

REVIEW

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# Primary immune regulatory disorders: Undiagnosed needles in the haystack?

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## Abstract

Primary Immune Regulatory Disorders (PIRD) describe a group of conditions characterized by loss of normal inflammatory control and immune tolerance mechanisms, with autoimmunity as a predominant clinical feature. PIRD can arise due to defects in the number or function of regulatory T-lymphocytes, defects in the immune mechanisms required to 'turn off' inflammation such as in perforin-dependent cytotoxicity or alterations in cytokine signalling pathways. Diagnosis of PIRD is a significant challenge to physicians due to their rarity, complexity, and diversity in clinical manifestations. Many of these individual conditions lack a genotype–phenotype correlation and display incomplete penetrance. However, establishing a diagnosis is integral in optimizing patient management, including the use of individualized treatment approaches. Increasing awareness among physicians is necessary as patients are likely to present to different subspecialties. Due to the rarity of these conditions, worldwide collaboration and data-sharing is essential to improve our knowledge of the clinical spectrum and disease course in PIRD, and to optimize therapeutic strategies including identification of which patients can benefit from hematopoietic stem cell transplant.

## Introduction

Historically, the term Primary Immune Deficiency (PID) described a genetic disorder causing loss or gain of function in an encoded protein with subsequent immune system dysfunction associated with susceptibility to recurrent, severe or opportunistic infections. Advances in our understanding accompanied by enhanced diagnostic tools over the last decade has made clear that the spectrum of clinical manifestations associated with PID is more extensive, encompassing autoimmunity, allergy, autoinflammation, malignancy and non-malignant lymphoproliferation as well as predisposition to infection leading to the term 'Inborn Error of Immunity' (IEI) to better describe this diverse collection of diseases. It is now recognized that certain IEIs exhibit a predominant phenotype of immune dysregulation; this group of disorders has been collectively termed 'Primary Immune Regulatory Disorders' (PIRD) which are characterized by

loss of normal inflammatory control and self-tolerance mechanisms within the immune system leading to autoinflammation, autoimmunity and lymphoproliferation (Table 1). Patients with these conditions may still develop infections but this is typically a less predominant feature and infections often occur as a complication of end organ damage as a consequence of immune dysregulation.

## Underlying pathophysiology in PIRD

T-lymphocyte tolerance in normal homeostasis is maintained by two mechanisms; central tolerance encompasses the processes of positive and negative selection within the thymus resulting in removal of autoreactive T-lymphocytes and the development of thymic-derived regulatory T-lymphocytes (Tregs), and peripheral tolerance which includes the suppression of effector immune responses by Tregs [1, 2]. Tregs are a specialized subset of T-lymphocytes that play a pivotal role in the maintenance of self-tolerance and limiting inflammation and can be divided into two main types based on their origin: natural and inducible. Natural Tregs (nTregs) are produced within the thymus whereas inducible Tregs

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**Table 1** Conditions characterized by immune dysregulation

