break of central tolerance mechanisms (as described in section 1 below). Also, some peripheral mechanisms, such as regula-

tory T cells activity and CTLA receptor function, are necessary to maintain tolerance and avoid the onset of autoimmunity (see section 2 below). Finally, proper functioning of many pathways is essential to ensure a balance between activation, proliferation and elimination of activated or exhausted cells (sections 3,4). All these conditions lead to clinical phenotypes characterized by different degrees of multiple autoimmunity, lymphoproliferation and malignancies. Lymphocytic infiltration and its consequences may involve the spleen, lungs, gut but also bones and the central nervous system, presenting with symptoms including hepatosplenomegaly, lymphadenopathy, interstitial disease and restrictive pneumopathy, gastritis, enteropathy, chronic diarrhea and weight loss and generalized seizures (6,7).

**1. Lymphocyte development and central tolerance:** RAG1 and RAG2 are recombinase enzymes essential in receptor constitution and early T/B lymphocyte development. While mutations in these genes that severely compromise their activity lead to a severe combined immunodeficiency (SCID) T-B-NK+, hypomorphic mutations, with residual enzyme activity, allow the differentiation of an oligoclonal repertoire of T and B cells. These autoreactive cells, reflecting impairment of central and peripheral T- and B-cell tolerance, are responsible for the frequent autoimmunity, lymphoproliferation and immune dysregulation associated with a variable degree of immunodeficiency characterizing atypical/leaky SCID (severe combined immunodeficiencies) or delayed onset CID (8). Clinical manifestations of these conditions include cytopenia, often multilinear, but also less frequently vasculitis, autoimmune endocrinopathy, enteropathy and lymphoproliferation. Moreover, granulomatous disease, involving skin, lungs, liver and bones are present in 25% of these patients (9,10).

**1.1 Impaired Thymic Central Tolerance:** Patients with DiGeorge syndrome (22q11.2 deletion) show among other symptoms an altered thymus development. This leads to a variable degree of T-cell deficiency ranging from normal T-cells number and function to a SCID phenotype. In this condition the thymic selection of autoreactive T-cells is also altered resulting in more frequent autoimmunity and immune

dysregulation (arthritis, hypothyroidism, and autoimmune cytopenias) described in 8.5% to 10% of patients with non-SCID phenotype (11).

Similarly, a mutation in AIRE (autoimmune regulator) gene leads to AIRE deficiency or autoimmunity–polyendocrinopathy–candidiasis–ectodermal dystrophy syndrome (APECED). This gene encodes a transcription factor involved in thymus development and in the expression of autologous antigens necessary for the induction of central tolerance by negative selection of T autoreactive lymphocytes. Autosomal-recessive mutations in this gene lead to the early onset of chronic mucocutaneous candidiasis (due to anti-IL17 antibodies) and autoimmune endocrinopathies including hypoparathyroidism, adrenal insufficiency, diabetes and gonadal dysfunction (due to autoantibodies directed against tissue antigens) (12).

**2. Peripheral tolerance and Tregopathies:** regulatory T cells (Tregs) expressing the transcription factor FOXP3 and the surface antigens CD3, CD4 and CD25 have a crucial role in ensuring peripheral tolerance. Tregopathies arising from mutations of these genes are therefore able to impair Tregs function or number, leading to multiple autoimmune diseases combined with an increased susceptibility to infections. Mutations of the FOXP3 gene, located on the X chromosome and controlling Treg cells function, lead to the lack of circulating Tregs (CD4+/FOXP3+ T-cells) in the periphery, while Tregs expressing other markers (CD25<sup>HI</sup>/CD127<sup>LOW</sup>, CTLA-4+) show reduced suppressive activity despite of normal cell count. These immunological findings are associated with the immunodysregulation, polyendocrinopathy and enteropathy (IPEX) syndrome. Indeed, IPEX patients present with severe and early onset enteropathy, typical eczema with hyper-IgE (with or without eosinophilia) and early-onset organ-specific autoimmunity (above of all type 1 diabetes). Onset of multiple autoimmune manifestations including cytopenia, hepatitis, and autoimmune disorders of the central nervous system can be observed later in time. Suggestive clinical symptoms in the absence of FOXP3 gene mutation should suggest an IPEX-like diseases (e.g. the CTLA4 haploinsufficiency and LRBA deficiency described later) (13).

