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Epstein–Barr virus infection in primary immunodeficiency

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ABSTRACT

Primary immunodeficiency (PID) is a group of genetic disorders which affects immune cell development, differentiation, and function. The affected individuals are highly susceptible to infection by a diverse array of pathogens. Epstein–Barr virus (EBV) infection is ubiquitous in humans and usually involves an asymptomatic or self-limiting clinical course. In rare cases, EBV can cause not only an acute infection but also a severe exaggerated immune response and lymphoproliferative disease.

Furthermore, EBV infection in patients with PID can lead to immune dysregulation and increased risk of malignancies, in addition to the severe course of the acute infection. Recognition of the different genetic defects and their effect on immunological pathways provide us with fundamental insights into the pathophysiology of EBV infection and associated disease, and may lead to developing better targeted therapies in the future. Here, we review all of PIDs with an abnormal response to EBV disease.

Statement of novelty: Here we provide a review of the current knowledge of all PIDs reported to be associated with abnormal response to EBV infection and associated disease, such as hemophagocytic lymphohistiocytosis.

Epstein–Barr virus (EBV) is 1 of 8 human herpesviruses that establish lifelong persistent infection in humans (Tangye et al. 2017). It is estimated that more than 90% of the global population are seropositive to EBV (Tangye et al. 2017). EBV belongs to the gamma-1 herpesvirus genus, whose members are distinguished by their restriction to primate hosts, B lymphoid tropism, and ability to drive B cell growth through expression of a unique set of latent cycle genes (Palendira and Rickinson 2015; Taylor et al. 2015).

EBV infection can manifest as either a lytic infection or a latent infection with expression of a very limited number of viral proteins (Cohen 2015). The initial EBV infection is acquired orally through the saliva (Palendira and Rickinson 2015; Tangye et al. 2017). It replicates as a lytic infection through the oropharynx with high levels of viral shedding in the throat (Palendira and Rickinson 2015; Taylor et al. 2015). It is thought that oral epithelial cells and possibly some infiltrating B cells at mucosal surfaces are the sites for viral replication during the lytic phase (Palendira and Rickinson 2015; Tangye et al. 2017). The mode of viral entry into B cells remains unclear (Tangye et al. 2017). The replicative cycle that follows viral entry results in sequential expression of lytic proteins involved in producing new viral particles and immune evasion (Tangye et al. 2017). Among these are proteins that interfere with antigen processing and presentation to CD8+ T cells, those that down regulate MHC class II, as well as a few that reduce expression of ligands for NK cell-activating receptors (Tangye et al. 2017).

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Following the spread of EBV infection through B cells, it then enters into the memory B cell pool where it can stay as a latent infection and recirculate between the blood and pharyngeal lymphoid tissue (Taylor et al. 2015).

Primary immunodeficiency (PID) is a heterogeneous group of hereditary diseases that are associated with compromised immune responses that reduce the ability of immune system to combat infectious pathogens, as well as the presence of malignant cells, and autoimmune state (Notarangelo 2010). In immunocompromised individuals, reactivation of EBV and persistence of proliferating latent growth-transforming EBV infected B cells are associated with severe pathologies that can have fatal outcome. These include hemophagocytic lymphohistiocytosis (HLH, also termed virus-associated hemophagocytic syndrome), non-malignant B cell lymphoproliferative diseases (LPDs), B cell lymphomas including Hodgkin's lymphomas, as well as non-Hodgkin's lymphomas such as Burkitt's lymphoma and diffuse large B cell lymphoma (DLBCL) (Kuehn et al. 2017).

In this review, we describe the PIDs associated with EBV-infection and disease. Identification of proteins important for control of EBV may help to identify new targets for immunosuppressive therapies.

Lymphoproliferative conditions and susceptibility to EBV

XLP-1: mutations in SH2D1A

X-linked lymphoproliferative disease (XLP-1) was first described in 1975 as a fatal immunodeficiency affecting 6 males from the Duncan family, which led to the original naming of this condition as Duncan's disease (Purtilo et al. 1975; Shabani et al. 2016). XLP-1 is caused by mutations in *SH2D1A*, encoding signaling lymphocytic activation molecule (SLAM)–associated protein (SAP). SAP is an SH2 domain containing intracellular adaptor protein that regulates signaling downstream of the SLAM family of surface receptors expressed on T cells, NK and NKT cells (Coffey et al. 1998; Nichols et al. 1998; Sayos et al. 1998; Rezaei et al. 2011). The most common clinical features of XLP-1 are EBV-induced fulminant HLH (~45%–70%), B cell lymphoma (~25%) out of whom the majority are Burkitt (Rezaei et al. 2011) and non-Hodgkin's type (Rezaei et al. 2011), and hypogammaglobulinemia (~50%) (Tangye et al. 2017).

SLAM activated pathways have important roles during regulation of antigen-specific proliferation and Th2-type cytokine production by CD4+ T cells (Cocks et al. 1995). As a result, SAP deficiency results in dysregulated patterns of TCR-induced cytokine production with preferential production of interferon (IFN)-γ and diminished interleukin (IL)-4 production (Cocks et al. 1995). SAP-deficient NK cells exhibit impaired cytolytic activity. As with NK cells, SLAM receptor functions are also impaired in SAP deficient CD8+ T cells (Palendira et al. 2011). SAP deficient CD8+ cells demonstrate poor response to antigen presenting B cells (Hislop et al. 2010). The impaired NK cytolytic activity along with reduced CD8+ T cell killing likely contributes to the increased susceptibility of affected individuals to EBV infection (Cocks et al. 1995).

It is thought that susceptibility to EBV infection, but not other viral infections, in XLP-1 patients is due to the affinity of EBV to B cells, while the T and NK cell interaction with B cells is impaired due to a defect in the SLAM receptor related pathway. Defects in humoral immunity observed in XLP patients are associated with reduced memory B cell count, poor germinal centre formation, and diminished immunoglobulin switching (Shabani et al. 2016).

The most recently published survival rate in XLP-1 is 71.4%, which is a marked improvement compared to the 25% survival rate reported in the 1980s (Booth et al. 2011; Schmid et al. 2011*a*). Early diagnosis and management have led to decreased mortality rates. Currently, hematopoietic stem cell transplantation (HSCT) remains the most effective curative treatment for SAP deficient patients (Marsh et al. 2014; Shamriz et al. 2014).

XLP-2: mutations in XIAP

In 2006, Rigaud et al. reported 12 males presenting with XLP-like syndrome (Nakanishi et al. 1993). These individuals had inactivating mutations in *BIRC4*, located on chromosome Xq25, which encodes the X-linked inhibitor of apoptosis (IAP) protein, XIAP (Nakanishi et al. 1993). Although initially described as XLP-2 due to similarities in clinical presentation to boys with SAP deficiency, it is now recognised as a more complex disorder of immune dysregulation with a wide spectrum of clinical manifestations (Worth et al. 2016). The main clinical features are HLH, splenomegaly, colitis, and periodic fevers (Schmid et al. 2011*b*;

Yang et al. 2012; Speckmann et al. 2013; Aguilar and Latour 2015). Unlike XLP-1, B lymphoma rarely occurs in XIAP deficiency, and when hypogammaglobulinemia is noted in XLP-2, it is usually transient (Filipovich et al. 2010; Marsh et al. 2010; Schmid et al. 2011*b*; Speckmann et al. 2013; Aguilar and Latour 2015). XIAP protein is 1 of 8 members of the IAP family and acts mainly as a potent inhibitor of caspases 3, 7, and 9; however, it is also involved in a variety of other signaling pathways, including nuclear factor kappa B (NF-κB) and c-Jun N-terminal kinase activation (Obexer and Ausserlechner 2014). It is not yet clear how the deficient protein leads to the clinical phenotype. A recent study of 1 XIAP kindred has identified individuals with persistent CD8 lymphocytosis and unusually large expansion of EBV-specific CD4+ and CD8+ T cells in the blood many years after symptomatic primary EBV infection. This supports the idea that hyperexpansion of the EBV-induced T cell response could underlie some of the cases of XIAP-associated HLH (Worth et al. 2016).

The mortality caused by XIAP deficiency is ~40%, with approximately one third of deaths resulting from HLH (Marsh et al. 2010; Schmid et al. 2011*b*). More recent report suggests a much lower mortality rate (Speckmann et al. 2013), indicating that, like XLP-1, survival has greatly improved since the discovery of the molecular lesion causing this condition (Tangye et al. 2017).

CD27 deficiency

CD27 deficiency is caused by homozygous mutations in the *CD27* gene, which encodes a tumor necrosis factor (TNF) receptor superfamily member that is present on the B cell membrane. CD27 is required for activating T cells as well as long-term maintenance of T cell immunity. The reported clinical presentation of these patients was symptomatic primary EBV infection/lymphadenopathy early in life, while some presented with chronic EBV viremia. About 50% of patients developed malignancy. Some patients had a history of severe infections with other viral etiologies including influenza virus, and herpesviruses such as varicella-zoster virus (VZV) or cytomegalovirus (CMV) (van Montfrans et al. 2012; Salzer et al. 2013; Alkhairy et al. 2015). The immunologic features in this disorder include lack of memory B cells (no CD27+ cells), normal T cell count, low NKT cells, and hypogammaglobulinemia (Alkhairy et al. 2015).

CD27 activity is dependent on its interaction with CD70. The CD27-CD70 interaction is crucial for regulation of T, B, and NK cell activity (Nolte et al. 2009). Furthermore, some studies revealed that the CD27-CD70 interaction is required for naïve T cell expansion and production of Th1-type cytokines (Kawamura et al. 2011), and together with CD28/CD80 and 4-1BB/4-1BBL interactions, is critical for activation of tumor-antigen specific T cells (Zeng et al. 2014). These data support the notion that CD27 deficiency can increase susceptibility to hematologic malignancies. Interestingly, it was reported that the survival of cancer patients increases following treatment with soluble CD27, which increases T cell activation and proliferation (Huang et al. 2013). In another study, it was proposed that this interaction also leads to NKT-dependent activation of CD8+ T cells (Taraban et al. 2008) and formation of memory CD8+ T cell clones that can protect against pathogens (van Gisbergen et al. 2011). Defects in these CD27-dependent processes may explain why CD27 deficient patients develop recurrent viral infections. CD70-dependant CD27 signaling is required for B cell proliferation and differentiation to CD38+ CD20- plasmablasts and antibody production (Sammicheli et al. 2012). Impairment in this function likely underlies the poor antibody production that is observed in CD27-deficient patients.

CTPS1 deficiency

CTP synthase 1 (CTPS1) deficiency is an autosomal recessive disease caused by a defect in de novo pyrimidine synthesis pathway due to the lack of CTPS1 enzyme. CTPS1 is essential for proliferation of lymphocytes, especially T cells, following activation by antigens. CTPS1 activity is induced following T cell receptor (TCR) activation, and deficiency results in a T cell proliferative defect despite normal TCR activation signaling. As a result, patients with CTPS1 deficiency have low T cell and low to normal B cell counts. Clinically, these patients present before 2 years of age with severe viral infections and encapsulated bacterial infection, suggesting both a functional defect of T cell cytotoxicity and T cell-independent B cell immunity. All 8 patients reported developed chronic EBV viremia, 50% (4/8) of patients developed severe infectious mononucleosis, and almost one third of them (3/8) developed CNS LPD. Six patients received an HSCT, and 4 remained alive, well and free of symptoms (Worth et al. 2016).

RASGRP1 deficiency

RASGRP1 encodes for a diacylglycerol (DAG)-regulated guanidine exchange factor (GEF) preferentially expressed in T and NK cells (Hogquist 2001; Kortum et al. 2013). RASGRP1 is a specific activator of the small G protein RAS that in turn activates the cascade of Raf-MEK-ERK kinases (also termed as the MAP kinases/MAPK cascade). TCR-mediated RAS-to-ERK activation is mainly dependent on RASGRP1 in human primary T cells (Roose et al. 2005; Warnecke et al. 2012). The clinical presentation of RASGRP1-deficient patients include recurrent infections, hepatosplenomegaly, lymphadenopathy, EBV-associated lymphoproliferation and B cell lymphoma, as well as autoimmune features including autoimmune hemolytic anemia, thrombocytopenia, and uveitis (Salzer et al. 2016; Platt et al. 2017; Mao et al. 2018; Somekh et al. 2018; Winter et al. 2018).

RASGRP1-deficient T cells exhibit defective MAPK activation and impaired proliferation, as well as decreased CD27-dependent proliferation towards CD70-expressing EBV-transformed B cells, a crucial pathway required for expansion of antigen-specific T cells during anti-EBV immunity (Winter et al. 2018).

CD70 deficiency

To date, 6 individuals from 4 unrelated families with homozygous mutations in CD70 were reported, affecting either expression of CD70 or its ability to bind CD27 (Abolhassani et al. 2017; Izawa et al. 2017; Caorsi et al. 2018). CD70 is the counter structure of CD27 (Lens et al. 1998; Borst et al. 2005). It is largely absent from naïve or resting leukocytes but is rapidly induced after activation of B cells, myeloid cells, and to a lesser extent T cells (Hintzen et al. 1994; Tesselaar et al. 1997, 2003; Lens et al. 1998; Borst et al. 2005; Izawa et al. 2017). Thus, CD70 is predominantly expressed by activated antigen presenting cells, with highest levels on B cells.

The clinical features of CD70 deficiency resemble those of CD27 deficiency, with all patients experiencing EBV viremia and most developing EBV-associated lymphoproliferation or B cell malignancy, hypogammaglobulinemia, and impaired specific antibody responses; infection with other herpesviruses also occurred in a few patients. However, unlike CD27 deficiency, all patients with CD70 deficiency are currently alive (Abolhassani et al. 2017; Izawa et al. 2017), suggesting mutations in CD27 cause more severe disease than those in CD70 (Tangye et al. 2017).

CARMIL2 (RLTPR) deficiency

Mutations in CARMIL2 (also known as RLTPR) have been recently reported by 4 independent groups to cause a novel autosomal recessive, primary immunodeficiency disorder with variable phenotypic presentations. CARMIL2 is a regulator of actin capping protein which is a critical component of cell motility. It is part of the human CARMIL family, of which at least the first 2 members have distinct cell migration functions that are nonredundant. CARMIL2 orchestrates cell polarity by modulating microtubules and intermediate filaments (Wang et al. 2016; Schober et al. 2017).

Currently, 21 patients with CARMIL2 deficiency have been reported, and the most common clinical presentations across all studies are dermatitis (80%), recurrent chest infections (80%), and skin abscesses (62%). Persistent uncontrolled viral infections such as warts, molluscum contagiosum, and EBV are also common (Sorte et al. 2016; Wang et al. 2016; Schober et al. 2017; Alazami et al. 2018). Defects in CARMIL2 is also a predisposing factor to severe mucocutaneous candidiasis and mycobactrial infections.

ITK deficiency

IL-2–inducible T cell kinase gene *(ITK)*, is a member of the tyrosine kinase expressed in hepatocellular carcinoma (TEC) family of nonreceptor kinases that is expressed by hematopoietic cells involved in proximal TCR signaling. By regulating PLCγ1 phosphorylation, ITK coordinates T cell activation and function (Readinger et al. 2009). Homozygous mutations in ITK were first recognized through its high prevalence of EBV-associated disease. The expression of ITK is restricted to T cells, NK cells, iNKT cells, and mast cells. In T cells, it is induced upon TCR activation or IL-2 stimulation and is thought to play a critical role in downstream signaling. Altogether, 13 patients from 8 families have been identified with loss-of-function biallelic mutations in ITK (Huck et al. 2009; Stepensky et al. 2011; Linka et al. 2012; Mansouri et al. 2012; Serwas et al. 2014; Cipe et al. 2015; Çağdaş et al. 2017). Most patients presented with EBV viremia, EBV-induced lymphoproliferation that frequently developed into lymphoma, CD4+ T cell lymphopenia, hepatosplenomegaly, and progressive hypogammaglobulinemia (Huck et al. 2009; Stepensky et al. 2011; Linka et al. 2012;

Mansouri et al. 2012; Ghosh et al. 2014; Bienemann et al. 2015; Cipe et al. 2015; Çağdaş et al. 2017). Several instances of severe VZV and CMV infections were also recorded among the above mentioned patients, thus the overall picture of viral susceptibility associated with ITK mutation remains unclear (Palendira and Rickinson 2015). Of the 13 reported ITK-deficient patients, 8 died, whereas 2 of 3 survived HSCT (Huck et al. 2009; Stepensky et al. 2011).