Group	Disorder	Gene	Inheritance	Typical features
Regulatory T-lymphocyte defect (absent/reduced Tregs and/or functional Treg defect)	IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked)	<i>FOXP3</i>	XL	Autoimmune enteropathy, early onset type 1 diabetes mellitus, eczema, thyroiditis, hemolytic anemia, thrombocytopenia, elevated IgE
	CTLA4 haploinsufficiency	<i>CTLA4</i>	AD	Autoimmunity particularly cytopenias, enteropathy, type 1 diabetes, lymphoproliferation, interstitial lung disease, recurrent infections, hypogammaglobulinemia
	LRBA deficiency	<i>LRBA</i>	AR	Autoimmunity particularly cytopenias, lymphoproliferation, recurrent infections, enteropathy
	STAT3 GOF	<i>STAT3</i>	AD	Autoimmunity, lymphoproliferation, infections, short stature
Autoimmune Lymphoproliferative Syndromes (ALPS)	CD25 deficiency	<i>IL2RA</i>	AR	Autoimmunity, lymphoproliferation
	ALPS-FAS (most common)	<i>FAS</i>	AD or AR	Lymphadenopathy, splenomegaly, hepatomegaly, autoimmune cytopenias, increased risk of lymphoma, defective lymphocyte apoptosis, elevated double negative T-cells. In ALPS-FAS, elevated soluble Fas Ligand, IL-10, vitamin B12, IgG
	ALPS-FASLG	<i>TNFRSF6</i>	AR	
	ALPS-Caspase10	<i>CASP10</i>	AD	
HLH – familial disorders	ALPS-Caspase8	<i>CASP8</i>	AR	
	Perforin deficiency	<i>PRF1</i>	AR	Early-onset HLH, fever, hepatosplenomegaly, cytopenias, decreased/absent NK and CTL activity
	Munc13-4 deficiency	<i>UNC13D</i>	AR	
	Syntaxin 11 deficiency	<i>STX11</i>	AR	
Munc18-2 deficiency	<i>STXB2</i>	AR		
HLH—IEI syndromes associated with increased incidence of HLH	Chediak Higashi syndrome	<i>LYST</i>	AR	Recurrent infections, partial oculocutaneous albinism, progressive neurological dysfunction, neutropenia, giant cytoplasmic granules
	Griscelli syndrome type 2	<i>RAB27A</i>	AR	Partial albinism, neutropenia, thrombocytopenia, neurological impairment
	Hermansky Pudlak syndrome type 2	<i>AP3B1</i>	AR	Oculocutaneous albinism, bleeding diathesis, neutropenia, hearing loss, pulmonary fibrosis
	Hermansky Pudlak syndrome type 10	<i>AP3D1</i>	AR	Oculocutaneous albinism, bleeding diathesis, microcephaly, neurodevelopmental delay
Immune dysregulation with colitis/inflammatory bowel disease	X-linked lymphoproliferative disease 1	<i>SH2DIA</i>	XL	EBV-associated HLH, lymphoma, aplastic anemia
	X-linked lymphoproliferative disease 2	<i>XIAP</i>	XL	EBV-associated HLH, colitis, hepatitis
	IL-10 deficiency	<i>IL10</i>	AR	Early onset severe colitis, respiratory tract infections, folliculitis, arthritis
	IL-10 receptor deficiency	<i>IL10RA</i> <i>IL10RB</i>	AR	
Innate	TGFB1 deficiency	<i>TGFB1</i>	AR	Recurrent viral infections, microcephaly, encephalopathy
	RIPK1 deficiency	<i>RIPK1</i>	AR	Recurrent infections, arthritis
	NFAT5 haploinsufficiency	<i>NFAT5</i>	AD	Colitis, recurrent respiratory tract infections
	STAT1 GOF	<i>STAT1</i>	AD	Chronic mucocutaneous candidiasis, recurrent infections, autoimmunity
Thymic disorder	NFKB2 deficiency	<i>NFKB2</i>	AD	Recurrent sinopulmonary infections, endocrinopathies, low immunoglobulins
	APECED	<i>AIRE</i>	AD or AR	Adrenal insufficiency, hypoparathyroidism, hypothyroidism, other autoimmunity, chronic mucocutaneous candidiasis

An increasing number of monogenetic disorders with immune dysregulation have been identified. These disorders may arise due to defects in Tregs, central tolerance, control of inflammatory responses and lymphocyte apoptosis, and in cytokine signalling pathways. This table is a non-exhaustive list of some of the more common PIRDs known to date