**3. Apoptosis defects:** After the eradication of infections, the immune cells that proliferated as a consequence of the infection should reduce in number through different apoptotic mechanisms. This prevents the onset of pathological phenotypes associated with immune system hyperactivation, lymphoproliferation and autoimmunity. An example of defective apoptosis is observed in ALPS (autoimmune lymphoproliferative syndrome) due to mutations of different genes (FAS, FASLG and the downstream signaling molecule CASP10, CASP8), all involved in the extrinsic pathway of apoptosis. ALPS is characterized by the expansion of lymphocytes, mainly T double negative (CD3+CD4-CD8- TCR  $\alpha/\beta$ ), increased IL-10 and vitamin B12 serum levels, lymphoproliferation with splenomegaly and lymphadenopathy, polyclonal hypergammaglobulinemia, organ autoimmunity and cytopenia. In these patients an increased risk of developing cancer, in particular B cell lymphoma, is also observed.

Somatic mutations of NRAS and KRAS genes coding for proteins involved in the intrinsic apoptosis pathway lead to an ALPS-like disorder also known as RAS-associated autoimmune leukoproliferative disease (RALD), characterized not only by lymphoproliferation and autoimmune manifestations, but also monocytosis, and increased risk of leukemia (14).

**4. Lymphocytes hyperactivation:** The phosphatidylinositol 4,5-bisphosphate 3-kinase  $\delta$  enzyme (PI3K $\delta$ ) is selectively expressed in leukocytes. Its role consists in transducing signals from different growth factors and cytokines activating receptors, through downstream effectors such as the serine/threonine kinase AKT. Heterozygous GOF mutations in the phosphatidylinositol 3-kinase C  $\delta$  (PIK3CD) and PIK3R1 genes, encoding for p110 $\delta$  and p85 $\alpha$  subunits of PI3K $\delta$ , result in increased activity of the PI3K-AKT-mTOR pathway, leading respectively to activated phosphoinositide 3-kinase  $\delta$  syndromes 1 and 2 (APDS1 and APDS2).

These conditions are therefore associated with immune overactivation characterized by increased proliferation of T-cells with prolonged effector function, accumulation of senescent T-cells and decreased number of naïve T-cells. Hypogammaglobulinemia IgG with normal/high IgM is also associated with an increased

number of transitional B-cells and reduced number of switched memory B-cells.

Clinically similar APDS1 and APDS2 are characterized by variable penetrance and expressivity presenting with susceptibility to respiratory tract infections, recurrent or persistent infections by herpes-virus systemic autoimmunity (multilineage cytopenias, bowel disease). In addition, in APDS patients particularly relevant is lymphoproliferation characterized by splenomegaly and/or hepatomegaly, lymphadenopathy, and organ infiltration with nodular lymphoid hyperplasia of the respiratory and gastrointestinal tract. A higher risk to develop B-cell lymphomas has also been reported (15,16).

Some newly described PIDs share immunological mechanisms and clinical features with APDS, IPEX (IPEX-like) and ALPS (ALPS-like), manifesting T-cells hyperactivation and T-regs impaired function. Cytotoxic T-lymphocyte antigen 4 (CTLA4) is constitutively expressed on T cells including Treg cells and plays an important role in their immune suppressive function. It competes as an inhibitory receptor for the same ligands (CD80/CD86) of CD28 that provides a co-stimulatory signal required for T-cell activation. CTLA4 therefore plays a crucial role in regulating DCs-Tcells cooperation, suppressing T-lymphocyte activation/proliferation and allowing T-regs regulatory function. Its haploinsufficiency (heterozygous germline mutations of CTLA4 gene) may lead to an immune activation syndrome characterized by multi-system autoimmunity (mostly multilineage cytopenia) and lymphoproliferation. Indeed, CTLA4 haploinsufficiency patients present with splenomegaly, lymphadenopathy, and T lymphocytic infiltration of target organs (lungs, bone marrow, nervous system and gastrointestinal tract), due to an expansion of effector T-cells, while showing impaired T-regs suppressor function and higher number of autoreactive CD21<sup>LOW</sup> B-cells. Finally, decrease in B-cell counts is responsible for hypogammaglobulinemia (17,18). A similar immunological and clinical phenotype is associated with biallelic mutations of LRBA (LPS responsive beige-like anchor protein) gene, where the encoded protein seems to act as an anchor or adapter for proteins directed to surface membranes and lysosomes. Binding to the cytoplasmic tail of CTLA4, LRBA prevents