MAGT1 deficiency

MAGT1 deficiency was recently identified through its impairment of EBV control. It is caused by loss of the X-chromosome–encoded Mg2+ ion transporter MAGT1, hence its definition as X-linked immunodeficiency with Mg2+ defect, EBV infection, and neoplasia (XMEN). The patients reported all had high EBV genome loads in the blood, and haematological malignancy is reported in all post-pubertal patients described, with many experiencing LPD early in life. These patients also have a history of recurrent respiratory infections, viral pneumonia, and severe pox virus (molluscum contagiosum) or herpesvirus (VZV and herpes simplex virus, HSV) infections (Chaigne-Delalande et al. 2013; Li et al. 2014). *MAGT1* encodes a ubiquitously expressed transmembrane Mg2+ transporter involved in the maintenance of free basal intracellular Mg2+ pools. However, MAGT1 also associates with the *N*-oligosaccharyl transferase complex and therefore may have a role in protein N-glycosylation (Cherepanova et al. 2016). In T cells, MAGT1 mediates the Mg2+ influx induced by TCR stimulation and, through downstream Mg2+ pathway signaling, also optimizes Ca2+ influx. MAGT1-deficient patients have low CD4+ T cell numbers, likely due to poor thymic output, whereas NK cell and CD8+ T cell (including EBV-specific CD8+ T cell) numbers appear normal (Li et al. 2014). However, both NK and CD8+ T cell cytotoxicity were partially impaired. That led to the finding that free Mg ions regulate these cells' expression of NKG2D, a membrane protein that engages its ligand, NKG2DL, on the target cell surface. As the name implies, NKG2D was first identified as an NK activating receptor but it also now appears to be required for optimal cytotoxicity by NKG2D-expressing CD8+ T cells. (Palendira and Rickinson 2015). Importantly, magnesium supplementation treatment *in vivo* and *in vitro* restored basal intracellular Mg2+ concentration, NKG2D expression, cell cytotoxicity, and immunity to EBV in MAGT1-deficient patients (Chaigne-Delalande et al. 2013).

Primary immunodeficiencies associated with EBV disease

Coronin 1A

Deficiency in the actin regulator coronin 1A (*CORO1A*) has been reported in 9 patients to date (Shiow et al. 2008, 2009; Moshous et al. 2013; Stray-Pedersen et al. 2014; Punwani et al. 2015; Yee et al. 2016). It was originally described as a thymic egress defect causing T- B+ NK+ severe combined immunodeficiency (SCID) (Shiow et al. 2008). Like other immunodeficiencies caused by actin cytoskeletal defects, coronin 1A deficiency impacts a wide range of lymphocyte processes, including development, survival, TCR signaling, immune synapse formation and migration (Föger et al. 2006; Mugnier et al. 2008; Mace and Orange 2014; Punwani et al. 2015). Impaired calcium flux and f-actin accumulation at the immune synapse result in increased T cell apoptosis and CD4 + lymphopenia. Patients with coronin 1A deficiency presented with severe infections, and 5 developed EBV-driven B cell lymphoma. Four patients had severe mucocutaneous immunodeficiency manifestations including epidermodysplasia verruciformis-human papilloma virus (EV-HPV). No patients have developed HLH or severe infectious mononucleosis (Shiow et al. 2008; Moshous et al. 2013; Punwani et al. 2015). Patients have an immunophenotype of absent or low naïve T-cells, severely impaired T proliferative responses, normal levels of total immunoglobulins, and impaired vaccine responses. Three patients were reported with neurological abnormalities including autism-like symptoms, which are likely explained by the role of coronin 1A in neurodevelopment reported in mice (Jayachandran et al. 2014).

STK4 deficiency

Serine threonine kinase 4 (STK4) deficiency, also known as mammalian sterile 20-like protein (MST1) deficiency, is an autosomal recessive combined immunodeficiency characterised by progressive CD4+ lymphopenia (Abdollahpour et al. 2012; Nehme et al. 2012). STK4 is a factor that regulates Treg and naïve T cell migration, homeostasis, and tolerance acting through Foxo proteins (Ouyang and Li 2011). STK4 regulates the stability of Foxo1/3 in lymphoid T cells, which are involved in FOXP3 induction, and therefore a deficiency in STK4 leads to impaired Treg development (Du et al. 2014). STK4 deficiency results in a naïve T cell survival defect and

also impairs homing of CD8+ cells to secondary lymphoid organs due to non-functional expression of the homing receptors CCR7 and CD62L (Nehme et al. 2012). Overall, the immune abnormalities lead to autoimmunity, EBV viremia, recurrent sinopulmonary and mucocutaneous infections mostly associated with herpesviruses as well as other viral (mulloscum contagiosum), fungal (candidiasis) and bacterial (Staphylococcal) infections (Abdollahpour et al. 2012; Nehme et al. 2012).

To date, 12 patients with STK deficiency of different origin have been reported, out of whom, 4 patients had developed EBV-lymphoproliferative disease during their disease (Abdollahpour et al. 2012; Nehme et al. 2012; Halacli et al. 2015; Dang et al. 2016).

APDS

Activated phosphatidylinositide 3-kinase delta (PI3Kδ) syndrome (APDS) is an inherited immune disorder caused by heterozygous gain-of-function mutations in the genes encoding the phosphoinositide 3-kinase delta (PI3Kδ) subunits p110δ or p85δ. Affected individuals develop combined immunodeficiency of variable clinical severity, characterised by recurrent sinopulmonary infections, increased susceptibility to viral infections, lymphoproliferation, bronchiectasis and an autosomal dominance inheritance pattern (Angulo et al. 2013; Deau et al. 2014; Lucas et al. 2014; Carpier and Lucas 2017). P110δ is the catalytic subunit of the lipid kinase PI3K-δ that generates the second messenger IP3 (inositol-3-phosphate) by degradation of PIP2 (phosphatidylinositol 4,5-biphosphate). PI3Kd is involved in downstream signaling from T and B cell antigen receptors, costimulatory receptors, cytokine receptors and some Toll like receptors (Okkenhaug 2013). Unregulated activity results in hyperactivation of the Akt-mTOR pathway, inducing excessive terminal differentiation of effector lymphocytes, impaired cytokine production and impaired immunoglobulin class switching in B cells (Angulo et al. 2013; Lucas et al. 2014). EBV infection is found in 30% of APDS patients and represents an important risk factor for the development of B cell lymphoma (occurring in 20% of EBV-infected APDS patients) (Carpier and Lucas 2017).

PRKCD deficiency

One patient with homozygous loss-of-function mutation in *PRKCD* (PKCδ) has been reported to date, and initially presented with recurrent otitis media and sinusitis, generalized lymphadenopathy, hepatosplenomegaly, B cell lymphocytosis, and persistent EBV viremia (Kuehn et al. 2013). The patient's serum had autoantibodies to several cellular proteins, and his NK cells had diminished cytotoxicity (Kuehn et al. 2013).

LPS-Responsive Beige-Like Anchor (LRBA) deficiency

LRBA deficiency is a PID caused by biallelic loss-of-function mutations in the *LRBA* gene. Affected individuals present with a variety of clinical symptoms including early onset hypogammaglobulinemia, recurrent infections, autoimmunity, and chronic diarrhea (Alkhairy et al. 2016; Gámez-Díaz et al. 2016). The main features of LRBA are typically a common variable immunodeficiency (CVID)-like phenotype, autoimmunity, and inflammatory bowel disease. The immunologic abnormalities in LRBA-deficient patients include decreased IgG antibody production, defect in specific antibody response, deficient T cell activation and proliferation, increased apoptosis, and decreased autophagy in B lymphocytes. The majority of LRBA-deficient patients have also low B-cell subset counts, mainly in switched memory B cells and plasmablasts (Lopez-Herrera et al. 2012; Azizi et al. 2017). Therefore, LRBA deficiency is a clinically variable syndrome with a wide spectrum of clinical and immunologic manifestations. LRBA deficiency is an autosomal recessive disease characterized by a CVID phenotype, autoimmunity with inflammatory bowel disease (Alangari et al. 2012; Alkhairy et al. 2016). One patient presented with EBV lymphoproliferative disease, elevated EBV DNA in the blood, and autoimmune pancytopenia (Alangari et al. 2012).

ALPS

Autoimmune lymphoproliferative syndrome (ALPS) consists of a group of immune dysregulatory disorders with different patterns of inheritance, and is caused by mutations that impair lymphocyte responses to apoptosis triggered by the Fas pathway, leading to impaired lymphocyte homeostasis. The most prevalent form of ALPS is ALPS-FAS with a heterozygous mutation in TNFRSF6, an intracellular domain of FAS (Pace and Vinh 2013). ALPS patients often present with hepatosplenomegaly, lymphadenopathy, EBV infection, cytopenia, and hypergammaglobulinemia. They demonstrate elevated double negative T cells and soluble

Fas-Ligand. The risk of developing lymphoma increases significantly with age, and at 30 years 15% of patients have developed lymphoma (Price et al. 2014). Estimates of the fraction of ALPS lymphomas that are EBV associated vary from 15% to 40% in independent surveys (Straus et al. 2001; Pace and Vinh 2013; Price et al. 2014). Two patients have also developed HLH (Bode et al. 2015). How deficiency in the Fas-mediated apoptosis pathway could increase the risk of EBV-associated lymphomas is unknown. However, 1 possibility is that during long-term virus carriage, one of the immune controls governing EBV in the B cell system involves Fas-mediated cell killing (Palendira and Rickinson 2015).

Familial HLH

Familial HLH (FHL) is a group of diseases associated with impaired cytolytic activity of CD8+ T cells and NK cells. These diseases are caused by gene defects in the perforin gene and the components of lytic granule exocytosis machinery (Sepulveda and de Saint Basile 2017). Four genes have been identified in which mutations cause FHL: *PRF1, UNC13D, STX11*, and *STXBP2*, which are responsible for FHL2, FHL3, FHL4, and FHL5, respectively.

Perforin is encoded by *PRF1* and is expressed in cytotoxic granules of cytotoxic T cells and NK cells. Mutations in perforin result in an autosomal recessive disorder known as FHL2. Perforin mutations result in impaired killing of target cells by cytotoxic T cells and NK cells. EBV-positive infectious mononucleosis followed by persistent splenomegaly and lymphadenopathy and chronic active EBV disease and HLH was reported in a boy with perforin mutation (Katano et al. 2003; Cohen et al. 2011).

UNC13D encodes munc13-4 which interacts with syntaxin-11 to change the conformation of syntaxin from a closed to an open conformation. The change allows priming of cytotoxic granules and ultimately results in fusion of the granules with the membrane of the cell, followed by exocytosis of granules. Mutations in munc13-4 result in an autosomal recessive disease referred to as FHL3 with impaired NK and T cell cytotoxicity. Mutations in munc13-4 were reported in 1 patient with chronic EBV viremia who had cerebral vasculitis, hypogammaglobulinemia, chronic hepatitis, splenomegaly, and recurrent respiratory infections (Rohr et al. 2010).

STXBP2 encodes munc18-2 which forms a bridge assisting in the docking of cytotoxic granules to the plasma membrane of cytotoxic T cells or NK cells. Mutations in munc18-2 result in an autosomal recessive disease referred to as FHL5. Deficiency in munc18-2 results in impaired binding of munc18-2 to syntaxin-11 reduced stability of both proteins, and impaired exocytosis of cytotoxic granules from cytotoxic T cells or NK cells (Côte et al. 2009; zur Stadt et al. 2009). Mutations in munc18-2 were reported in 4 patients with chronic active EBV disease, and 2 of them developed HLH after primary EBV infection (Rohr et al. 2010).

ZAP70

ZAP70 deficiency is a combined immunodeficiency disorder with a profound presentation of CD8 lymphopenia. Although the count of CD4 T cells in patients with ZAP70 deficiency appears to be normal, they function abnormally due to impaired TCR signaling (Au-Yeung et al. 2009). The B cell and NK cell compartments seem unaffected (Turul et al. 2009). ZAP70 is a nonreceptor tyrosine kinase that is a key component of the TCR signal transduction pathway. Upon TCR stimulation, ZAP70 is recruited to the CD3ζ chain where, after its phosphorylation by Lck, it phosphorylates a number of downstream targets to initiate the signaling cascade (Turul et al. 2009). Although the clinical presentations of patients with ZAP70 deficiency have been heterogeneous, generally, they show increased susceptibility to recurrent bacterial, fungal, or viral infections in the first 2 years of life and a failure to thrive. Of the viral infections, HSV, molluscum contagiosum, and HPV are the most pathogenic. The EBV status of many patients was often not determined. However, 1 infant with normal numbers of B cells and CD4+ T cells, but a near absence of CD8+ T cells, developed an aggressive EBV-positive diffuse large B cell lymphoma (Newell et al. 2011).

TYK2

Tyrosine kinase 2 (TYK2), is a Janus kinase associated with the receptors of type I interferons, IL-6, IL-10, IL-12, and IL-23, and plays a central role in the signal transduction of these cytokines (Nemoto et al. 2018). TYK2 deficiency presents with symptoms of hyper-IgE syndrome (HIES) with susceptibility to various pathogens, including *Staphylococcus*, mycobacteria and HSV (Nemoto et al. 2018). Recently, 2 siblings with TYK2 deficiency were described, who presented with T cell lymphopenia characterized by low naïve

CD4+ T cell counts and who developed EBV-associated B cell lymphoma (Nemoto et al. 2018).

Non-homologous DNA end-joining deficiencies (radiosensitive SCID)

Defects of the non-homologous DNA end-joining mechanism result in T- B- NK+ SCID, but the clinical severity of defects in this pathway are heterogeneous as several patients have been described with a hypomorphic phenotype. Hypomorphic DNA ligase IV (*LIG4*) and Artemis (*DCLRE1C*) gene mutations demonstrate susceptibility for EBV-driven LPD or diffuse large B cell lymphoma. However, HLH has not been reported (Moshous et al. 2003; Enders et al. 2006; Toita et al. 2007; Woodbine et al. 2014). Although there are a low number of affected individuals described for each of these conditions, the incidence of EBV LPD seems to be between 20% and 50% of described patients (Worth et al. 2016).

NF-κB1 and NF-κB2

NF-κB1 insufficiency predominantly affects maturation, survival, differentiation, and T cell-independent immunoglobulin class switching of B cells, but can also lead to impaired T cell function. NF-κB1 is a member of NF-κB transcription factor family, called p50. It triggers transcription of inflammatory cytokines and immune response genes through the canonical pathway by creating a heterodimer with RelA, another member of NF-κB family. EBV-driven LPD and recurrent EBV infection have been reported in patients with heterozygous mutations in the NF-κB1 gene (Boztug et al. 2016; Schipp et al. 2016; Lougaris et al. 2017).

A recent report describes a patient with a heterozygous NF-κB2 precursor-skipping mutation that resulted in a constitutive presence of p52. The mutation was shown to cause CID with severe EBV infection (Kuehn et al. 2017).

CARD11 gain-of-function mutations

Patients with gain-of-function mutations in CARD11 present with B cell lymphocytosis, splenomegaly, lymphadenopathy with florid follicular hyperplasia, recurrent sinusitis, and otitis media (Snow et al. 2012). Some patients have chronic EBV infection. The disorder is also referred to as B cell expansion with NF-κB and T cell anergy (BENTA) (Outinen et al. 2016). CARD11 is a scaffold protein that is essential in the activation of the canonical NF-κB pathway in B and T cells (Cohen 2015). It is not yet understood whether the EBV infection is a contributor to the lymphocytosis or a result of the immune abnormality observed in this entity.

NK cell abnormalities and EBV

There are currently three genetically defined conditions with selective loss of NK cell function. All three are associated with increased susceptibility to various pathogens, including herpesviruses such as HSV-1 and VZV; however, EBV is also implicated on occasion (Rickinson et al. 2014).

The most NK-specific deficiency involves patients with a homozygous missense mutation in Fc-γ receptor 3A (CD16), the NK cell activating receptor for antibody-dependent cell cytotoxicity (ADCC). Interestingly, the mutation does not affect NK cell development or ADCC function but does impair spontaneous NK-mediated cytolysis (Rickinson et al. 2014). Although only three patients have been described with homozygous mutations in the gene coding for CD16, two developed EBV-related severe complications, prolonged infectious mononucleosis (de Vries et al. 1996), and EBV-associated lymphadenopathy (Grier et al. 2012). Patients with CD16 deficiency have normal numbers of NK cells but impaired NK cell cytotoxicity. Affected patients suffered from severe viral infections, particularly VZV and HPV in addition to EBV (Cohen 2015).

Another condition affecting NK cell development arises from homozygous mutation of the gene encoding minichromosome maintenance 4 (MCM4), a helicase component of the DNA replication complex (Eidenschenk et al. 2006; Hughes et al. 2012). Patients with mutations in MCM4 present with adrenal insufficiency, growth retardation, low numbers of NK cells, and absent CD56dim NK cells (Gineau et al. 2012). Among the few MCM4-deficient kindreds studied to date, one child developed EBV-positive B cell lymphoproliferative disease. Notably, this child was the only one of four siblings who also failed to mount a T cell dependent EBNA1 antibody response to EBV infection (Rickinson et al. 2014).