(iTregs) develop from naïve T-lymphocytes in the periphery upon antigen exposure. FOXP3 is a transcription factor that plays a critical role in both Treg development and suppressive function [3]. The immune regulating mechanisms of Tregs are multifarious and include suppression of effector T-lymphocyte proliferation and activation (such as by expression of CTLA4 and LRBA proteins), production of inhibitory cytokines (such as IL-10, TGF $\beta$  and IL-35) and their effect on other cell subsets such as dendritic cells and B-lymphocytes [4]. Deficiency in Treg number or function leads to a group of disorders known as ‘Tregopathies’, the prototype condition being IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) caused by mutations in *FOXP3* gene. An essential component of central tolerance is the expression of Autoimmune Regulator (AIRE) transcription factor by medullary thymic epithelial cells which permits the unique ability of intra-thymic ectopic expression of a wide range of peripheral tissue-restricted self-antigens (TRAs) and to form a ‘molecular mirror of peripheral self’ [5, 6]. During the process of central tolerance, developing T-lymphocytes that react with high affinity to TRAs are deleted as they harbour the potential to elicit autoimmunity if released into the circulation. Disruption to the *AIRE* gene leads to impaired negative selection and multi-organ autoimmunity, manifesting in humans as the rare condition autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED) [7].

Immune dysregulation can also arise from an inability of effector immune cells to ‘switch off’ inflammation or due to alterations in cytokine signalling pathways. Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyperinflammatory syndrome caused by impaired down-regulation of activated macrophages by natural killer (NK) cells or cytotoxic T-lymphocytes. NK cells and cytotoxic T-lymphocytes normally eliminate macrophages by formation of the immunologic synapse, insertion of a pore into the macrophage membrane, and delivery of cytotoxic granules leading to cell death. Failure to remove activated macrophages results in excessive production of inflammatory cytokines and subsequent tissue destruction. Primary HLH is caused by biallelic mutations in genes encoding proteins involved in formation of the membrane pore (Perforin, *PRF1* gene) and in cytotoxic granule formation and release mechanisms including *UNC13D*, *STX11* and *STXB2* which encode Munc13-4, Syntaxin 11 and Munc18-2 proteins respectively. Other IELs are associated with an increased risk of developing HLH including Chediak Higashi syndrome, Griscelli syndrome type 2 and Hermansky Pudlak syndrome [8]. Defects in immune signalling involving the JAK-STAT or NF- $\kappa$ B pathways alters the level of

T-lymphocyte or B-lymphocyte receptor activation which impacts their development and survival and can be associated with increased autoimmunity, for example, in *STAT1* and *STAT3* gain of function mutations, and loss of function mutations in *NFKB2* presenting with an autosomal dominant combined variable immunodeficiency (CVID) phenotype. In IL-10 receptor deficiency, effector T-lymphocytes are unresponsive to the regulatory cytokine IL-10 resulting in severe early onset enteropathy [9]. Finally, inflammasomes, multiprotein complexes within the cytoplasm, constitute part of the innate immune system leading to activation of proinflammatory caspases and subsequent release of IL-1 $\beta$  and IL-18, and pyroptosis [10]. Dysregulated inflammasome activity is associated with monogenic autoinflammatory disorders such as familial Mediterranean fever, hyperimmunoglobulin D syndrome and chronic infantile neurological, cutaneous, and articular syndrome (CINCA).

### A diagnostic challenge

With recent huge advances in diagnostic technologies, new IEL molecular diagnoses are being discovered at an impressive rate [11]. The most recent classification from the International Union of Immunological Sciences includes 430 monogenic IEL defects with 64 new disorders described in the preceding two years with a growing proportion of these new defects falling within the PIRD category [8]. PIRD present a significant diagnostic challenge to physicians for multiple reasons. They are rare conditions; the estimated prevalence of IELs is 1/1000–1/5000, with PIRDs representing of a subset of this. Genetic defects in the immune system are well known by physicians to be associated with serious, recurrent and/or unusual infections. However, autoimmunity, or other manifestations of immune dysregulation, either without co-existing infections or before the occurrence of infections, often does not trigger early consideration of a potential IEL, leading to a delayed or missed diagnosis. Patients may be labelled with a diagnosis such as Common Variable Immune Deficiency prior to identification of their true underlying monogenic diagnosis which may impact their management and prognosis.

There is often no correlation between genotype and phenotype and/or there is incomplete clinical penetrance in PIRD, further complicating diagnostic accuracy [12, 13]. Organ-specific autoimmunity can differ between patients with the same genetic defect. Different management strategies may be needed for individuals within the same family, for example in heterozygous loss of function mutations in cytotoxic T-lymphocyte antigen-4 (CTLA4) where family members of individuals with CTLA4-associated disease carry the same mutation and have evidence of reduced CTLA4 function but have no clinical

evidence of disease, although there is potential for late onset of symptoms [12, 14, 15].