CTLA4 degradation in the lysosomes while providing its recirculation onto the surface of T lymphocytes. Therefore, LRBA mutations impair surface recycling of CTLA4 leading to immunological and clinical features similar, and usually more severe, than CTLA4 haploinsufficiency (19,20).

### Targeted therapies in Immune Dysregulation Disorders

Treating immune dysregulation in patients affected by the aforementioned PIDs is sometimes challenging. Clinical manifestations frequently result in resistance to immunosuppressive therapies that have to be balanced with the risk of infections. If untreated, most of these diseases are fatal early in life, and severe complications are observed in patients who survive beyond childhood.

Understanding the pathophysiological mechanisms underlying PIDs, especially when associated with autoimmune manifestations or lymphoproliferation, offer the chance to identify therapeutic targets and treat both conditions at once. Some of these targeted therapies, also used in combination, can turn out to be more effective and with a reduced toxicological profile compared to standard of care (steroids or other immunosuppressants). They can control disease progression and improve clinical conditions in view of hematopoietic stem cell transplantation (HSCT). Furthermore, these approaches might improve the understanding of the underlying pathogenesis for a variety of these PIDs (5,21,22).

For example, following the identification of dysregulated pathways as hyperactivation of AKT/mTOR signaling, Sirolimus/Rapamycin (an inhibitor of mTOR kinases) has been used, showing good effectiveness in many PIDs associated with autoimmunity and lymphoproliferation. In many conditions like APDS(16,23), CTLA4-LRBA deficiency (24,25), IPEX (26) and ALPS syndrome (27) this treatment also displayed a significant patients' improvement in non-neoplastic lymphoproliferative disease, a variable response for related cytopenias and gastrointestinal disease, combined with a good tolerability profile. For some PIDs specific targeted therapies are now avail-

able or under evaluation (phase 2/3 trials). For example, PI3K inhibitors like Leniolisib or Nemoralisib (28) for patients with APDS or Abatacept (or Belatacept) (24,25) (a fusion protein constituted by the extracellular domain of CTLA4 and the Fc region of an IgG) are under evaluation. These molecules have proved effective in controlling autoimmune manifestations such as cytopenias and lymphoproliferation, with an overall improvement of clinical and immunological phenotypes. In particular when a fully matched donor is available, in patients who cannot tolerate therapies or with treatment-refractory manifestations, hematopoietic stem cell transplantation (HSCT) could represent the definitive therapy. However, to date, data are limited to small cohorts of patients and outcomes are currently suboptimal. Many variables need to be considered like the correct timing and control of immune dysregulation pre-HSCT, the intensity of conditioning regimen, the prophylaxis and treatment of infections and graft-versus-host-disease (29).

### Conclusions

In several PIDs, increased susceptibility to infections and immune dysregulation might be considered two sides of the same coin with many overlapping areas: each clinical manifestation should therefore alert the clinician for the possible presence of the other. An increased knowledge of the clinical phenotypes and molecular mechanisms underlying these conditions allowed the development of therapeutic approaches targeting the pathophysiological mechanisms of the disease, leading to improvements of the clinical conditions and quality of life in PIDs patients.

**Conflict of interest:** Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Received: 10 July 2020

Accepted: 31 July 2020

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