Patients with mutations in GATA binding protein 2 (GATA2) can have various signs and symptoms including acute myeloid leukemia, myelodysplastic

syndrome, autoimmune disease, pulmonary alveolar proteinosis, and primary lymphedema. Patients with GATA2 mutations have presented with chronic active EBV disease, and persistent EBV viremia (Hsu et al. 2011; Spinner et al. 2014). Two of those patients had an EBV-associated disease, and both involved unusual EBV-positive malignancies of mesenchymal cells. One was of spindle cell origin, whereas the other was a leiomyosarcoma (Spinner et al. 2014). In addition to EBV, these patients also are susceptible to other severe herpesvirus infections as well as severe HPV, fungal, and non-tuberculous mycobacterial infections. *GATA2* encodes a transcription factor important for hematopoiesis; accordingly, patients with mutations in GATA2 often have low numbers of B cells, CD4 T cells, NK cells, dendritic cells, red blood cells, neutrophils, monocytes, and platelets (Cohen 2015).

Syndromes with CID and severe EBV infection

Wiskott–Aldrich syndrome (WAS)

Wiskott–Aldrich syndrome (WAS) is an X-linked immunodeficiency caused by mutations in the gene encoding the WAS protein. Mutations in the *WAS* gene located at Xp11.22-23 results in defective WASP function, which acts as a scaffold for actin polymerization, leading to impaired hematopoietic cell growth and functions (Thrasher and Burns 2010). More than 300 mutations have been identified with different effects on WAS expression or function (Massaad et al. 2013). However, the classical syndrome, associated with complete absence of WAS protein, is characterized by small platelets, thrombocytopenia, and increased susceptibility to bacterial, viral, and fungal infections. Among several immune defects are a progressive T cell lymphopenia, near absent iNKT cell numbers, and impaired NK and T cell effector function due to defective immune synapse formation. About 13% of WAS patients develop malignancies, mainly B cell lymphomas, with an average age of onset of 9.5 years (Sullivan et al. 1994). EBV infection appears to increase the lymphoma risk. EBV-induced LPDs observed in patients with WAS include lymphomatoid granulomatosis (Sebire et al. 2003), non-Hodgkin's lymphoma (Gulley et al. 1993; Yoshida et al. 1997; Du et al. 2011), Hodgkin's lymphoma (Sasahara et al. 2001; Du et al. 2011) and other LPDs (Nakanishi et al. 1993).

Ataxia telangiectasia (AT)

AT is an autosomal recessively inherited syndrome characterised by progressive cerebellar ataxia, oculomotor dyspraxia, oculocutaneous telangiectasia, immunodeficiency and susceptibility to malignancy. It is caused by mutations in the ATM protein, which plays an integral role in DNA repair and cell cycle checkpoint control (Paull 2015). Immune defects vary between patients, but typically present as mild impairment of both humoral and cell-mediated immunity. Low levels of serum antibodies and reduced numbers of B cells are common features, whereas NK cell numbers appear normal. CD4+ T cell lymphopenia has been reported in some patients, but T cell function remains broadly intact. Patients are particularly susceptible to bacterial sinopulmonary infections early in life, and many develop chronic lung disease (Chopra et al. 2014). A recently published French registry study demonstrated a 19.1% incidence of lymphoma in patients with AT by 20 years of age. Approximately one-third of these lymphomas were Hodgkin's lymphomas (all tested were EBV-related), and the remaining two-thirds were non-Hodgkin's lymphomas (50% EBV positive) (Suarez et al. 2015). HLH, severe infectious mononucleosis or chronic EBV viraemia has not been described in AT.

WHIM

Warts, Hypogammaglobulinaemia, Immunodeficiency, and Myelocathexis (WHIM) syndrome is characterised by a susceptibility to severe papilloma virus and herpesvirus infections (Gorlin et al. 2000). WHIM syndrome results from gain-of-function mutations in CXCR4, the receptor for CXCL12, which is critical for regulating the release of neutrophils from the marrow during inflammation and maintaining circulating neutrophil homeostasis (Gorlin et al. 2000). The WHIM syndrome-associated CXCR4 mutations cause impaired receptor internalization leading to increased signaling (Balabanian et al. 2005; Bachelerie 2010; Dotta et al. 2011). Two cases of EBV-associated LPD (fatal in 1 case) have been described (Chae et al. 2001; Imashuku et al. 2002).

Chediak–Higashi syndrome

Chediak–Higashi syndrome (CHS) is an autosomal recessive disorder, caused by a defect of *LYST*, a gene that encodes a regulator of lysosomal trafficking. Mutations affect the size, structure, and function of

lysosomes and other secretory granules, resulting in abnormally large organelles in virtually all granulated cells (Kaplan et al. 2008). The most important features of the immunodeficiency are neutropenia and NK cell dysfunction. Patients suffer recurrent life-threatening bacterial and viral infections.

Furthermore, without HSCT, almost 85% of affected children will progress to an accelerated disease with lymphoproliferative infiltration of major organs and HLH (Kaplan et al. 2008). Although there has been no detailed analysis, EBV infection has been postulated as a trigger. In one early report, 6 of 9 unrelated patients were EBV seropositive, and 3 of these were reported to have signs of chronic active EBV infection post-infectious mononucleosis (Merino et al. 1986). Another more recent report has shown a clearer association between primary EBV infection and CHS acceleration (Ogimi et al. 2011).

22q11.2 deletion syndrome (Di George)

22q11.2 deletion syndrome (22q11.2DS), also known as Di George syndrome (DGS), is the most common chromosomal microdeletion disorder. It was initially described as a clinical triad of immunodeficiency, hypoparathyroidism, and congenital heart disease. The syndrome is now known to have a heterogeneous presentation that includes multiple additional congenital anomalies and later-onset conditions, such as palatal, gastrointestinal and renal abnormalities, autoimmune disease, variable cognitive delays, behavioural phenotypes, and psychiatric illness (McDonald-McGinn et al. 2015). Thymic hypoplasia in partial DGS or complete aplasia in classic DGS is the major factor generating the T cell immunological defects. Besides severe age-specific T cell lymphopenia through infancy, there is additionally a decline in functional CD4+ CD25+ regulatory T cell counts and decreased expression of the AIRE gene (Shabani et al. 2016). EBV-associated lymphoproliferation was reported to date in 3 patients with 22q11.2 deletion syndrome (Shabani et al. 2016).

Conclusion

EBV infection can manifest in a variety of ways: asymptomatic/mild infection, infectious mononucleosis, HLH, or a variety of malignancies. These clinical features are most prominent in immunocompromised individuals resulting from coincident infections, iatrogenic therapies, or gene mutations. Our understanding of the host-virus interaction in EBV infection and better characterised the pathophysiology of severe and aberrant EBV infection has increased significantly over the past 2 decades. By identifying new genes associated with immunodeficiency and predisposition to EBV viremia and associated disease, the underling cellular, biochemical, and molecular mechanism involved in the immunity to EBV are starting to unravel. This understanding will hopefully allow identification of targeted biological, cellular, or small molecule therapies for managing these patients effectively and safely in the future.

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Leukocyte adhesion deficiency-I caused by a novel mutation in *ITGB2* presenting with pyoderma gangrenosum

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ABSTRACT

Background: Leukocyte adhesion deficiency (LAD) syndromes are primary immunodeficiency disorders caused by defects in adhesion molecules on leukocytes resulting in impaired migration into tissues. Common cutaneous manifestations of LAD include bacterial infections, omphalitis with delayed separation of the umbilical cord, impaired pus formation and poor wound healing. LAD is associated with significant morbidity and mortality, making early diagnosis and management integral in the care of these patients.

Methods: Molecular testing and flow cytometry for expression of CD18 were performed on 2 siblings presenting with cutaneous lesions including pyoderma gangrenosum (PG).

Results: We describe 2 siblings with a novel homozygous mutation in *ITGB2* (c.2070del, p.Asp690Glufs*25) resulting in an atypical presentation of LAD-I with PG.

Conclusion: LAD should be considered in patients presenting with unexplained PG, even in the absence of significant infections or umbilical cord complications.

Statement of novelty: To the best of our knowledge, we describe a novel homozygous mutation in *ITGB2* (c.2070del, p.Asp690Glufs*25) resulting in LAD-I. Patients with LAD-I may present with unexplained PG and may lack classic symptoms including umbilical cord complications.

Introduction

The leukocyte adhesion cascade system allows for leukocyte accumulation at sites of tissue inflammation and infection. Adhesion molecules including selectins, integrins, and members of the immunoglobulin superfamily of proteins, are expressed on leukocytes and vascular endothelial cells and are required for leukocytes to migrate from the vasculature into the tissues (Schmidt et al. 2013). Leukocyte adhesion deficiency (LAD) syndromes are primary immunodeficiency disorders caused by defects in adhesion molecules on leukocytes resulting in impaired migration into tissues (Al-Herz et al. 2011). LAD is divided into 3 subgroups including LAD-I (beta-2 integrin defect), LAD-II (fucosylated carbohydrate ligands for selectins are absent) and LAD-III (activation of all beta integrins is defective) (Al-Herz et al. 2011). LAD-I is an autosomal recessive syndrome caused by a mutation in the integrin beta-2 gene (*ITGB2*) resulting in deficiency and/or defects in CD18, the common beta chain of the beta-2 integrin family, and the inability of leukocytes to adhere to the endothelium and migrate into tissues (van de Vijver et al. 2012). LAD-I has also been associated with

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impaired inhibition of interleukin-23 and interleukin-17 resulting in a hyperinflammatory response and chronic inflammation (Moutsopoulos et al. 2014). Patients with severe LAD-I, defined as less than 2% of CD18 expression, typically present in early infancy with recurrent, life threatening infections that are frequently fatal before age 2 years old without hematopoietic stem cell transplant (HSCT) (Almarza Novoa et al. 2018). Patients with mild to moderate LAD-I, defined as 2%–30% of CD18 expression, tend to have fewer significant infections and often survive into adulthood without HSCT (Almarza Novoa et al. 2018). Common clinical manifestations of LAD-I include delayed separation of the umbilical cord with omphalitis, recurrent bacterial infections especially of skin and mucus membranes, impaired wound healing, absent pus formation, periodontitis, and leukocytosis (Hanna and Etzioni 2012). We describe 2 siblings with LAD-I caused by a novel mutation in *ITGB2* and atypical presentation including pyoderma gangrenosum (PG).

Methods

Molecular genetic evaluation

DNA collected from blood was submitted to Fulgent Diagnostics for sequencing. DNA was barcoded and enriched for the coding exons of targeted genes using hybrid capture technology. Prepared DNA libraries were sequenced using Next Generation Sequencing technology. Following alignment, variants were detected and interpreted manually using locus specific databases, literature searches and other molecular biological principles. All variants were confirmed by Sanger sequencing. Patient consent was obtained and recorded through the Canadian Primary Immunodeficiency Evaluation Study (C-PRIMES).

Flow cytometry

Flow cytometric immunophenotyping of peripheral blood was performed by Mayo Medical Laboratories to evaluate the presence of the CD11/CD18 complex using monoclonal antibodies directed against the CD11 isoforms, CD11a and CD11b, and CD18 antigens.

Results

Patient presentation

A female patient born to non-consanguineous Caucasian parents presented at age 7 years old with multiple painful, erythematous and necrotic papules on the inner thigh and intermittent fevers. She was admitted for intravenous antibiotics following a failed course of oral cephalexin. Wound and blood cultures were negative. She had a significant lymphocytosis, 35.1×10^9/L, with predominate neutrophils, 28.4×10^9/L. Past infectious history included 1 previous cutaneous infection following an abrasion and molluscum contagiosum. She did not have delayed separation of the umbilical cord. Partial evaluation of the immune system was performed at age 7 years old and reported normal lymphocyte subsets ($CD20^+$, $CD3^+$, $CD3^+/CD4^+$, $CD3^+/CD8^+$, and $CD3^-/CD56^+CD16^+$), normal immunoglobulin levels, normal vaccine titers, and normal neutrophil oxidative burst index. The patient had a persistent leukocytosis ranging from $38.8–10.8 \times 10^9$/L and neutrophilia ranging from $32.6–11.3 \times 10^9$/L.

Fifteen months later, she presented to the emergency room with papules that rapidly progressed to necrotic ulcerations on the thighs and fever requiring another admission for intravenous antibiotics (Figures 1–4). Dermatology was consulted and a skin biopsy was

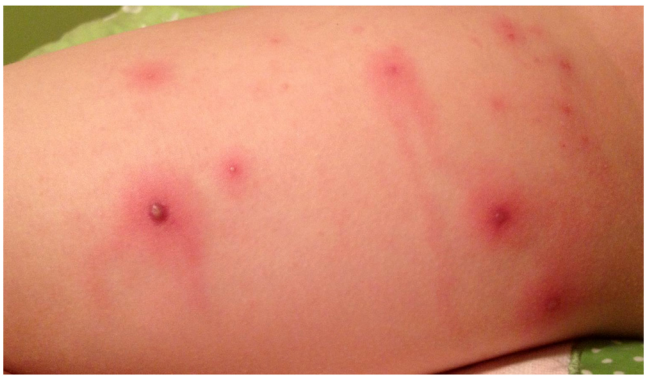

Figure 1: Patient 1, Day 1

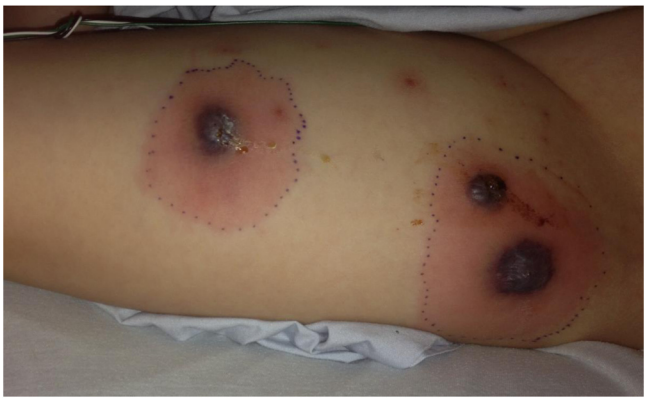

Figure 2: Patient 1, Day 3

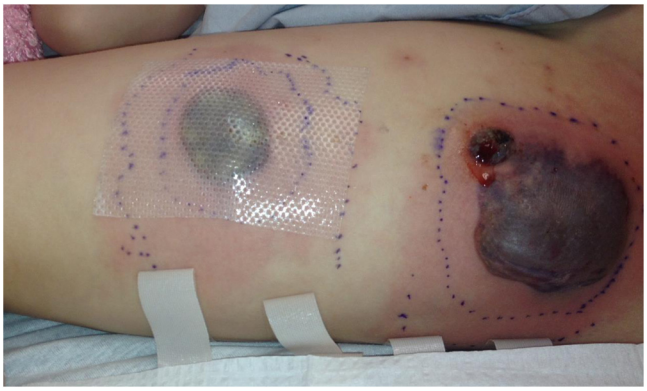

Figure 3: Patient 1, Day 5

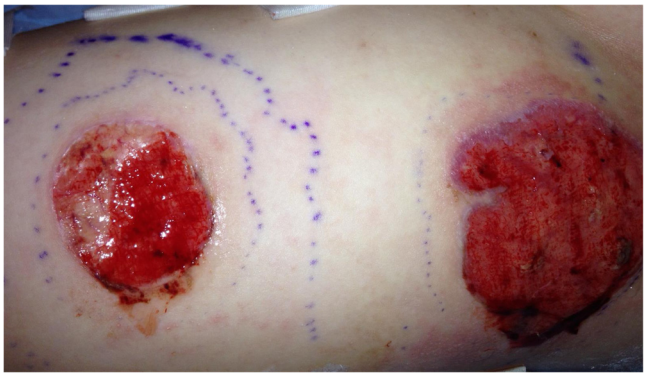

Figure 4: Patient 1, Day 7

performed. The skin biopsy reported epidermal necrosis with superficial dermal abscess with neutrophils and eosinophils consistent with superficial granulomatous pyoderma. Evaluations for inflammatory bowel disease, including colonoscopy with biopsies, and rheumatologic disorders were negative and the patient was treated for presumed idiopathic PG with oral prednisone and cyclosporine. Genetics was consulted due to the persistent nature and difficult to treat skin lesions. A literature review led to the article by Madkaikar et al, describing patients with PG in the setting of persistent neutrophilia who were diagnosed with LAD-1. Given this, at age 9 years and 6 months, molecular genetic testing was arranged and a homozygous frameshift mutation was identified in the *ITGB2* gene (c.2070del, p. Asp690Glufs*25) located on 21q22.3. This was predicted to result in premature truncation of the protein and according to the American College of Medical Genetics (ACMG) guidelines the variant was classified as pathogenic. A heterozygous variant in *NOD2* (c.3019dup, p.Leu1007Profs*2) was also identified and was classified as a susceptibility factor for Crohn's disease. Flow cytometry reported absent expression of CD18 on lymphocytes with significantly reduced expression on granulocytes. There was normal expression of CD11a on lymphocytes and monocytes and absent expression on granulocytes. CD11b was absent on lymphocytes, significantly reduced in monocytes, and close to normal on granulocytes. The patient was diagnosed with LAD-I. Immunosuppressive medications were discontinued and she was started on prophylactic antibiotics. The PG has resolved since starting prophylactic antibiotics with amoxicillin. She has had intermittent skin lesions that resolve with a course of oral antibiotics.