The diverse spectrum of clinical problems associated with PIRD, the frequent overlap of manifestations between different PIRD conditions and the evolution over time with gradual accrual of additional problems also contribute to the difficulty to diagnose these disorders in a timely manner. Varying degrees of activity in the residual amount of the affected protein can occur depending on the type of mutation and predisposes to significant phenotypic variability. Loss of function (LOF) or hypomorphic mutations result in a reduction or loss of protein function, whereas gain of function (GOF) or hypermorphic mutations cause enhanced protein activity. As our knowledge of monogenic IEs expands, we can increasingly appreciate the true extent of the breadth and severity of the PIRD clinical spectrum. The classical clinical triad of IPEX syndrome (severe enteropathy, eczema and type 1 diabetes mellitus presenting early in life) is now recognized to not always be present, and patients may present with only one of these manifestations, at a later age, or with alternative autoimmune manifestations [16–18]. This diverse clinical spectrum in immune dysregulation, often predominantly involving autoimmune manifestations including cytopenias or endocrinopathies, means that patients may present to different subspecialists who may be unfamiliar with the emerging spectrum of immune dysregulatory genetic diagnoses, and patients may therefore not be offered the most appropriate treatment. This reinforces the importance of considering the ‘whole’ patient and considering an underlying IEI to be responsible for a combination of disorders afflicting the patient. Collaboration with an immunologist can help in connecting a range of medical problems to an underlying genetic defect.

First line immunological investigations including T-lymphocyte and B-lymphocyte subsets, immunoglobulin levels and vaccine responses may be normal or non-specifically abnormal in PIRD. Abnormalities in autoantibodies or inflammatory markers are also non-specific. Certain laboratory immune parameters can be suggestive of a particular disorder but not absolute in diagnosing a disease. In Autoimmune Lymphoproliferative Syndrome (ALPS) characteristic abnormalities include increased circulating  $CD3^+TCR\alpha/\beta^+CD4^-CD8^-$  double-negative T-lymphocytes which is highly sensitive but can also be detected in other conditions such as Systemic Lupus Erythematosus or Juvenile Idiopathic Arthritis [19–21]. Certain investigations important in the diagnosis of PIRD are not routinely available to clinicians or are performed only at a research level, which can impede the time to reach a diagnosis. Definitive

diagnoses depend on genetic sequencing to identify the causative defect in PIRD.

Herein we present an overview of selected PIRDs to demonstrate the spectrum of clinical features, aspects that impede diagnosis particular to these disorders and current management strategies.

### **IPEX syndrome**

Hemizygous mutations in *FOXP3* located on the X chromosome result in IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) classically presenting in the first few weeks of life with severe enteropathy, eczema and type 1 diabetes mellitus [18]. However, prenatal presentation and late onset have been reported [17, 18, 22]. It is now recognized that patients with IPEX syndrome can present with a range of other manifestations, may be atypical with a mild phenotype, or be asymptomatic and picked up during family screening [18, 23]. Although mutations in the *FOXP3* repressor domain are associated with a more severe clinical phenotype compared to those in the leucine-zipper and forkhead domains, the same genetic defect in two different individuals can cause a different clinical presentation, suggesting the involvement of other variables that can impact the disease course and severity [23]. In a study of 96 patients with IPEX syndrome, the median time to diagnosis was 14 months after the onset of symptoms but ranged up to a maximum of almost 24 years [18]. The classical triad of enteropathy, type 1 diabetes and eczema are the most common presenting features in those with neonatal onset disease but are not always present particularly in those who present at an older age and there is a wide variety of other clinical manifestations including nephropathy, hepatitis, autoimmune hemolytic anemia, thrombocytopenia, neutropenia, thyroiditis, alopecia, food allergies and arthritis that can occur [18, 23]. First line immunological investigations may be unremarkable with the possible exception of increased eosinophils and IgE levels. T-lymphocyte and B-lymphocyte subsets are often normal. Although CD4 T-lymphocyte expression of *FOXP3* may be reduced or absent, *FOXP3* expression can be normal but with diminished or absent function [13]. Through international multicentre collaborative efforts, strategies to optimally manage patients with IPEX syndrome are better understood. Information from the cohort of 96 patients identified rapamycin as the preferred choice for immune suppression. In addition, this study demonstrated that although there was no difference in overall survival between those on long-term immune suppression and those who underwent allogeneic hematopoietic stem cell transplant (HSCT), transplanted patients had superior disease-free survival with

stable or resolved disease, while those on immune suppression experienced disease progression [18].

### **CTLA4 haploinsufficiency**

CTLA4 is a negative immune regulator of T-lymphocyte proliferation and differentiation, essential in normal Treg function and maintenance of self-tolerance homeostasis [24]. CTLA4 competes with CD28 for the co-stimulatory ligands CD80 and CD86 expressed on antigen presenting cells thereby blocking co-stimulatory-dependent T-lymphocyte activation [25, 26]. Heterozygous *CTLA4* mutations cause an autosomal dominant disorder of immune dysregulation with Treg dysfunction and hyperactivation of effector T-lymphocytes [12, 14, 15] and is characterized by a highly variable clinical phenotype with different organ systems affected and incomplete clinical penetrance (approximately 70%) independent of the underlying mutation and age [15, 27]. Diagnosis is by gene sequencing followed by measuring CTLA4 protein expression and CTLA4-mediated transendocytosis. Although most affected mutation carriers present in the first two decades, late onset can also occur [15]. Initial manifestations of CTLA4 haploinsufficiency are diverse, including cytopenias, enteropathy, type 1 diabetes, skin, respiratory or neurological problems, thyroid disorders and arthritis, highlighting the likelihood of patients with this condition presenting to different specialities [15]. Autoimmunity and non-malignant lymphoproliferation are commonly found but affect different organ systems including lymph nodes, skin, lung, brain, gut, spleen and kidneys. Immunology findings are also variable but hypogammaglobulinemia is commonly observed [15, 27]. Patients with CTLA4 haploinsufficiency can benefit from targeted treatment with CTLA4 fusion proteins (abatacept or belatacept) or with mTOR inhibitors (such as sirolimus) which block CD28 signalling. Some patients undergo curative allogeneic HSCT, the most common indication being cytopenias [27, 28]. Establishing a molecular diagnosis of CTLA4 insufficiency is also important to facilitate careful monitoring of these patients who are at increased risk of malignancy [29].

### **LPS-responsive beige-like anchor protein (LRBA) deficiency**

LRBA deficiency is caused by biallelic mutations in *LRBA* gene, resulting in loss of or reduced expression of LRBA protein. LRBA deficiency and CTLA4 haploinsufficiency share a similar underlying pathophysiology; LRBA plays an essential role in recycling CTLA4 from intracellular vesicles to the cell surface and therefore impairs the availability of CTLA4 in Treg-mediated immune regulation [30]. Like other PIRDs, there is significant variability in the clinical phenotype and a lack of

genotype–phenotype correlation [30–34]. Patients with residual protein expression exhibit a less severe clinical course compared to those with absent LRBA protein [34]. LRBA deficiency typically presents in the first two decades of life, although the timing of onset varies widely during this period [32]. Autoimmunity is the most common clinical manifestation, with splenomegaly, pneumonia, lymphoproliferation and enteropathy being other common clinical features [31–33, 35]. Autoimmune cytopenias are a prominent feature, but a wide spectrum of autoimmune diseases including type 1 diabetes mellitus, hepatitis, thyroid disease, and arthritis are reported and patients often exhibit polyautoimmunity [32, 33, 36]. Reduced class-switched memory B-lymphocytes, Tregs and immunoglobulin levels are the most common abnormalities in immune parameters [35]. As expected, due to the shared pathogenetic mechanisms, the phenotype of LRBA deficiency overlaps significantly with that of CTLA4 haploinsufficiency, although severe infections appear to be a more prominent feature in LRBA deficiency [34, 37]. A recent analysis of long-term outcomes in a large cohort of patients with LRBA deficiency demonstrated the superior effect of targeted therapy with abatacept or sirolimus with decreased disease activity scores compared to conventional immune suppression [34]. Treatment with allogeneic HSCT can result in complete disease remission, but higher disease burden pre HSCT is associated with a worse outcome [34].