Patient 2, the brother of patient 1, presented at age 13 years with small painful papules on the lateral aspect of upper thigh. Past infectious history was remarkable for molluscum contagiosum. He did not have delayed separation of the umbilical cord. Based on the sibling's diagnosis of LAD-I, molecular genetic testing was completed and he was confirmed to have the same homozygous mutation in *ITGB2* (c.2070del, p.Asp690Glufs*25). The parents of patient 1 and patient 2 also had molecular genetics testing completed and were both found to be carriers of the mutation in *ITGB2*. The patient was diagnosed with LAD-I and was started on prophylactic antibiotics to prevent the development of cutaneous infections. The papules have resolved since starting prophylactic antibiotics.

Discussion

LAD-I is a rare primary immunodeficiency caused by defects in adhesion molecules on leukocytes resulting in impaired migration into tissues. To the best of our knowledge, we describe a novel homozygous variant in *ITGB2* (c.2070del, p.Asp690Glufs*25) resulting in LAD-I. Common cutaneous and mucus membrane manifestations of LAD-I include bacterial infections, omphalitis with delayed separation of the umbilical cord, gingivitis, oral ulcers, impaired pus formation, and poor wound healing. In particular, necrotic skin lesions have been reported in more than 10% of patients with LAD-I (Almarza Novoa et al. 2018). PG is an ulcerative skin disease characterized by sterile, painful, necrotic ulcers and is commonly associated with systemic conditions including inflammatory bowel disease, hematologic disorders, and rheumatic disorders (Wollina 2007). Patients with LAD-I presenting predominately with PG have been reported (Van de Kerkhof and Weemaes 1990; Bedlow et al. 1998; Hinze

et al. 2010; Nord et al. 2011; Thakur et al. 2013; Madkaikar et al. 2015). Similar to patient 1, most of these reported patients did not have significant infections prior to presenting with PG and therefore the diagnosis of LAD-I was often delayed. The histopathology of classic PG shows predominant neutrophil infiltration. In contrast, the histopathology of LAD-I ulcerating lesions typically has an absence of neutrophils (Bedlow et al. 1998; Nord et al. 2011). The skin biopsy of patient 1 reported the presence of neutrophils suggesting partial neutrophil recruitment into the tissues. Similar histopathology findings have been described in patients with LAD-I and PG (Hinze et al. 2010; Madkaikar et al. 2015). This case report highlights the importance of considering LAD-I in patients presenting with PG, even in the absence of significant infections.

Other atypical features of our LAD-I patients include lack of umbilical cord complications and older age of presentation. Umbilical cord complications such as delayed separation of the umbilical cord and/or omphalitis are early manifestations of LAD-I. Umbilical cord complications are more common in patients with severe LAD-I (84% of patients) compared to mild to moderate LAD-I (58% of patients) (Almarza Novoa et al. 2018). Neither of our patients had umbilical cord complications. In addition, our patients presented later in childhood, at ages 7 and 13 years old, compared to the majority of other LAD-I patients described in the literature. Almarza Novoa et al. 2018 reported the median age of presentation was 1 month old (range 0.03–18 months) for severe LAD-I and 6 months old (range 0.03–192 months) for mild to moderate LAD-1. The lack of umbilical cord complications and older age at the time of presentation may have contributed to the delayed diagnosis of LAD-I in patient 1. In addition, it could be hypothesized that this particular mutation results in a mild phenotype explaining the delayed onset of presentation.

We present 2 siblings with a novel homozygous mutation in *ITGB2* (c.2070del, p.Asp690Glufs*25) resulting in LAD-I with atypical presentation including PG. LAD-I should be considered in patients with unexplained PG, even in the absence of classic symptoms such as umbilical cord complications. An early diagnosis of LAD-I is imperative to prevent life-threatening complications such as significant infections and for consideration of treatments including HSCT.

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Original Article

Gastrointestinal defects and immunodeficiency syndrome with normal in vitro IgG production

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ABSTRACT

Background: Gastrointestinal defects and immunodeficiency syndrome (GIDID) is a severe neonatal disorder usually fatal within the first months of life. We report a case presenting with intestinal atresia, combined immunodeficiency, and a novel association with hypothyroidism and cardiac malformations. The immune phenotype was remarkable for agammaglobulinemia, lymphopenia, and mildly decreased lymphocyte proliferation. We present here the unique phenotype as well as studies to determine if the agammaglobulinemia was due to an intrinsic B lymphocyte defect.

Methods: Peripheral blood mononuclear cells from the patient and a healthy control were isolated by Ficoll-Hypaque centrifugation and stimulated with anti-CD40, IL-4 and IL-21 for 7 days. Total IgG production was measured by ELISA in the supernatant of the stimulated sample on day 7. Cells were stained for CD19, CD27, IgM, CD11b, CD11c, and CD14.

Results: At day 7, supernatant from the patient stimulated cells contained levels of total IgG comparable to the control (755 ng/mL vs. 658 ng/mL, respectively). B cell maturation appeared impaired, as morphologically the patient sample demonstrated fewer B cell clones and cells with dendritic projections.

Conclusions: Despite this typical severe clinical picture of GIDID with agammaglobulinemia, IgG production was detected under optimal stimulation for induction of plasma cells. This suggests that there may not be an inherent defect in class switching and antibody production in B cells in this disorder. It is possible that the in vivo physical or cytokine milieu may be defective for optimal B cell function. Further studies assessing the function of the immune cells as well as possible gastrointestinal loss of immunoglobulins are needed in this disease.

Statement of novelty: Despite much improvement in understanding the effects of *TTC7A* mutations in GIDID, the root cause of hypogammaglobulinemia in these patients is still unclear. The work portrayed in this study furthers the current knowledge. It suggests that when appropriately stimulated in vitro, this patient's B cells were capable of adequate immunoglobulin production. Moreover, to the best of our knowledge, this patient is the first with this genetic defect to be reported with hypothyroidism and cardiac malformations.

Background

Gastrointestinal defects and immunodeficiency syndrome (GIDID), previously called multiple intestinal atresia with combined immunodeficiency (MIA-CID), has been reported in unrelated families of multiple ethnicities. The first identification of the genetic defect was determined by whole exome sequencing in a cohort of

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French-Canadian infants (Samuels et al. 2013). They identified mutations in the tetratricopeptide repeat (TPR) domain 7A gene (*TTC7A*). Other groups also confirmed this finding (Chen et al. 2013; Bigorgne et al. 2014). This neonatal disorder is usually fatal within the first months of life. These infants classically present with intestinal obstruction prenatally or at birth leading to multiple organ failure. Surgical interventions are mostly palliative (Chen et al. 2013; Bigorgne et al. 2014). The associated immunodeficiency can be mild or severe. It is characterized by hypogammaglobulinemia with B and T cell lymphopenia. On pathologic evaluation, patients often present thymic hypoplasia, poor corticomedullary demarcation and a paucity of lymphocytes and Hassall's corpuscles (Bigorgne et al. 2014; Fernandez et al. 2014). *TTC7A* mutations have also been associated with autoimmunity and multi-organ involvement (Fernandez et al. 2014; Lemoine et al. 2014*a*, 2014*b*), early-onset inflammatory bowel disease (Avitzur et al. 2014), combined immune deficiency with dendriform lung ossification (Ngan et al. 2014), and ichthyosis (Leclerc-Mercier et al. 2016). *TTC7A* plays an important role in actin cytoskeleton organization by interacting with other proteins in the RhoA pathway. Thus, it is thought to have a crucial role in the apicobasal polarization of intestinal epithelial cells and perhaps in the differentiation, proliferation, and survival of lymphocytes (Bigorgne et al. 2014; Lemoine et al. 2014*a*, 2014*b*). Hypogammaglobulinemia has been reported in the literature but it has not been well characterized in this disorder. It is postulated to result from a primary failure of immunoglobulin production by B cells or from the severe protein-losing enteropathy associated with this defect. Given the rapid loss of immunoglobulins after intravenous and subcutaneous replacement in our patient, we hypothesized that the intestinal loss played the major role in our patient's agammaglobulinemia. We report a case of GIDID with novel features and assess immunoglobulin production in vitro under appropriate stimulation.

Methods

Cell culture

Peripheral blood mononuclear cells (PBMCs) from this patient and one healthy adult control were isolated by Ficoll-Hypaque centrifugation from 5 mL blood samples. PBMCs were resuspended in complete medium [RPMI 1640 supplemented with 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 15 mM HEPES buffer (pH 7.0), and 100 U/mL penicillin/streptomycin]. The isolated PBMCs were cultured at 10^6 cells/mL/well in a 12-well non-treated tissue culture plate in medium alone (unstimulated) and in medium with activating conditions to enhance the induction of antibody-producing memory B cells and plasma cells (stimulated). The activating conditions were achieved with anti-CD40 antibodies (Clone G28.5, ATCC, Manassas, Virginia, USA, 1 µg/mL), interleukin-4 (IL-4, R&D Systems, Minneapolis, Minnesota, USA, 200 U/mL), and IL-21 (Peprotech Inc., Rocky Hill, New Jersey, USA, 50 ng/mL) incubated at 37 °C and 5% CO_2 for 7 days (Desjardins et al. 2018). Culture medium was replenished at days 3 and 5 without addition of cytokines.

B cell class switch analysis

Cell staining was done on day 7 of culture. Antibodies included allophycocyanin (APC)-conjugated mouse anti-human CD19 (BioLegend, San Diego, California, USA), efluor450-conjugated mouse anti-human CD27 (eBioscience, Thermo Fisher Scientific, Waltham, Massachussetts, USA), phycoerythrin (PE)-conjugated mouse anti-human IgM (BD Pharmingen, BD Biosciences, San Jose, California, USA), peridinin-chlorophyll-protein complex (PerCP)-conjugated rat anti-human CD11b (BioLegend, San Diego, California, USA), fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CD11c (BioLegend, San Diego, California, USA), and phycoerythrin-cyanine 7 tandem (PE-Cy7)-conjugated mouse anti-human CD14 (BioLegend, San Diego, California, USA). Appropriate isotype controls were performed using APC IgG_1 k, efluor450 IgG_1 k (eBioscience, Thermo Fisher Scientific, Waltham, Massachussetts, USA), PE IgG_1 k (BD Pharmingen, BD Biosciences, San Jose, California, USA), PerCP IgG_{2b} k (BioLegend, San Diego, California, USA), FITC IgG_{2b} k (BioLegend, San Diego, California, USA), and PE-Cy7 IgG_1 k (BioLegend, San Diego, California, USA). Flow cytometry analyses were performed using an LSRII Cytometer (Becton Dickinson, Mississauga, ON, Canada) and FlowJo software.

IgG quantification by ELISA

On day 7, supernatants were collected for total IgG measurement by ELISA. A 96-well plate (Costar, Corning Corp, Acton, Mass) was coated with goat anti-human IgG (Bethyl, Montgomery, Texas, USA, 1 mg/mL, working dilution of 1:500) in 0.5 M carbonate-bicarbonate buffer, pH 9.6 and stored overnight at 4 °C. The plate was washed 3 times with PBS/0.1%

Tween-20 and blocked with blocking buffer (TRIS 30 mM, NaCl 0.14 M, and 1% BSA) for 30 minutes at room temperature. It was washed again and incubated for 1 hour with serial dilutions of human IgG standards (Bethyl, Montgomery, Texas, USA, 4.4 mg/mL) or cell culture supernatants in duplicates. After washing, HRP-conjugated goat anti-human IgG (Bethyl, Montgomery, Texas, USA, 1 mg mL, working dilution of 1:50 000) was added and incubated for 1 hour. After incubation and washing, tetramethylbenzidine (Invitrogen, Thermo Fisher Scientific, Carlsbad, California, USA) was added, and the plate was incubated for 10 minutes. The reaction was stopped with NH_3PO_4 and the optical density was measured with an ELISA plate reader (Tecan Infinite M1000) at 450 nm. The limit of IgG detection was 0.586 ng/mL.

Genetic analysis

Sequencing of *TTC7A* was performed by Next Generation Sequencing at Fulgent Laboratories Diagnostics. The DNA was barcoded and enriched for the coding exons using hybrid capture technology. Prepared DNA libraries were sequenced using Next Generation Sequencing technology. Following alignment, variants were detected in regions of at least 10× coverage. For this specimen, 100% of coding and splicing junctions of *TTC7A* have been sequenced with coverage of at least 10× and 20×, respectively.

Results

Case report

A French-Canadian pre-term boy of 35 weeks gestation presented with intestinal obstruction on his first day of life (Figure 1), requiring urgent surgical intervention. He was born to non-consanguineous parents. The pregnancy was only remarkable for late-onset polyhydramnios. The family history was significant for a paternal grandfather who underwent a pneumonectomy at age 9 and received intravenous immunoglobulin (IVIg). The parents reported that this paternal grandfather passed away from pneumonia at the age of 47 years.

During surgical laparotomy, he was found to have pyloric atresia, antenatal transverse colon perforation, and meconium cyst with meconium peritonitis. His clinical course worsened with secretory diarrhea, intermittent bloody stools, and poor weight gain despite parenteral nutrition and regular albumin infusions.

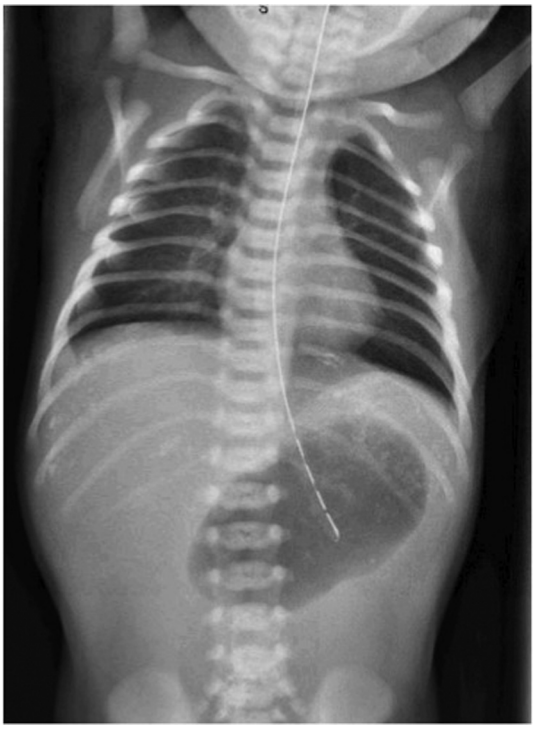

Figure 1: Abdominal X-ray performed on first day of life. Absence of gas below the gastric outlet suggests obstruction at the level of the gastric outlet or proximal duodenum. Clusters of calcifications in the left and right upper quadrants indicate prior antenatal intestinal perforation or meconium peritonitis.

At 2 months of life, he had persistent feeding difficulties, intermittent fevers without focus of infection, severe normocytic anemia, intermittent leukocytosis, persistent neutrophilia (47×10^9/L), monocytosis (7.2×10^9/L), and lymphopenia (1.3×10^9/L). He had undetectable levels of IgG, IgA, and IgM despite normal albumin levels and a mildly elevated stool alpha-antitrypsin (0.76 g/L, normal 0–0.72 g/L). Upon further evaluation, he had decreased B and CD8 T cell counts: CD19+ 120×10^6 cells/mm (5%), CD4+ 1778×10^6 cells/mm (73%), CD8+ 200×10^6 cells/mm (8%), CD4/CD8 ratio 8.9, CD16_56 268×10^6 cells/mm (11%). B and T lymphocyte phenotyping revealed a high proportion of immature B and T cells (Table 1) with absent switched memory B cells. The lymphocyte stimulation to mitogens was normal (data not shown). A T cell receptor V-beta repertoire showed normal variability without oligoclonal expansion (data not shown).

The agammaglobulinemia persisted despite IVIg and subcutaneous immunoglobulin (SCIg) infusion (Figure 2). The IgG level increased to 11.44 g/L (normal

Table 1: **B and T lymphocyte phenotyping.**

Cells	Absolute count $\times 10^6$ cells/μL (%)	Reference (%) (10th–90th P)
CD4+/CD45RA+ (%CD4)	1679 (88%)	80% (77–94)
CD4+/CD45RO+ (%CD4)	172 (6%)	8% (3–16)
CD45RA/CD45RO ratio	9.8	
CD8+/CD45RA+ (%CD8)	178 (96%)	94% (85–98)
CD8+/CD45RO+ (CD8%)	3 (1%)	3% (1–7)
CD45RA/CD45RO ratio	59.3	
CD19+/sIgM+ (%CD19)	126 (95%)	50% (34–66)
CD19+/CD5+ (%CD19)	72 (55%)	39% (24–54)
CD19+/CD27+ (%CD19)	16 (12%)	23% (11–35)
CD19+/CD27+/IgD– (%CD19)	0 (0%)	

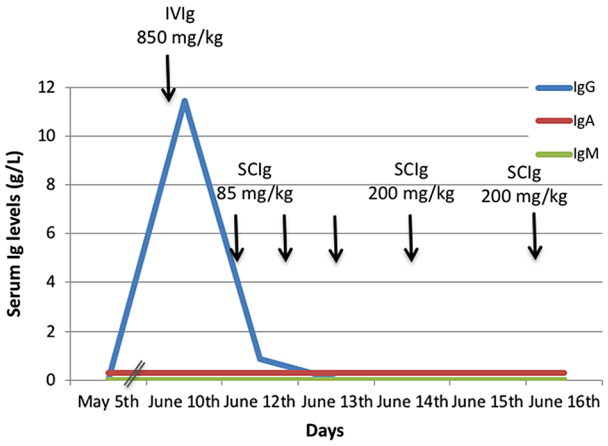

Figure 2: Evolution of immunoglobulin levels with replacement therapy.

range for age 2.22–5.84 g/L) with a loading dose of 850 mg/kg of IVIg but dropped to 0.86 g/L 48 hours later and was undetectable by 72 hours despite daily SCIg doses of 85 mg/kg initiated the day after the IVIg. SCIg doses as high as 200 mg/kg every other day were tried without success. This was attributed to the gastrointestinal losses, although concomitant decreased IgG production remained a possible contributor.

The infant developed rapidly progressive hepatosplenomegaly and displayed dysautonomic symptoms including hyperthermia, tachycardia, and variable blood pressure. Further investigations including a transesophageal echocardiogram showed subaortic stenosis and moderate mitral regurgitation. He developed clinically significant hypothyroidism at 4 months of age, with a thyroid stimulating hormone (TSH) level as high as 72.39 mIU/L (normal 0.34–5.6 mIU/L). His neonatal screening had previously been reported as normal at 5.37 mIU/L (normal <10 mIU/L) and antithyroperoxidase antibodies were absent.

Given his clinical features, gastrointestinal defects and immunodeficiency syndrome (GIDID, MIM 243150) was suspected. Molecular investigations by Next Generation Sequencing revealed compound heterozygous variants in the *TTC7A* gene (Figure 3). One of those variants, NM_001288951.1:c.1001+3_1001+6del(p.?) causes a frameshift in exon 7 leading to a premature stop codon. This has been reported in the homozygous or compound heterozygous state in French-Canadian patients with multiple intestinal atresia (Chen et al. 2013; Samuels et al. 2013). The second variant, NM_001288951.1:c.518G>A(p. Gly173Asp), had not been previously reported. No other variants including deletion or duplication were detected. Based on his clinical features and the fact that each parent was a heterozygous carrier of one of the two variants identified in the patient, the c.518G>A *TTC7A* variant is presumed likely causative and pathogenic.

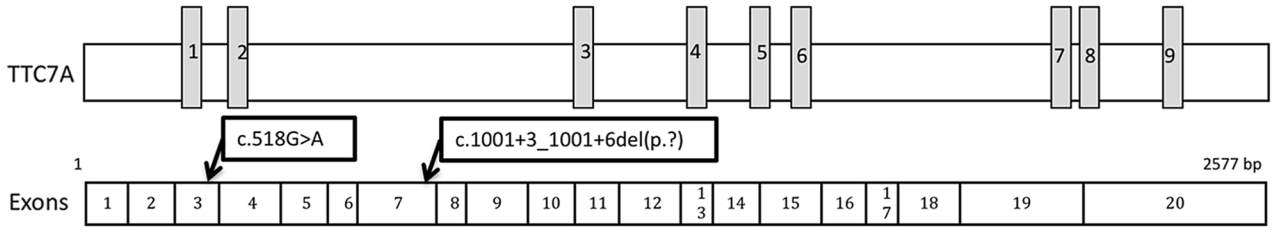

Figure 3: Schematic representation of *TTC7A* gene and the patient's compound heterozygous variants where grey zones represent TPR domains.

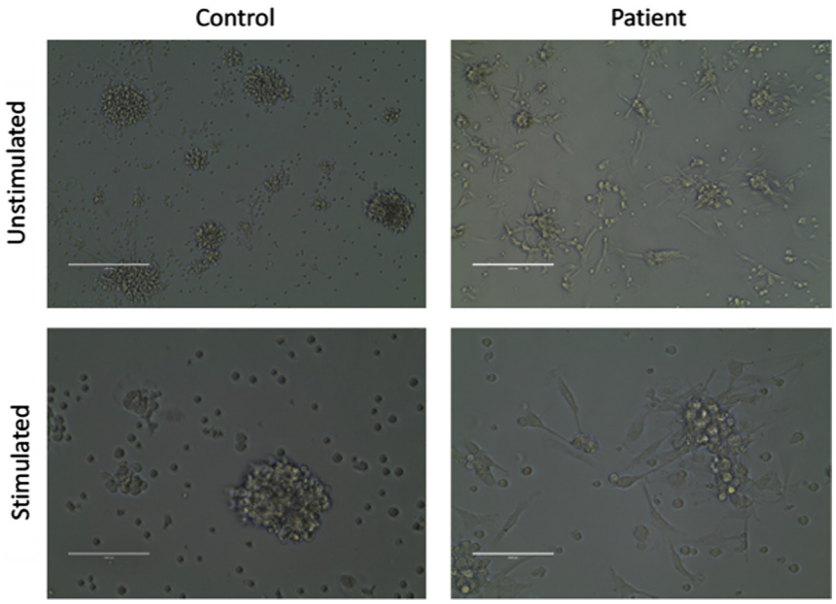

Figure 4: Microscopic findings: Day 7 of cell culture with and without stimulation with anti-CD40+IL-4+IL-21. Patient cells demonstrate very few B cell clones with an abundance of dendritic-looking cells compared to control.

Given the predictable poor prognosis of this condition, the family elected to pursue with palliative care and declined bowel and hematopoietic stem cell transplantation. In the following weeks, the patient developed worsening respiratory distress and hypoxemia and passed away at 5 months of age.

B cell class switch analysis

The patient's PBMCs were cultured for 7 days with or without stimulation with anti-CD40+IL-4+IL-21. At day 7, the microscopic appearance of the patient's PBMCs appeared very different than control cells, with very few B cell clones and an abundance of dendritic-looking cells (Figure 4). The unstimulated patient's cells on day 7 had higher $CD14^+$ cells (3.43%) than control (0.46%), which was lower with stimulation at 1.14% and 0.23%, respectively (Figure 5). The day 7 unstimulated patient cells also had a larger $CD11b^+$ cell population (8.62%) that was not present in the control cells (0.27%). Upon stimulation, this $CD11b^+$ population decreased to 2.26% yet remained relatively unchanged in the control. There was a population of double positive $CD11b^+CD11c^+$ cells in the unstimulated patient cells, which was also not seen in the control cells (8.85% vs. 0.98%, respectively) (data not shown).

After 7 days of culture, the patient developed a population of $CD19^+CD27^+$ B cells that was larger than control (Figure 6). Under stimulation with anti-CD40+IL-4+IL-21, the patient demonstrated 11.0% $CD19^+CD27^+$ cells compared to 2.71% in the control. Within the $CD19^+CD27^+$ cells, the patient also had a greater $CD19^+IgM^+$ population than the control cells (9.48% vs. 5.78%, respectively).

The supernatant obtained after stimulation with anti-CD40+IL-4+IL-21 at day 7 contained comparable levels of total IgG as the control (755 ng/mL vs. 658 ng/mL, respectively).

Discussion

We report here for the first time a case of GIDID presenting with typical multiple intestinal atresia and combined immunodeficiency but also demonstrating novel features. He presented with acquired hypothyroidism with a TSH level as high as 72.38 mUI/L at 4 months of age, with a previously normal thyroid newborn screen and absence of anti-thyroperoxydase antibodies. He also displayed cardiac anomalies, including subaortic stenosis and moderate mitral regurgitation. To our knowledge, thyroid involvement has not been described in previous patients with GIDID. One paper reported a subject with probable cardiomyopathy (Fernandez et al. 2014) but no clear cardiac malformations. This highlights the multi-systemic involvement of the disease also suggested by previous authors (Fernandez et al. 2014).

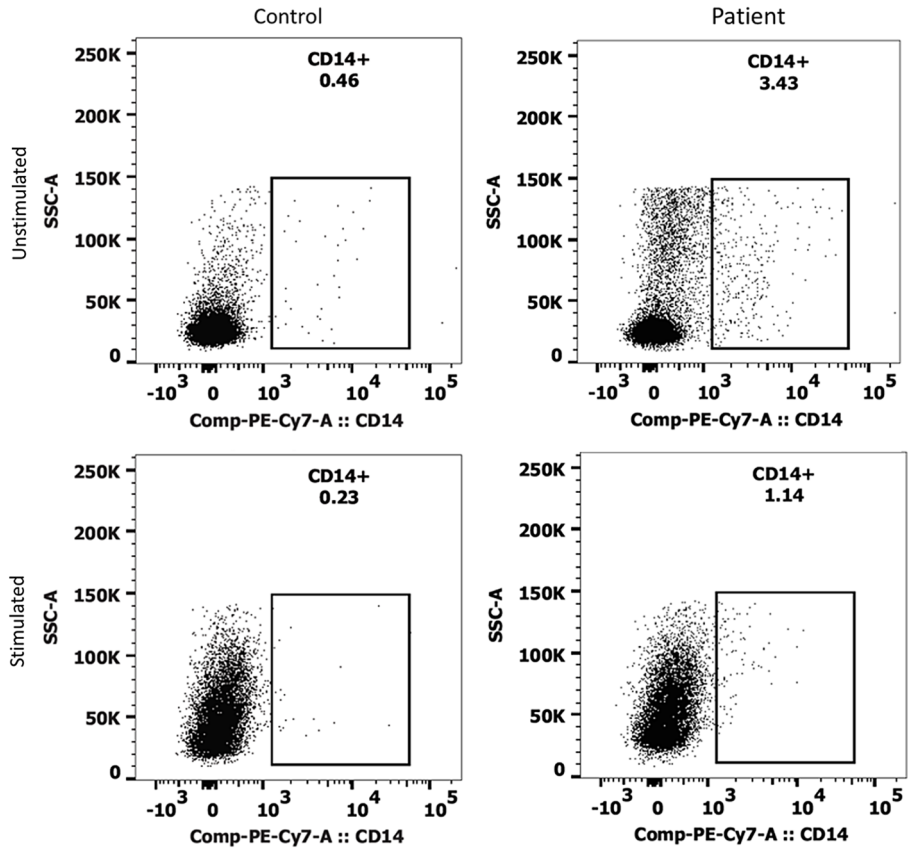

Figure 5: Flow cytometry on day 7 demonstrates an abundance of CD14+ expressing cells with and without anti-CD40+IL-4+IL-21 stimulation in the patient compared with the control.

Moreover, we present a patient with compound heterozygous mutations with a novel amino acid substitution in *TTC7A* (Figure 3). The variant NM_001288951.1:c.1001+3_1001+6del(p.?) causes a frameshift in exon 7 leading to a premature stop codon. This has been reported in the homozygous or compound heterozygous state in French-Canadian patients with multiple intestinal atresia (Chen et al. 2013; Samuels et al. 2013). The father is from Bas-du-Fleuve, an area of Canada where a founder effect has been previously described for this *TTC7A* variant (Samuels et al. 2013). The second variant, NM_001288951.1:c.518G>A (p. Gly173Asp), had not been previously reported. No other variants including deletion or duplication were detected. This rare variant has an extremely low allele frequency of 4.063×10^{-6} and had been reported only in individuals of Non-Finnish European descent (Lek et al. 2016). The glycine at position 173 is highly conserved across species. It is located within the first tetratricopeptide repeat (TPR) domain of the TTC7A protein. TPR domains are structural motifs important for protein–protein interactions. Based on his clinical features and the fact that each parent was a heterozygous carrier of one of the two variants identified in the patient, the c.518G>A *TTC7A* variant is presumed likely causative and pathogenic.

Further, our clinical experience and in vitro results suggest that agammaglobulinemia in this case may be due to gastrointestinal loss rather than a defect in antibody production. This patient presented with the classical features of GIDID with significant and secondary immune deficiency. As shown above, he exhibited agammaglobulinemia and displayed in vivo B cell lymphopenia with absent switched memory B cells. Replacement with high doses of SCIg or IVIg was unsuccessful in achieving normal levels of immunoglobulins. This clinically suggests gastrointestinal loss as opposed to a B cell defect.

In vitro studies showed that, under optimal conditions and stimulation, the patient's B cells were able to produce levels of immunoglobulins comparable to the control when measured in the supernatant obtained after 7 days of culture with anti-CD40+IL-4+IL-21 stimulation. The patient's PBMCs were compared to

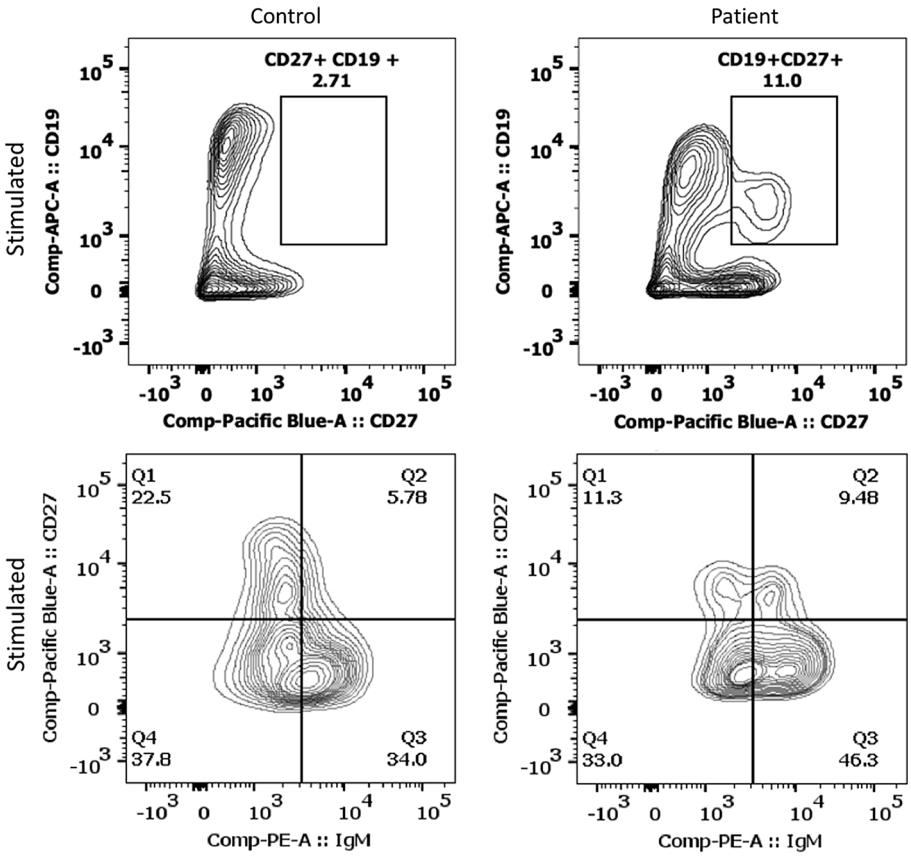

Figure 6: Flow cytometry upon 7 days stimulation, PBMC from the patient show an increase in the proportion of CD19+CD27+. Within the CD19+CD27+ cells, the patient cells had a greater IgM+ population than the control.

an adult control given the inability to obtain an age-matched control. Flow cytometry on day 7 show a larger population of $CD19^{+}CD27^{+}$ cells in the patient when compared to control (Figure 6). This is in keeping with the hypothesis that the agammaglobulinemia is secondary to the gastrointestinal losses instead of an intrinsic B cell defect in class switching and antibody production. However, the higher number of $CD19^{+}CD27^{+}$ cells in the patient is probably a reflection of the age difference between the patient and control.

The cellular arm of his immune system was also severely affected with lymphopenia and an abnormal CD4/CD8 ratio of 8.9. This may have been the result of a thymic production defect due to poor cellular polarization, as for the GI epithelium, or preferential gastrointestinal loss of $CD8^{+}$ T cells.