### **STAT3 gain of function**

Signal Transducer and Activator of Transcription (STAT) 3 belongs to a transcription factor family involved in the transmission of cytokine signals including IL-6, IL-10 and IL-23 from the cell membrane to the nucleus via Janus kinases [38]. Autosomal dominant GOF *STAT3* variants cause increased transcriptional activity, impaired cytokine signalling by other STAT molecules and reduced Treg number and function [40]. Clinically it is characterized by early onset polyautoimmunity and lymphoproliferation, with immune deficiency a less prominent feature [38–40]. The clinical phenotype is diverse with incomplete penetrance and although onset is in childhood, the age at initial presentation is variable. Immunological parameters are also variable and may include T-lymphocytopenia, hypogammaglobulinemia, decreased Tregs and elevated double-negative T-lymphocytes [40]. Targeted treatment with Jakinibs (tofacitinib and ruxolitinib), small molecule inhibitors inhibiting JAK activation, has shown to be successful in *STAT3* GOF disease [41]. In addition, targeted blockade of IL-6 with the anti-IL6R monoclonal antibody therapy tocilizumab has shown to be effective in *STAT3* GOF [39]. Allogeneic HSCT has been used in some patients although data is

limited on the effectiveness of HSCT in this specific condition [42].

### **Autoimmune lymphoproliferative syndrome (ALPS)**

ALPS is a disorder of immune regulation due to disruption in the process of Fas-mediated lymphocyte apoptosis. It can be caused by autosomal dominant or autosomal recessive deficiencies in several different proteins involved in this pathway; Fas (caused by germline or, less commonly, somatic mutations in *FAS* gene), Fas ligand, Caspase 8 or Caspase 10 [19, 43, 44]. There is no genotype–phenotype correlation, although later age at onset is observed in ALPS-FAS patients with a combined germline *FAS* mutation and a somatic mutation affecting the second *FAS* allele [45]. Variable clinical penetrance and differing phenotypes in patients from the same family harbouring the same mutation are observed [45, 46]. Although most cases develop symptoms in the first few years of life, adult-onset disease can also occur [45]. Patients typically present with lymphadenopathy, hepatosplenomegaly, and autoimmunity, most commonly cytopenia [45, 46]. Immunological abnormalities include elevated CD3<sup>+</sup>TCRα/β<sup>+</sup>CD4<sup>-</sup>CD8<sup>-</sup> double-negative T-lymphocytes, polyclonal hypergammaglobulinemia, and increased plasma IL-10 levels [47]. Vitamin B12 is also increased. In ALPS-FAS patients, elevated Fas ligand levels are detected [47]. Whilst non-malignant lymphoproliferative disease abates with increasing age, the risk of developing autoimmune disease and lymphoma persists. Treatment is usually with high dose immunoglobulin and steroids, often with addition of a steroid-sparing agent such as sirolimus. Splenectomy is not recommended due to the lack of a sustained therapeutic response and the risk of post-splenectomy sepsis [44, 45].