Conclusion

Our patient presented with a classical and severe clinical picture of GIDID confirmed by compound heterozygous variants in *TTC7A*. The immunologic phenotype was remarkable for evidence of agammaglobulinemia, decreased B cells and CD8 T cells, defects in lymphocyte maturation, and mildly decreased proliferation to mitogens. Interestingly, under the proper stimulation in vitro, the IgG production was comparable to control. Whether this environment is attainable for the B cells in vivo is unknown. There were other obvious differences in cell populations in our patient compared to control, as seen in the increased number of dendritic cells and macrophages, as well as IgM+ memory B cells. There may not be an inherent defect in class switching and antibody production in B cells, however the physical and cytokine milieu may be defective for cell–cell communication and signaling. Our findings support that the humoral impairment related to *TTC7A* mutations may be secondary to gastrointestinal loss more than a primary defect. The cellular defects may be more directly related to the TTC7A function. Further studies addressing the function of both the B and T cells are needed in this disease. It is also crucial to assess the role of hematopoietic stem cell transplant,

intestinal transplant, and gene therapy in the management of these critically ill patients.

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Original Article

Physician–patient shared decision making in the treatment of primary immunodeficiency: an interview-based survey of immunologists

Christopher C. Lamb[a,b*]

ABSTRACT

Background: Patient–physician shared decision making (SDM) can result in better care as well as reduced treatment costs. A better understanding of the factors predicting when physicians implement SDM during the treatment of primary immunodeficiency (PID) could provide insight for making recommendations to improve outcomes and reduce healthcare costs in PID and other long-term chronic conditions.

Method: This study made use of grounded theory and was based on the interview responses of 15 immunologists in the United States. It focused on their decision making in the diagnosis and treatment of PID, how they interact with patients, and the circumstances under which they encourage SDM with patients.

Results: All invited immunologists took part in the interviews and were included in the study. All but one had 10 or more years of experience in treating PID. The study found that SDM is bounded/limited by "nudging" bias, power balance considerations, and consideration of patient health literacy alignment. Immunologists also reported that they were mainly responsible for coordinating care and for allowing sufficient time for consultations.

Conclusion: SDM occurs between the physician and patient throughout the treatment of PID. The study also shows the ways physicians influence SDM by guiding patients through the process.

Statement of novelty: Little is known about the factors that influence SDM in the long-term management of chronic diseases. The present study investigated the extent to which immunologists experienced in the treatment of patients with PID include SDM in clinical practice. Findings such of these may be of use when formulating treatment guidelines and improving the effectiveness of long-term management of PID.

Introduction

Long-term immunoglobulin G (IgG) supplementation by the intravenous or subcutaneous route is indicated and recommended for the most common types of primary immunodeficiency (PID). Optimal dosing needs to be determined on an individual basis due to the high costs of IgG as well as to minimize the risk of adverse reactions (Bonilla et al. 2015; Betschel et al. 2017). As with any treatment for a lifelong chronic condition, the benefits of challenges or therapeutic options should, whenever possible, be discussed with the patient on an ongoing basis. Such a process of shared decision making (SDM) (Friesen-Storms et al. 2015) has been defined as an "approach where clinicians and patients share the best available evidence when faced with the task of making decisions, and where patients are supported to consider options, to achieve informed preferences" (Elwyn et al. 2010). SDM has been promoted at policy level in many countries whether for enhancing patient involvement as a desirable goal in itself or for benefits incurred, such as greater treatment adherence

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and the preferences of some patients for more conservative and lower-cost treatment options (Elwyn et al. 2010; Barry and Edgman-Levitan 2012; Lee and Emanuel 2013). Moreover, the Institute of Medicine report on healthcare inefficiencies in the US made 2 recommendations aimed at improving healthcare delivery while reducing costs: (*i*) that greater consideration should be given to patient preferences and (*ii*) that the care of chronic diseases should be seen as the most effective means to reduce overall costs in the healthcare system (Barry and Edgman-Levitan 2012; Gerteis et al. 2014). However, despite the existence of developed and defined SDM models, the implementation of SDM in most forms of chronic care is limited and little is known about how it can serve as a useful and practical way for doctors and patients to interact (Légaré et al. 2014; Couët et al. 2015). Because PID is a lifelong chronic condition that must be managed over the long term, it is an excellent model to study SDM because of the complex and challenging dimensions of the disease and complex ongoing treatment decisions. As with most chronic diseases (Noonan et al. 2017), the use and effectiveness of SDM have not been adequately studied to date. The study described here specifically addressed the knowledge gap in SDM in the treatment of PID by means of a qualitative survey of 15 immunologists experienced in the treatment of PID focusing on their decision making, including the extent to which they included patients in decision making.

Methods

Qualitative approach and research paradigm

The study was conducted from a constructivist and interpretivist point of view, applying grounded theory. A qualitative method was chosen in this initial study as a way of obtaining rich detail and explanatory theories on the topic of treatment decisions in PID, a topic on which little is known. The study was based on interviews with immunologists currently treating patients with PID and thus making use of specialists' extensive experiences and acquired knowledge. Questions aimed to be open ended and focused on treatment decision making in the immunologists' clinical practice including the level of shared decision making with patients. Simultaneous data collection and analysis facilitated the identification and pursuit of themes that shaped data collection and framed the emerging analysis (O'Reilly et al. 2012). Under grounded theory, data collection and analysis proceeds in stages, data collection, open-coding for preliminary analysis and labeling of data, axial coding for grouping of open coded labels and focused coding for constructing a formal framework within a variable (Corbin and Strauss 2008; Weick et al. 2008; Charmaz 2014).

Concepts used in developing the questionnaire

Dual Process

Under dual process theory, decisions may be reached in 1 of 2 ways (Tversky and Kahneman 1986; Scott 2000): heuristic decision making (System 1), the faster of the 2, relies on experience and recognition (Croskerry 2009; Kahneman 2011; Gigerenzer 2015); rational thinking (System 2), is a slower, more effortful process of problem solving by conscious analysis (Kahneman 2011; Gigerenzer 2015). Dual process theory is applicable to medical decision making processes (Djulbegovic et al. 2012).

Uncertainty

Physicians are usually confronted with 3 types of uncertainty when making clinical decisions: limitations of medical knowledge, the physician's perception of the gaps in his or her medical knowledge, and the tolerance of uncertainty (Jones 1992). Knowledge limitations often require the need to use clinical skills and judgement when there is incomplete empirical support of a decision (Flynn 2003). Any conflicts between research evidence and the physicians own experience can add to uncertainty (Timmermans and Berg 2010). A greater tolerance to uncertainty may increase a physician's willingness to deviate from standard protocols to accommodate the patient's lifestyle and preferences (Flynn 2003). Chronic care requiring complex management, decision making and coordination, as well as management of comorbidities can be a source of uncertainty (Whitson and Boyd 2016). However, dual process theory implies that greater uncertainty requires System 2 thinking.

Bias (and nudging)

Bias consists of flawed evaluations of initial information. For example, a physician may treat a new patient with the same methods and drugs as previous patients on the basis of similar symptoms and insurance

coverage (anchoring, availability, money-priming, and status quo bias). Training as a resident may drastically affect how physicians think and potentially reinforce bias: initial exposure to real patients is often the link between knowledge and experience (Patel et al. 2002, 2009). Perhaps the most important bias related to SDM is how word framing can alter decision making. For example, in deciding whether to have a hypothetical drug administered, the advice "This treatment has a 95% survival rate" is met with more favorably by patients than "This drug has a 5% death rate." Words have power and verbal primes impact decision making (Topol 2015).

Both biological and behavioral theories support the hypothesis that how doctors frame a treatment has profound effects on how patient receives, interprets and experiences that treatment. One method to combat bias is for the physician to practice metacognitive "slow" thinking, or to study how they reach conclusions (Klein 2005). A bias may be considered either a negative attribute or a "nudge" aimed to encourage advantageous decisions (Sunstein and Thaler 2008).

Power imbalance

Power dynamics are a fundamental aspect of human relationships and the physician–patient dyad is no exception (Fiske et al. 2016; Mirowsky 2017). Physicians are likely to categorize themselves and colleagues as highly educated when compared to patients. Asymmetries of information may explain why physicians are slow to adopt SDM (Tapscott 2010). Their interactions with patients, who are regarded as being in different category, affect the physician decision process depending on the particular patient. These categories, or identities, lead to a power imbalance in the physician–patient relationship and likely a reduction in SDM.

Traits (physician and patient)

Trait theory predicts that decision making is influenced by both physicians' and patients' background and sociodemographic characteristics (Kaplan et al. 1996). The following traits are thought to most influence physician decision making: age, gender, race, experience, trust, culture, and family (Hawley and Morris 2017). Participants in the medical dialogue bring with them all of their personal characteristics which affect patient–provider communication (Cooper-Patrick et al. 1999; Cooper and Roter 2003; Cooper et al. 2012).

Experience

Experience refers to the cumulative knowledge and frequency a physician has treated patients for the particular disease state. Experienced physicians, who tend to make greater use of intuition to make decisions based on patterns, could decrease the potential for SDM with patients (Marinova et al. 2016).

Trust

It has been hypothesized that trust between physicians and patients is necessary for SDM to occur. It is well-documented that trust improves patient compliance, satisfaction, and outcomes (Cook et al. 2004; Schoenthaler et al. 2014) and, conversely, lack of trust is associated with non-adherence to medication (Bauer et al. 2014). Research has shown that the use of decision aids may facilitate the SDM process by increasing the level of trust (Nannenga et al. 2009).

Organizational

Physicians and patients interact within a larger healthcare system. Rules determine how much time a physician can spend with a patient. Feedback is an organizational process that influences future behavior. Patient care frequently needs to be coordinated between providers. Other decisions are required on how care is paid for and who must approve treatment costs (DeMeester et al. 2016).

Policy

The selection of 1 treatment approach over another can be based on various factors such as efficacy, safety, and cost (Liras and García-Trenchard 2013; Peyvandi et al. 2014). The physician's practice or institution functions in a larger macro system, which includes regulatory agencies, patient advocacy groups, and accepted standards of care. Policies are thought to reduce decision making between a patient and physician and limit SDM options (McMurray et al. 2011).

Rules and time

Time constraints can limit decision making in care of chronic diseases (Légaré et al. 2012; Légaré et al. 2013; Légaré and Witteman 2013).

Coordination

Poor coordination of care is thought to be a barrier to SDM due to sub-optimal information flow between physicians. Conversely, good coordination of care supports SDM (Joseph-Williams et al. 2014).

Feedback

Effective feedback methods can help improve how the physician and healthcare organization meet the patient's

needs (NORC 2014). One study has shown that physicians and patients agree that patients can evaluate healthcare providers based on infrastructure, staff, organization, and interpersonal skills but are not able to effectively evaluate technical skills (Rothenfluh and Schulz 2017). In the instance of Medicare accountable care organizations, measures, feedback and auditing methods incentivize physicians to use SDM (CMS 2016).

Colleagues

The extent of colleague influences on the physician decisions process has not been extensively described in the literature. A colleague's advice can sometimes be sought for a decision that is out of their expertise, for confirmation, or on ethical matters (Hickner et al. 2014; Godager et al. 2016; Rothman 2017).

Reimbursement

Reimbursement of healthcare costs can limit the physician's decision process. Insurance companies in many instances apply their own cost effectiveness analyses to determine what is covered. Reimbursement policies are widely perceived as limiting the physician's decision process (Casto and Layman 2006) and have been shown to limit choices in SDM (Scalone et al. 2009; Wilson et al. 2014).

Reflexivity

Reflexivity was maintained by the research team through the analysis and writing by recording, discussing, and challenging established assumptions. The author conducted all interviews and discussion groups. The author was familiar with PID through a long association with the development and commercialization of IgG products. Only one of the 15 study participants was acquainted with the author prior to undertaking the study.

Participants and interview

A purposeful homogeneous sampling method was used to identify potential participants with diverse perspectives. In this type of sampling, participants are selected or sought after based on pre-selected criteria based on the research question. For example, the study may be attempting to collect data from a particular region of the US. The sample size may be predetermined or based on theoretical saturation, which is the point at which the newly collected data no longer provide additional insights.

Board certified immunologist physicians who treat PID were primarily identified through the Immune Deficiency Foundation (IDF), a US based nonprofit patient advocacy organization, or through the author's network. Data were collected using individual, in-depth, semi structured interviews, which were conducted by both the author in person, mainly at an IDF conference in May 2017, or at the immunologists' offices. Two interviews were conducted by phone. The interviews were conducted by May and July of 2018, with each lasting between 30 to 90 minutes depending on scheduling and the flow of conversation. Questions related to the physicians' approach to diagnosis and treatment of PID, with emphasis on the ways in which patients were involved in decision making. Questions were designed to be open ended and time was included for follow up questions if needed. The full questionnaire guide is shown in Appendix A.

Reliability and validity were addressed based on Silverman's guidance (Silverman 2015). The initial interview protocol was revised based on feedback from pilot interviews. All interviews were recorded and transcribed to ensure fidelity of the data.

Each interview was transcribed by a third-party transcription service (Rev.com, San Francisco, CA, USA). Responses were coded both by the author and his assistant, Ryan Dagenais, using a multi-stage open coding procedure. Codes were derived from participants' words and were added or modified as necessary when new meanings or categories emerged. All interviews were recorded and transcribed to ensure fidelity of the data. Exact quotes from participants were used to state findings. To enhance reliability, the interview protocol was first pilot tested with 3 healthcare professionals who treat PID (non-specialists), and assessed for clarity, appropriateness, and relevance of the interview questions. The interview protocol was revised based on this feedback. Data were extracted using NVivo, a software and data analysis tool specifically designed for qualitative research.

Ethics

This study received approval from the Institutional Review Board committee of Case Western Reserve University before participants were contacted. All participants provided written informed consent in order to participate in this study. Persons' names and any other potentially identifying information such as employers or academic institutions were redacted from interview transcripts.

Table 1: **Study participants.**

Category	Number	%
Total	15	100.0%
Male	12	80.0%
Female	3	20.0%
White	11	73.3%
Non-white	4	26.7%
Age: 40s	1	6.6%
Age: 50s	9	60.0%
Age: 60s	5	33.3%
PhD	5	33.3%
North East	4	26.7%
South East	1	6.7%
Midwest	8	53.3%
West Coast	2	13.3%

Results

Participants

Participants' characteristics are shown in Table 1. Twelve were men and 3 were women. The participants all treated patients with PID, and their patient numbers ranged from approximately 100 to 4000. All participants were highly educated; all with an MD, and 5 also having a PhD. The average number of years practicing was approximately 15 years with a standard deviation of 7 years. Planned recruitment had been for up to 20 participants based on previously published recommendations (Morse 1994; Creswell 2013). Enrolment was stopped at 15 participants when it was evident that data saturation had been reached and no further themes were emerging.

Interview development

After pilot testing with 3 non-specialist healthcare professionals, the questionnaire was revised based on the themes that emerged. No subsequent revisions were made during the study. All invited immunologist participants successfully completed the interview.

Interview findings

Table 2 summarizes the findings from the interviews with study participants for the 12 key categories identified.

Long diagnosis period

PID is notorious for long diagnosis time frames averaging about 5 to 7 years. All physicians who I asked about diagnosis timelines (7 of 7) confirmed this and described the problem in context. The physicians attributed diagnostic delays to imperfect data, insufficient screening for PID by primary care providers, and lack of awareness of PID. One participant discussed the context of the 7-year diagnosis average across regions and an imperfect research method:

> It varies from one region to another. In rural areas, yes. In major cities like NY, Toronto, LA, no. The moment you start lumping up different regions, you are not going to solve well what is behind it. I would say that if there is a delay of treatment I don't see very much of this in our place. We need to study it more carefully. Nothing is simple. If you say a delay of diagnosis in [IA], I say no. if you say overall PID, possibly yes; not because only knowledge, but progress of the field. We identified [IA] that had infections for a long time that never had PID, but even if we did, I doubt we would have managed to label that way because our diagnostic tools are much better today. Also do you include autoimmunity in that category? Or cancer? The delay in diagnosis for PID is hard for me to accept 7 years. It was a survey, not a study. Part of pushing the enzyme issue. We all support it, but it is not studied. (P07)

Another participant felt that the disease is overlooked in the primary care setting:

Table 2: **Summary of findings.**

#	Key category	Summary of interview findings
1	Rational decision making	Pattern recognition > Evidence-based medicine
2	Bias/nudging	Physicians provide options they approve of first.
3	Power balance	Patients go to the physician with high expectations because they were unsatisfied with previous care.
4	Health literacy	Patient health literacy must align with the physician.
5	Trust	Trust is assumed.
6	Culture	Culture can change the entire interaction with the patient.
7	Coordination of care	Most act as a coordinator of care
8	Rules (Time)	—
9	Reimbursement	Insurance does not affect decisions or participation.
10	Performance reviews	No reliable performance feedback.
11	Cost	No reliable/consistent cost information.
12	Electronic medical records	Helpful, yet inconvenient.

> Often times that's because people don't get in to see a specialist in primary immunodeficiency. I think that many patients who talk about these diagnostic delays will talk about, "Oh, it was this breath of fresh air when I got to see Dr. X." Well Dr. X was just somebody who's trained in this process of true pattern recognition, and has the 90 minutes to go through and do it, as opposed to community-based allergist that's trying to fit this into an otherwise 20-patient workday. I also think that … That's one reason for diagnostic delay, getting to the true specialist. The other is that some of these diagnoses do evolve over time, so that when you see someone at point A, the laboratory tests may not necessarily have caught up to what their history is, and some of that evolution does happen over time as well. So those two reasons. (P12)

Lastly, one participant (P11) believed that the lack of awareness of PID contributes to the delay:

> I think, again, another kudos to the advocacy networks like Jeffrey Modell Foundation, Immune Deficiency Foundation. I mean, they get the word out to inform people and put placards up in airports, community areas that have a lot of traffic, to tell people about these conditions. As physicians, we don't do a good job of that. So people are becoming more aware. But I think there's still an awareness gap. I do think patients are coming, and I've seen it frequently. I mean, I just saw a patient who's 67 years' old who actually makes absolutely no antibody whatsoever; none whatsoever; makes no antibody-producing cells; was actually diagnosed 35 years ago and put on IgG replacement therapy, but then stopped due to faulty information, and has been on antibiotics time, and time, and time again, essentially, continually for 35 years. *[How did they get to you?]* She ended up seeing a very good colleague in the community who was like, "Whoa, you've got a big problem. You need to go to the center where they're used to taking care of this." So they came over. Gave her her first infusion of IgG, and bridged her with some antibiotics because she was sick, and then hopefully she's going to do well. (P11)

Rational/slow thinking

Participants were more in favor of pattern recognition and experience than evidence-based thinking. Although they expressed the importance of following the literature for quality control purposes, they pointed out the inherent flaws in approaching patients with a data-driven mindset; many study results are adequate for the population, but not the individual.

> I think that evidence-based medicine is, it's there to provide some type of quality control and some type of guidance towards where we want to move. But we always have to understand where evidence-based medicine comes from. (P08)
>
> I have a general idea of how much gamma globulin I want to give somebody based on data but I can tell you that individual patients don't respond the way the median response in a paper, so I can tell you that lots of people will do fine with a gamma globulin replacement of about let's say 500 milligrams per kilogram per month and there are other patients with exactly the same kind of characteristics that may do fine with 400 and others who may need 1000. (P01)

Bias and nudging

Nudges are the subtle suggestions in the decision process; methods or strategies to compel limited responses. Kahneman (2017) has described nudges as "explicitly paternalistic" because they set the "choice architecture" by setting predetermined options. The physicians were aware of their potential bias but knew there are some situations where nudging the patient towards a certain treatment pathway is necessary. For instance, one physician discussed the importance of using encouragement to help patients choose treatments, rather than forcing options:

> I guess it's listening to the patient and offering things in a fair and objective way. I think those are the most important factors. So if I can understand someone and lay things out fairly and help guide them, 'cause I'm sure that I'm biased with what I think is right, but I don't want to ever force someone to do something, because I think it'll backfire. I'd rather encourage them and tell them why I think they should do something and have them agree and buy in, otherwise you don't get the compliance and outcome you want. (P03)

Another physician described a subtler approach to nudging patient decisions:

> As a pediatrician, dealing with the issues, that what you did not want to have is a scenario set up where anyone would perceive blame. So you don't want the physician to be blamed for whatever is done, you don't want a parent, either parent, to be blamed or feel blame. And so, what's done is a collective decision making. Now there's sometimes when the cost of your medical knowledge, you believe the decision should be in a certain direction. And if the parent wants, or the patient wants, are counter to that, you try to use, for want of better words, savvy psychology to help them understand why that may be a preferred route to what they're thinking. Many people have mixed perceptions of things or read testimonies that are incorrect because someone has a grudge on one or the other. And so what you do is you lay out the perspectives. If they're equally good, you don't add any bias to it. (P02)

One participant acknowledged bias as inevitable. However, they believe that experience helps mitigate the issue of bias:

> I'm very sensitive to this issue. I would say if I'm unbiased, no way. Everyone has their own ideas and experiences. Everyone is biased one way or another. We try to present in an unbiased way. It is just human nature. You just try and find the best way. I found that you get better over time in dealing with being challenged by patients and ideas and being open to new ideas. Experience gives you flexibility. I think it also has to do with egos as well. I am definitely better than 25 years ago. (P07)

Findings related to SIT and agency theory

Power balance

When asked about how their authority affects the patient, many participants (7/15) stated that their authority is helpful and makes the interaction more comfortable for the patient. They described the patients coming to them for advice, to validate their experiences with a specialized and professional opinion.

> I think when they come here to us, already they have the highest expectations because either they can come here because they didn't get the satisfactory treatment or approach elsewhere or they came here for unique things we do for newborn screening, or they're just referred to us because the other part didn't know what to do. (P15)

The physicians were open to SDM for treatment decisions, but not for the diagnosis. In particular, the choice of treatment administration routes is a shared decision to best fit the patient's lifestyle.

> I would, usually, emphasise to the patient that in order to progress along this path of diagnosis to treatment, we need to do x, y, z. In order to understand the problem more clearly. There isn't usually very much of a discussion about the pro's and con's, and risk, benefit, cost, et cetera. Most of the time, during that process. There are circumstances where, specifically, cost will become an issue. (P04)
> I mean sometimes somebody would say, "I want to try facilitated subcutaneous, because I heard about it." That's fine. That's great. If their insurance will let them have it, we'll get that for them. If somebody's on IV and wants to go to subcu, that's great too. (P12)

Physicians are aware of the power balance. Some physicians try to mitigate any intimidation by reading body language or maintaining a humble persona. However, they try to keep a professional distance to maintain some power in the relationship for the more difficult decisions.

> That's an area that I've thought a lot about and kind of very conscious about, so I never address an adult patient of mine by their first name, no matter, I've known people for 30 years, I've gone to their kids' weddings or whatever, I never ever ever address an adult patient of mine except as Mr. or Mrs. or Ms. and I do it because I think I need to maintain a certain degree of professional distance, maybe part of that is to protect myself but part of that is there are certain times in a doctor–patient relationship when you have to say to somebody, you have to give somebody bad news or you have to say to them "I know this is what you want to do but I think this is really wrong". (P01)

Health literacy

The physicians mentioned how modern access to medical information is a double-edged sword, meaning it can be beneficial or detrimental to SDM. To mitigate this risk, many participants had taken steps to have literature, links, decision aids, and other health information on-hand that they personally approve. One participant described going as far as to correct and manage Wikipedia pages to ensure their patients are exposed to the most relevant information.

> So, there are people who have read x, y, and z on the 'net and they may consider themselves to be health literate but they're getting a lot of misinformation which can really cause problems. Because now you've gotta sort of undo what they've read and redirect them to what the actual reality is of the treatment. (P05)
> I think it's helpful; in general, I do. I think the internet is a great resource for people. Problem is when they go to chat rooms and hear weird things from different people, it means nothing. You have to use reputable sites, so we actually give out our list of reputable sites for information on immune deficiency for patients, so they can read at their leisure and look things up. (P03)

The physicians often (7/15) mentioned that there are no guarantees of patient adherence, whatever their education and health literacy levels. Some participants described examples of educated patients having poor outcomes due to non-adherence, while uneducated adherent patients had better outcomes. Participant P06 stated, "In fact, sometimes people who are very highly educated go out there and make up their own mind what they want to do, and it was a pretty dumb decision."

Patient networks are the personal and professional connections and programs that assist in providing access to the information and care to the patient population. Networks catering to the needs of particular groups such as teens may be particularly effective (Shama and Reid 2018). All participants of this study praised the patient and family support that patient networks, especially those through IDF and JMF, can provide. Some participants refer their patients to such networks as a resource for the more personal needs; physicians may not always appreciate some of the details of daily life with PID.

> I feel like those patients feel like they have a plan. That they can go and find people … I think humans, by nature, are herd animals. When they find like-minded people that are going through the same thing, and they don't feel so isolated, then you don't have the anxiety, depression, and all of the other issues related to treating a child with a chronic disease or having a child with a chronic disease. They can find like-minded people that have been through the same things and can help them with the day-to-day things that I don't necessarily have advice for because I don't live with it every day. (P13)

Findings related to traits

Patient and physician traits inherently influence decision making. For instance, physicians may approach

patients as either individuals or examples of the disease (e.g., a patient with PID or a PID patient, respectively). Participant P02 made this distinction:

> I think it's really good, but I always keep telling them, take everything with a grain of salt, because each patient is really their own individual disease process. We look at each patient as their own individual experiment. And so what's solely true for this other patient, and because you think the symptoms are the same, there only telling you a fraction of the process. And while that fraction may seem to match up, may not be the direction towards getting the right answer type of thing. So it's very helpful, but it also helps the patients to get guided to the right physicians to help with the thing. (P02)

Trust

Trust in the patient–physician relationship is built quickly or present from the beginning for immunologists, and is not built over multiple visits. Some participants credited their professional distance to the patient; usually by their formal or informal persona:

> I think for my personality, and again, coming as a pediatrician, my patients call me by my first name, and because their parents call me by my first name, and so some of the kids will start calling me by my first name. And I've never been pretentious about that issue, and never tried to correct people on that. And so I think I come across, for myself, less intimidating, and so there's a trust that builds up because I'm not trying to snow them, I'm not trying to pull the wool over their eyes, I'm not trying to intimidate them, and so I think happens is because they realize I'm well educated, many actually want me to help more in the decision, not realizing that I'm psychologically trying to help them in the process. They're actually wanting more of my input. Even physicians I've dealt with. (P02)

Other physicians recognize that some patients will try to take advantage of trust or need to earn it depending on their intentions:

> I use their noncompliance, or the dishonesty as evidence in a very professional and transparent way, as to why I feel the way I feel, and then I'm very clear that I'm going to document that this is my recommendation, and they don't take it, that they're going against medical advice. That it's their choice to go against medical advice, and if their child gets sick, there are consequences for that. I'm very clear about that and because when you set that expectation, in a patient that has good intentions, they will work with you. They will understand that they have been at fault, but if their intentions are good, and there's no secondary gain, then 9 times out of 10, they will actually comply with you. When there is secondary gain, then I have protected myself, and told them what the consequences would be and set expectations. When they fail to meet my trust again, then I can take recourse to protect the child. *[What would be the secondary gain?]* "My child is my proxy-ish" kind of thing. I don't know. Parents like the attention. That's what we consider secondary gain. For everybody, I don't know what that would be. (P13)

Culture/family

Cultural differences between the patient and physician influence the decision-making process to take an alternative approach to care. One physician pointed out encountering this differences with cultures such as Middle Eastern, African American, and Hmong patients:

> And so, for example, I've dealt with individuals from the Middle East, and so one of the things we learn as being a pediatrician, obviously, is to make contact with the individual. And so shake their hand, depending on the severity of the process and things that are going on. Perhaps hold their hand a while. Usually a mother, or a female, or the child type of thing for that. Especially a child. Have them sit in your lap, you know younger children, sit in your lap while you're doing all this and hold them, so that you reach out. Some of the Middle Eastern cultures, you know, it's very offensive for a male to touch a female, for example. And so you have to learn to know that you can't use the same context for connecting. *[Is that something you just learned over time?]* Part of that was learning, but part of it was also when having interpreters and others that would help explain the cultural differences on there. In the U.S., African American tends to be more concerned about … So the African American, there is more distrust of the healthcare system. With good reason for a variety of the things that have occurred. And so you have to develop that trust from the very beginning, and honesty from the very beginning on there. And establish the fact that you recognize they're African American. You point out there's specific items and issues that are more unique towards African Americans than to Caucasians. And you, again, you create these, not boundaries, but openness to that where you can generate that trust, you know?! That "I'm not gonna be perpetrating on you things that are against your will, or that otherwise would be harmful. That color of your skin is not a barrier to being able to achieve the healthcare that you need." Hispanics, different cultural things. (P12)

This participant continued into an example of the decision-making process incorporating entire families:

> Hmong believe in a lot of tribalism. From Southeast Asia. Gypsies. Gypsies are very interesting … they're always very distrustful of anyone because they think everyone's always out to get them. And usually, with the gypsies, you are in a room of ten people, because they bring in the elders and everybody to all that. (P12)

Findings related to organizational context

Coordination of care

Some participants adopted the role of coordinator of the patient's care because the patient–physician relationships are often long-term when treating PID. These immunologists, with a highly specialized knowledge-base, would assist in health-related appointments that could have implications for the management of PID.

> … I'm a pediatrician so there I'm much more of a coordinator, but the continuity doesn't change, I think that's one of the really big things that we offer in our clinic is from the time that I started and I was the junior person with two people in the clinic, we decided the way we were going to operate is that each of us was just going to take new patients, we would split them up or whatever way it worked but once you had seen the patient that patient was just going to be your patient and whenever they came into the clinic for a follow-up, if it was my patient I would see that patient, if they were admitted to the hospital, I would go to see them in the hospital. (P01)

Other physicians prefer not to be considered the coordinator, but rather a temporary coordinator at most. Participant P06 was adamant about treatment roles between specialists:

> … when it comes down to the therapy that I'm proposing; for example, immunoglobulin, or gamma interferon, or Rituxan, or antibio, or anything else, naturally, I'm going to coordinate and make that happen. I'm going to make sure it happens. If it's a person who has a gastrointestinal condition, it's not me. I'm not a gastrointestinal doctor. So I'm going to help them see that other doctor. So I'm going to coordinate on one hand, and do continuity on the other. Mine's continuity; the things that I have suggested. If I have to send them to a rheumatologist, or to a pulmonary doctor, their pulmonary hypertension, or their hematology, I have to send that person over to the other quadrant for all of that. (P06)

Rules related to time

Most of the participants specified that they treat patients over a long period of time (10/15) ranging from 10 years to a lifetime (5/15). Before receiving adequate chronic care, which can take approximately 5 years, the participants compared their meeting time with patients to the short appointments of primary care physicians. Participant P02 stated, "When I schedule patients, initial visit's an hour, an hour and a half when I was doing outpatient, and the follow-ups were 45 minutes to an hour depending on the needs of things". They emphasized the need for prolonged meetings to explore the patient's illness and lifestyle. The same participant drew concern to the state of medicine today:

> … in the ideal world, as I think you're eluding to, what we'd have are primary care physicians that would be set up in a scenario where they would not be having to see 50 patients a day, but be able to see 25 patients a day. (P02)

Reimbursement

A common limitation of SDM is the reimbursement method; patients can only afford a limited selection of treatment options. Physicians are constantly battling with insurance companies regarding the treatment of patients with chronic diseases. Many have incorporated strategies to "never lose" arguments with insurance, whereas others prefer to pick their battles.

> Yeah, I don't win all the time. The big issue is that the insurance companies out there are not aware of what the evidence shows in the literature. Most of the time what they do is they rely on "physician review" and these physicians have absolutely no understanding of any particular field. … Most of those cases get approved after a lot of fighting. Even then sometimes insurance companies are like no we're not doing this. (P14)

Findings related to feedback

Performance reviews

The physicians described the presence of feedback systems, but few found them useful. One physician stated the benefits of extended conversations with the patient, which allow for direct feedback. This direct feedback increased patient confidence in the physician:

> I don't think I'm an intimidating presence. That's not my style at all. I mean, I will tell somebody if I think they're making a bad decision, certainly. But, I think because I really do try to make this a discussion, you know, a lot of feedback from the patient. I think most of the time they tell me they feel much more comfortable with the diagnosis, much more comfortable with the treatment plan, because we've had this conversation. And I do get a lot of referrals from people who come in and see me specifically. So, I think they feel like they've talked to somebody who's got a lot of experience, a lot of expertise. (P05)

However, most of the feedback is not useful or ignored. In most cases, the feedback comes from the patients in the form of surveys, wherein patients motivated enough to participate are often displeased with the physician; the conflict may be related to their illness or irrelevant topics. One physician describes the system of feedback and why they rarely view it anymore:

> *[Is there any systematic feedback?]* There's not any systematic feedback. Usually … I've been fortunate enough that no one has lodged a complaint against me where administration and patient advocacy have had to get involved. I have gotten feedback when patients have said nice things to me, nice things about me. I don't get consistent feedback and I think the only consistent mechanism, by way that the hospital has the patients rate us is through the Press Ganey surveys. After that incident where I had that horrible review online, I have stopped googling myself. I'm a caretaker, I'm a feeler. I take those things really personally because I don't want other people, and other patients, who I take care of to read something like that and then lose their trust in me because of something they read on the internet. These last two years is a perfect example of seeing things on the internet that aren't really true or not the full side of the story. They rely on that too much… (P13)

Cost

One aspect of feedback that physicians are lacking is cost. Most physicians do not have direct feedback

regarding cost outcomes to treatment. One physician mentioned that the insurance companies will occasionally inform them of costs.