### **Hemophagocytic lymphohistiocytosis (HLH)**

HLH is characterized by life-threatening uncontrollable hyperactivation of the immune system resulting in excessive inflammation and tissue damage, typically with multi-organ involvement. It can be triggered by a variety of factors including infections and malignancy and can occur due to underlying genetic defects as described above, resulting in failure of NK cells and cytotoxic T-lymphocytes to eliminate activated cytokine-secreting macrophages. Other IEs have also been associated with increased incidence of HLH including Griscelli syndrome type 2, Chediak-Higashi syndrome and X-linked lymphoproliferative disease. Hemophagocytosis describes the engulfment of blood cells by activated macrophages and can be detected in tissue or bone marrow biopsies. Although finding it in tissues supports the presence of HLH, it is not pathognomic [48]. Diagnosis of HLH is

challenging, particularly establishing an early diagnosis; clinical manifestations are diverse and associated with a variety of triggers, and features overlap with other potential causes such as sepsis, hepatitis, encephalitis, or Kawasaki disease [49]. Diagnosis is determined by the presence of a confirmed HLH-associated genetic mutation or the presence of at least 5 of the following 8 features: fever  $\geq 38.5$  °C, splenomegaly, cytopenia (in at least 2 lineages), hypertriglyceridemia and/or hypofibrinogenemia, hemophagocytosis in bone marrow or tissue, low/absent NK activity, ferritin  $> 500$  ng/mL and elevated soluble CD25 [50]. Many of these features are characteristic of an intense inflammatory response, which may not be due to an underlying genetic defect. NK cytotoxicity assays and measurement of soluble CD25, as well as other helpful immunology tests such as perforin expression and NK degranulation assays using CD107a, are not readily available in all centres and results of confirmatory genetic testing can take weeks or months in a condition where time is critical to commence treatment [49, 51]. Patients may also not meet all the defined diagnostic criteria but display clinical evidence of HLH and require HLH-targeted treatment. A high index of clinical suspicion of HLH is necessary to allow prompt diagnosis and initiation of treatment which is critical for patient survival. Conversely, the diagnosis of other inflammatory disorders or malignancy must be carefully considered and looked for to avoid inappropriate immunosuppressive treatment. First line therapy of HLH consists of etoposide and dexamethasone, with intrathecal therapy for those with CNS involvement followed by HSCT in those with an underlying predisposing genetic defect, persistent or relapsing disease [50, 52]. Alternative regimens are less toxic and utilize alemtuzumab and ciclosporin.

### **Immune dysregulation with inflammatory bowel disease**

Symptomatic inflammatory bowel disease (IBD) before the age of 6 years, termed ‘very early-onset IBD’ (VEOIBD), can be characterized by a severe, treatment-refractory clinical course, with predominant colonic involvement, and is more likely to have an underlying monogenic defect [53]. However, diagnosing monogenic defects causing VEOIBD is challenging due to their rarity; although the number of monogenic defects associated with VEOIBD has increased in recent years, the majority of patients with VEOIBD lack a genetic diagnosis. In addition, there is a diverse phenotypic spectrum of the IEs associated with IBD, and currently there is a paucity of knowledge regarding many of these genetic defects. Clinical features that are more often associated with monogenic forms of IBD include young age at onset (particularly younger than two years of age), very severe

disease, resistance to conventional therapies, family history of IBD or immunodeficiency, consanguinity, recurrent infections, associated autoimmunity or malignancy, or features suggestive of HLH [54]. IL-10 is an important anti-inflammatory cytokine and plays a critical role in the maintenance of immune homeostasis in the gastrointestinal tract. Loss of function mutations in IL-10 and IL10 receptor genes typically cause VEOIBD within the first few months of life, often with severe perianal involvement and is resistant to conventional immunosuppressive therapeutic approaches [55]. Allogeneic HSCT has been demonstrated to successfully cure this disease [56]. RIPK1 (receptor-interacting serine/threonine-protein kinase) is an essential signalling molecule involved in regulating cell death and inflammation. Loss of function *RIPK1* mutations are a rare cause of VEOIBD associated with a combined immunodeficiency and polyarthritis [57]. Other IEs that are associated with colitis include IPEX syndrome, X-linked inhibitor of apoptosis protein (XIAP) deficiency, Chronic Granulomatous disease, Wiskott-Aldrich syndrome and nuclear factor  $\kappa$ B essential modulator (NEMO) deficiency.

#### **Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED)**

Loss of functioning AIRE protein leads to defective removal of self-reactive T-lymphocytes and autosomal recessive homozygous or compound heterozygous *AIRE* mutations give rise to APECED syndrome characterized by multi-organ autoimmunity. There is no clear genotype–phenotype correlation [58] with significant clinical variability even among affected siblings. The classic triad of APECED syndrome includes mucocutaneous candidiasis, hypoparathyroidism and adrenal insufficiency. However, recent studies demonstrate that the clinical spectrum of APECED is broader, encompassing more non-endocrine manifestations than previously appreciated with many patients presenting with clinical features outside the classic triad resulting in delayed diagnosis [59]. Less frequent endocrinopathies include hypothyroidism, primary ovarian or testicular failure, growth hormone deficiency and hypopituitarism. Non-endocrine manifestations include urticarial eruption, hepatitis, intestinal malabsorption, gastritis, tubulointerstitial nephritis, Sjogren's-like syndrome and pneumonitis. Chronic mucocutaneous candidiasis (CMC) typically develops in the first 1–3 years of life, manifesting as recurrent, severe and chronic *Candida* infections of the skin, nails and mucous membranes but not as invasive fungal infections. A wide range of tissue-specific autoantibodies may be detected in patients with APECED and antibodies against type 1 interferons are highly sensitive and specific (>95%) [60]. Immunological abnormalities

may include increased total T-lymphocytes, CD4<sup>+</sup> T-lymphocytes, CD4/CD8 ratios and reduced Treg numbers and function. Immunosuppression is needed in established autoimmune disease to prevent the development of irreversible end-organ damage, however, the evidence to guide optimal treatment strategies is limited due to the small number of patients. There is no role for HSCT in APECED syndrome as the defect originates in thymic epithelial cells and therefore will not be corrected with replacement of hematopoietic stem cells. There is no evidence supporting the routine use of prophylactic immunosuppression to prevent the development of autoimmune disease. Treatment of CMC is required to prevent complications such as oesophageal strictures, although development of resistance to oral anti-fungal agents can make long-term management of CMC challenging.

#### **Conclusion**

Diagnosis of IEs including PIRD is increasing largely due to continued discovery of new gene defects and improved understanding of disease presentations. Timely diagnosis of PIRD is pivotal to individualize appropriate management strategies, guide prognosis and allow genetic counselling, but is a challenge due to their rarity, diversity, and complexity. Early recognition of HLH is imperative to initiate life-saving treatment.

The diversity of presenting clinical manifestations highlights the importance of raising awareness of PIRD among different specialists, including hematology, respiratory, gastroenterology, dermatology, endocrinology, rheumatology, nephrology and neurology physicians. Specific patterns of autoimmune disorders can be associated with certain PIRD, and awareness of these patterns can help with earlier recognition or consideration of a possible monogenic cause. Clues that should alert clinicians to a potential underlying PIRD include development of autoimmunity at an age that is younger than expected, the development of multiple autoimmune processes affecting different organ systems, and/or a family history of autoimmunity or lymphoid malignancy. Early recognition and treatment of autoimmunity is essential to optimize quality of life and reduce the rate of associated complications.

Management is difficult in PIRD because of the need to balance the frequent requirement for immunosuppression and the co-existing increased risk of infection. Some PIRD such as HLH are fatal without prompt treatment. Ongoing detection of new PIRD diagnoses and worldwide collaboration and pooling of data is essential to improve our knowledge of individual rare PIRD; their underlying pathophysiology and molecular mechanisms, the clinical spectrum, natural history,

and response to treatments including HSCT. Identifying those with monogenic causes allows the opportunity to use precision targeted treatment for example with the use of abatacept in CTLA4 haploinsufficiency and LRBA deficiency. As well as identifying successful therapeutic strategies, this approach also informs physicians regarding inappropriate therapeutic options for example splenectomy in the management of cytopenias which fails to result in a sustained therapeutic benefit in CTLA4 haploinsufficiency [30]. Continuing this collaborative data-sharing approach will facilitate improved individualized patient management, support development of future precision therapies and aid in identifying which patients may benefit from HSCT as well as providing important insight into the complexities underlying immune regulatory processes.

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