> *[In the 300 patients that you have, is there any way that someone looks at the cost of treating them over the course of the year and the outcomes? Is there any way that someone says, "Wow, here's the 300th." And why not?]* You know, what I'll say is that there's no systematic way that is intentionally done for my individual patients that gives me feedback. I do hear from payers, from time to time, to say, "This patient on X asthma therapy," again, not talking about immunodeficiency, but kind of an easier disorder to talk about because there are just much more metrics in place to track quality and so forth, "you might consider stepping them down from drug X to drug Y." And I think that's based on guidelines. It's also based on cost. For sure. There's no doubt. I mean, let's face it. We all get that. So there are some of those things. There's also, even within our own system, like, our health plan covers women and children who are of lower income. It's a medical assistance, Medicaid-based payer. So we will get feedback on use of expensive medications and lower-cost alternatives. There are some of those things out there, but there is nothing that tracks, "Yeah, here's your 300 patients. 250 of them are optimally treated based on these outcome measures. You're providing as cost-effective care as you can based on the complexity level of the patient, available resources, et cetera" (P11)

Electronic medical records

The physicians' opinions were split regarding electronic medical records (EMRs). Some were enthusiastic because they could lead to better care coordination between physicians, whereas the others mentioned flaws such as accidentally nullifying clinical trial participants. Every participant that discussed EMRs described benefits and issues:

> *[Are electronic data records a good thing?]* Oh yeah. I think so because I can read everybody's notes. *[Any downside to electronic records?]* Sometimes, you need to be careful what you really need to write. For example, some of the patients, but we do sometimes research testing, of course after getting consent, it's not … But some other sub specialist that's taking care of the same patient for another thing, they … If parents tell that to that doctor and they put it down, that's a problem because research data cannot be in the medical records for clinical care. They happen to me a couple of times which is difficult because then you have to addend. But, it's not good to disappear completely. If the medical records wants to go back and look at it, they can see it. It's going to go to the record that the other provider will see, but it will be in the records see, there is no way you can correct it or get rid of it. [In general terms of caring for the patient] It makes it very easy. (P15)

Discussion

Although SDM is widely advocated for being a patient-centric approach to care that is best used for the care of chronic conditions, the study findings suggest that SDM in the management of PID is also influenced by "nudging" in toward the physician's choice of treatment. It was also evident that individual physicians have their personal thresholds for adopting SDM. For some of these thresholds, the physicians try to encourage particular choices while guiding the patient in SDM, such as having decision aids available for patient learning. Therefore, contrary to the literature, the paternalistic approach is not entirely obsolete but has adapted to the patient-centric movement of the last 30 years. Participants specifically mentioned use of SDM for the choice between subcutaneous and intravenous routes. In this context, the current Canadian guidelines for IgG replacement endorse patient choice when deciding on the route of administration (Betschel et al. 2017).

Other limitations of this study involve the sample, which was not demographically diverse and was recruited based on participant availability rather than being a truly random sample, thereby exposing the study to potential bias, although there is no evidence that the sample participants did not accurately represent the population of immunologists in the US. Future research might purposefully recruit a larger sample across the widest demographic range, in order to obtain insights about treatment decisions by female and minority physicians. Furthermore, all but one of the participants had more than 10 years' experience in the immunology specialty. It may well be that immunologists with less than 10 years' experience make treatment decisions differently. The study did not consider physicians outside the immunology specialty who treat patients with PID.

In conclusion, this study offers an analysis of physician decision-making for the treatment of PID. It may also be useful as an example of how treatment decisions are made in the management of a lifelong chronic disease that incurs high treatment costs. It also expands on the literature by characterizing the boundaries in which SDM exists. Immunologists are open to incorporating SDM as long as the patient is aligned with the suggested treatments; the physicians likely nudge the patient to that alignment if necessary. Understanding physician perspectives of SDM and how they guide it in clinical practice has practical implications for the care of patients with chronic illnesses. Forming an environment which favors SDM can lead to better treatment outcomes and reduce overall costs and, for this reason,

policy makers and departments responsible for setting treatment policies—such as hospital standards or insurance reimbursement—may also benefit from a greater understanding of how SDM works in real life settings.

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Appendix A

Interview guide

Introduction and Explanation—read to the interviewee before proceeding

a. Greeting → "Hello [*name of participant*]. Thank you for taking the time to meet with me today. Your participation is greatly appreciated. Before getting started, there are a couple things I would like to cover."
b. Purpose and Format of Interview → "As a current student in the Case Western Reserve University Doctor of Management (DM) program, I am interested in developing a greater understanding of the factors that influence physician decision making for the treatment and management of PID. I will ask you a series of open-ended questions on this topic, and I will ask one or more follow-up questions as you respond. The interview will last approximately 60 to 90 minutes."
c. Confidentiality → "Everything you share in this interview will be kept in strictest confidence, and your comments will be transcribed anonymously—omitting your name, anyone else you refer to in this interview, as well as the name of your current organization and/or past organizations. Your interview responses will be included with all the other interviews I conduct."
d. Recording → "To help me capture your responses accurately and without being overly distracting by taking notes, I would like to record our conversation with your permission. Again, your responses will be kept confidential. If at any time, you are uncomfortable with this interview, please let me know and I will turn the recorder off."
    a. "Do you have any questions before we begin?"

Introduction

1. Name
2. Education
3. Current job title and responsibilities
4. Years of experience (total + specialty as an immunologist)
5. Involved with research (clinical or otherwise)
6. Practice setting/site of care (multiple?)
7. How many PID patients have you treated in your career/how many now?
8. What is the average length of time with the patient?
9. Most have co-morbidities?
10. Are all receiving IG: IV or SC?

Focus in on factors that determine how you make decisions

1. Describe a typical patient
    a. How do they get to you?
    b. Diagnosis to treatment to maintenance
2. Describe the types of decisions you make?
    a. SC vs IV
3. Do you use decision aids (describe/evidence-based?)
4. Do you find that your patients are educated or well educated on self-management?
    a. High levels of self-efficacy (I am confident I can manage my situation)
    b. High levers of self-activation
    c. Do you think more informed patients result in fewer health resources and better outcomes?
    d. Are you patients actively involved in patient networks (IDF, JMF, internet networks such as patients-like-me)?

   e. Do you have a patient portal where patients can review their health history?
5. What seeing a patient: look for patterns that match past experience?
   a. Tend to quickly assess symptoms and diagnosis or it it's a slow painstaking process that has lots of complexities (particularly with comorbidities)
   b. Tend to spend more time on protocol or treatment
   c. Would you describe yourself as patient-centric or evidence centric (Evidence-based medicine)?
6. If you were to guess: do you as the physician make the final treatment decision or leave it up to the patient
   a. Protocols
   b. Drugs
7. Does your DM style vary based on the patients' level of understanding and interest?
   a. Does it vary based on complexity or uncertainty?
8. Do you think your status as a physician or authority figure influences how patients respond to you in a clinical setting?
   a. Is Intimidated or encouraged to share information or
   b. More likely to tell you their treatment preferences or express an option on treatment options?
9. Do you routinely ask about patient preferences: lifestyle and how treatment will affect patient goals and values?
10. When discussing pros and cons of a potential treatment (protocol or drugs) do you tend to lead with the pros or cons
11. How much and in what way do cost or reimbursement influence your decision making
   a. Protocol
   b. Drug
12. Do patients want to play an active role in their decisions
   a. Function of health literacy
   b. Function of health numeracy
13. Do you see yourself a continuation of care or coordinator of care?
   a. Is coordination of care an issue for your PID patients
   b. Are patients actively looking for you to coordinate their care?
   c. Do your PID patients have problems accessing healthcare services or getting adequate treatment?
14. Have you ever made a mistake/misdiagnosis?
   a. Proper follow-up
   b. Diagnostic test
   c. Adequate history
15. Prefer face-to-face or is phone or internet possible and how often
16. Trust → impact on patient participation
17. Does DM change over time as uncertainty changes
   a. Less shared decision making
18. Provider number
19. Are you rated? Do you recall your score?
20. How would you assess or describe the quality of your communication style?
   a. Outgoing
   b. seeking
21. Do patients tend to speak up or does it depend of the context and your relationship
22. Impact of the organization on your decision making
   a. IOM: patient-centered care: patient perspectives are now being factored into Medicare value-based payments to hospitals

Gene therapy for PNP deficiency protocol

Linda Vong*

ABSTRACT

Purine nucleoside phosphorylase (PNP) is a key enzyme required for the degradation of purine nucleosides into uric acid or their salvage into nucleic acids. Patients who are deficient in PNP suffer from progressive T cell immunodeficiency, with increased susceptibility to infections, autoimmunity, and neurologic abnormalities. In the absence of successful treatment to restore immune function, these patients rarely survive to adulthood. Hematopoietic stem cell transplantation is the only known cure for PNP deficiency. Use of an HLA-matched donor is preferable as the outcome with alternative donors have been variable; however, this option is rarely available.

Gene therapy represents a therapeutic option that bypasses the need for a donor, and thus associated complications. Although first generation γ-retroviral vectors have been successful in some immunodeficiencies, in others, evidence of insertional mutagenesis prompted a halt in their use. More recently, the introduction of safer lentiviral vectors holds promise in offering a viable option to treat immunodeficiency.

Here, we present a clinical trial protocol utilizing self-inactivating lentiviral vectors to treat PNP deficiency. Patients will be evaluated up to 3 years post-transplantation to determine the safety of lentiviral-treated stem cell infusion, as well as the extent of immune reconstitution.

Statement of novelty: This protocol describes the novel treatment of PNP deficiency using lentiviral-based gene therapy.

Study summary

Purpose

This is a Phase I/II clinical trial to study the safety and efficacy of gene therapy in purine nucleoside phosphorylase (PNP) deficiency. $CD34^+$ stem cells derived from PNP-deficient patients will be transplanted after the transduction of normal PNP cDNA using lentiviral vector.

The primary outcome is to determine the safety of infusing lentiviral-treated stem cells, while the secondary outcome will be assessment of reconstitution of mature lymphocytes carrying the normal PNP gene.

Rationale

Profound T cell immunodeficiencies are life-threatening conditions which require urgent assessment and consideration of curative measures, such as hematopoietic stem cell transplantation. Many of these infants, but not all, can be identified shortly after birth by screening for T cell receptor excision circle (TREC) levels (Buckley 2012), an indication of thymic function and T cell development (Douek et al. 1998). The most studied subgroup of these patients are infants born with severe combined immunodeficiency (SCID). Prior to the implementation of newborn screening (NBS), and in jurisdictions where NBS is unavailable, infants with SCID typically present in the first year of life with viral or fungal lung infections, chronic diarrhea, and failure

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to thrive (McWilliams et al. 2015). Immune evaluation in these patients reveals profound T cell lymphopenia according to the underlying genetic condition afflicting these patients (Shearer et al. 2014).

Over the past two decades there has been a growing recognition that profound T cell immunodeficiency (combined immunodeficiency, CID), unlike typical SCID, can result in significant residual immune function and sometimes normal numbers of circulating lymphocytes, as well as variable in vitro and in vivo immune function (Roifman et al. 2012). Some of these patients may present clinically like SCID while others can present later in childhood with recurrent infections, autoimmune manifestations, or atopic features. Unfortunately, many of these conditions cannot be detected by TREC-based NBS. The treatment of choice for SCID and for most cases of CID is hematopoietic stem cell transplantation (Pai et al. 2014). Use of an human leukocyte antigen (HLA)-matched related donor has previously been reported to result in the best outcome (Caillat-Zucman et al. 2004). In the absence of such a donor, matched unrelated donors (Dalal et al. 2000; Grunebaum et al. 2006) and sometimes mismatched related donors have also been used successfully (Beatty et al. 1985; Haddad et al. 1998).

PNP deficiency (OMIM #613179) is an autosomal recessive progressive immunodeficiency characterized by susceptibility to opportunistic infection, neurologic abnormalities, and autoimmunity (Markert 1991). The onset may be delayed beyond infancy. Immune evaluation varies from patient-to-patient as well as over time in the same individual. T cell lymphopenia is progressive, while in vitro function as well as TREC levels may be low or within normal range. This CID is caused by mutations in the PNP gene at 14q13.1, which encodes a key enzyme involved in the purine salvage pathway (George and Francke 1976). The features observed in this immunodeficiency are likely caused by the consequent accumulation of purine metabolites (Mitchell et al. 1978; Ullman et al. 1979).

The only known curative treatment for PNP deficiency is hematopoietic stem cell transplantation, preferably with an HLA-matched related donor (Delicou et al. 2007). Experience with alternative donors has been inconsistent, with only few reports suggesting successful engraftment after matched unrelated cord or bone marrow transplants, albeit after repeated transplants (Grunebaum et al. 2013; Brodszki et al. 2015).

It is fair to assume that, similar to adenosine deaminase (ADA) deficiency, the more common purine metabolism defect, transplants other than full sibling HLA-matched donors may pose a great challenge (Hassan et al. 2012). This prompted the recent effort to offer a safer alternative by using gene therapy (Fischer et al. 2013). Gene therapy for ADA deficiency, using γ-retroviral vector based gene transfer, has been studied for more than two decades and was found to be safe and frequently effective (Candotti et al. 2012). However, whether full immune reconstitution occurs as well as the durability of transduced stem cells remains unknown. In contrast, use of γ-retroviral vectors was found to induce leukemia in other conditions, leading to termination of these studies (Hacein-Bey-Abina et al. 2008; Howe et al. 2008). Lentiviral vectors might be safer as they can be self-inactivating through deletion of viral regulatory elements, as well as allowing the use of cellular promoters (Aiuti et al. 2002; Gaspar et al. 2006; Candotti et al. 2012).

We study here for the first time the safety and efficacy of lentivirus-based PNP gene transfer for the treatment of PNP deficiency. We have previously demonstrated that lentivirus transduction of the PNP gene into murine PNP-deficient stem cells was effective (Liao et al. 2008).

Principles of screening and enrollment

1. Only patients who have no HLA-matched sibling donor will be eligible for enrollment.
2. Study protocol will be described to family members by the Principal Investigator.
3. Patients' guardians will sign informed consent.
4. Patients must meet inclusion criteria.

Inclusion criteria

1. Low PNP activity in patient red blood cells and a PNP gene mutation
2. Evidence of combined immunodeficiency including lymphopenia and abnormal phytohemagglutinin (PHA) responses (stimulation index <300)
3. Older than 6 months of age
4. Absence of HLA-identical sibling donor.

Exclusion Criteria

1. Age greater than 6 years
2. Known sensitivity to Trisulfan
3. Availability of a related HLA-identical donor
4. Refusal to sign consent forms by guardians.

Study plan

Screening

1. History and physical examination
2. Renal and liver function tests
3. CBC with differential and blood film
4. Prothrombin time (PT)/Partial thromboplastin time (PTT)
5. Viral screen for HIV-1, hepatitis B, hepatitis C, cytomegalovirus (CMV), Epstein-Barr virus (EBV) and parvovirus (by DNA polymerase chain reaction)
6. Pregnancy test
7. Urine chemistry and culture
8. Immune evaluation including flow cytometry, PHA response, TCR-Vβ, TREC analysis
9. Electrocardiogram
10. Echocardiogram
11. Chest x-ray
12. Skin biopsy (growth of fibroblasts) or alternatively skin obtained during administration of central venous device.

Procedure

1. Placement of a central venous access device.

Study treatment

Stem cell harvest

Patients will be treated with erythrocyte colony stimulating factor (ECSF) at a dose of 10mg/kg for 4 days. Stem cells will be then isolated with a target of $>7 \times 10^6$/kg. Ideally, $2–3 \times 10^6$ cells will be used for gene transduction and the rest will be stored for future backup use if required.

Transduced cells will be approved for administration only if:

1. Cell count is $\geq 7 \times 10^6$ $CD34^+$ cell/kg
2. Cell viabilitiy is better than 75%
3. Endotoxic assessment
4. Bacterial and fungal stains negative
5. Culture medium shows no microbial growth.

Conditioning

Trisulfan will be given in a single intravenous administration. Trisulfan will be administered only if harvest is sufficient and $CD34^+$ cells are approved for administration. A dose of 4mg/kg will be administered intravenous 24 hours before the administration of transduced $CD34^+$ cells.

Follow up

Patients will be evaluated monthly for the first 4 months after transplantation and quarterly thereafter up to 3 years.

Follow up includes:

1. Physical exam and history
2. CBC, liver and renal function tests
3. Viral PCR tests
4. Monitoring for insertional leukemia (twice a year only)
5. Flow cytometry, PHA responses, responses to antigens, TRECs, TCR-vβ, immunoglobulins, specific antibodies
6. Analysis of vector integration site
7. PNP enzymatic activity in red cells and measurement of the frequency of cells containing inserted PNP in peripheral blood mononuclear cells (PBMC) or T cells.

Safety assessment

The records from each follow up will be used to determine adverse events. Clinically significant adverse events will be based on and compared to the National Institute of Allergy and Infectious Disease (NIAID) Pediatric AIDS toxicity evaluation to determine the grade of the adverse event.

Administration of back-up stem cells will be triggered by failure to engraft transduced cells by day +120 after autologous transplant infusion based on lack of T cell recovery.

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