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Gain-of-function mutations and immunodeficiency: at a loss for proper tuning of lymphocyte signaling

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Abstract

Purpose of review—To present recent advances in the discovery and characterization of new immunodeficiency disorders linked to gain-of-function (GOF) mutations in immune signaling molecules.

Recent findings—In the past two years, extensive cellular and molecular studies have illuminated the root causes of pathogenesis for several new monogenic primary immunodeficiency disorders (PIDs) linked to GOF mutations in signaling molecules. Here we discuss on two disorders (BENTA and APDS/PASLI) featuring shared clinical presentation (e.g. lymphoproliferation, selective antibody deficiencies, recurrent sinopulmonary infections). These findings highlight an emerging theme: both loss and gain-of-function mutations in key molecules can disrupt finely-tuned immunoreceptor signaling modalities, resulting in the dysregulation of lymphocyte differentiation and impaired adaptive immunity.

Summary—Continued research on the molecular pathogenesis of PIDs defined by hyperactive signaling molecules will better distinguish these and related disorders, and pinpoint tailored therapeutic interventions for “retuning” the immune response in these patients.

Keywords

BENTA; CARD11; APDS; PASLI; PI-3K

Introduction

Incredible advances in next-generation sequencing have accelerated our ability to pinpoint definitive molecular and genetic diagnoses for previously undefined monogenic primary immunodeficiency diseases (PIDs) (1, 2). Genetic alterations including missense/nonsense point mutations, insertions and deletions can bestow gain of function (GOF) or loss of function (LOF) activity on proteins expressed in hematopoietic cells. Such mutations can affect various functions of the immune system, including leukocyte survival and differentiation, antibody production, and/or cytokine signaling, compromising our ability to combat specific infections. Although the majority of PIDs are attributed to LOF alleles inherited in an autosomal recessive fashion, autosomal dominant GOF alleles have been

implicated in several PIDs over the past decade (3). Although these GOF mutations are all hypermorphic, disease manifestations are often paradoxically linked to a greater susceptibility to infection or malignancy. This review highlights salient discoveries related to two recently described PIDs linked to definitive GOF mutations in key signal transduction molecules. Herein we examine underlying molecular mechanisms and shared pathological features that provide new insights on these distinct diseases and their clinical management. In doing so, we emphasize a common theme: immune signaling pathways are finely tuned, such that loss *or gain* of signal transduction capability can ultimately manifest as immunodeficiency. Although other GOF mutations are linked to multiple autoimmune and inflammatory disorders often classified as PIDs (3), these will not be discussed here.

BENTA Disease

Our group recently discovered and characterized a novel congenital B cell-specific lymphoproliferative disorder termed B cell Expansion with NF- κ B and T cell Anergy (BENTA) disease (4–6). Excessive B cell lymphocytosis presenting in early childhood is the cardinal feature of BENTA disease, with associated splenomegaly and lymphadenopathy. Immunologic phenotyping consistently reveals striking accumulation of both immature transitional (CD10⁺ CD24^{hi} CD38^{hi}) and mature naïve (IgM⁺IgD⁺) polyclonal B cells, while T cell numbers typically fall within normal pediatric ranges. However, overt autoimmune manifestations are usually absent in these patients. Moreover, BENTA patients exhibit several hallmarks of primary immunodeficiency. Recurrent sinopulmonary and ear infections are common, and other “opportunistic” viral infections (chronic Epstein-Barr virus, molluscum contagiosum, BK virus) have been noted in some patients. In most patients, insufficient humoral immune responses are observed in response to pneumococcal and meningococcal polysaccharide-based vaccines. Other patients display low antibody titers to other vaccines containing T-dependent antigens, such as measles and varicella zoster virus. Impaired humoral immunity is also evidenced by (a) extremely low frequencies of circulating class-switched and memory B cells, (b) poor immunoglobulin secretion and plasma cell differentiation when tested *in vitro*. Consistently, most patients exhibit low serum IgM levels, with IgA and IgG levels in the lower end of normal range. In addition, BENTA patient T cells are typically hyporesponsive to *in vitro* stimulation, with impaired proliferation and IL-2 secretion. Hence BENTA disease constitutes a mild combined immunodeficiency.

At present, BENTA disease is genetically defined by germline-encoded, heterozygous GOF mutations in *CARD11* (also known as *CARMA1*). *CARD11* is expressed primarily in lymphocytes and functions as a critical scaffold molecule that bridges engagement of the antigen receptor (AgR; i.e. B cell receptor or T cell receptor) with several downstream signaling outputs, including c-Jun N-terminal kinase (JNK), mechanistic target of rapamycin (mTOR), and the canonical NF- κ B pathway (7–9). Upon stimulation of the AgR and/or other immune receptors (e.g. TNF receptor, Toll-like receptors), the NF- κ B family of transcription factors induces the expression of many genes involved in immune cell survival, proliferation, and effector functions (10). In lymphocytes, AgR ligation triggers the phosphorylation of an inhibitory “linker” domain in *CARD11* that normally maintains the protein in a closed, inactive conformation (11, 12). Linker phosphorylation “opens” the

CARD11 molecule to recruit BCL10 and MALT1, which nucleates the formation of dynamic multi-protein signalosomes responsible for activating inhibitor of κ B kinase (IKK) (13–15). IKK-mediated phosphorylation marks the inhibitor of κ B (I κ B) for ubiquitination and proteosomal degradation, thereby facilitating the nuclear translocation of NF- κ B for gene transcription.

Since our initial report in 2012, five distinct *CARD11* mutations have been confirmed in a total of twelve BENTA disease patients (4–6) (unpublished data). Similar to somatic GOF mutations described in ~10% of diffuse large B cell lymphomas (DLBCL) (16), all BENTA-associated mutations reside within the N-terminal portion of CARD11 containing the CARD, LATCH and coiled-coil domains. These domains are responsible for CARD11 oligomerization and recruitment of BCL10 and MALT1, making this region a “hotspot” for GOF mutations (17). When expressed ectopically in B and T cells, GOF CARD11 mutants can spontaneously aggregate to form active signaling clusters with BCL10, MALT1, and active IKK, triggering constitutive NF- κ B activation without AgR stimulation (4–6). Just as DLBCL tumors harboring GOF CARD11 mutations are dependent on constitutive NF- κ B signaling for continued growth and survival, enhanced NF- κ B activity in resting B cells is likely a major driver of excessive B cell accumulation in BENTA patients. This may predispose B cell clones to malignant transformation as additional mutations are acquired over time. Indeed, two BENTA patients developed B cell tumors in adulthood. Moreover, transgenic expression of a constitutively active form of IKK β (caIKK β) promotes the survival and proliferation of mature murine B cells *in vivo*, although it is not enough to induce outright lymphomagenesis (18).

Whereas overactive NF- κ B signaling is commonly associated with lymphomagenesis (19), defects in NF- κ B activation clearly contribute to immunodeficiency. This is best illustrated by a rare form of anhidrotic ectodermal dysplasia and immunodeficiency (EDA-ID) caused by GOF mutations in I κ B α itself. These GOF mutations prevent I κ B from being phosphorylated and degraded in response to stimuli (20–25). As a result, hypermorphic I κ B perpetually retains NF- κ B complexes in the cytosol, severely compromising both innate and adaptive immune responses in these patients. Although a connection between defective NF- κ B signaling and impaired immunity seems intuitive, our studies of BENTA patients may suggest that constitutive activation of NF- κ B may also result in immunodeficiency. This phenomenon could relate to T cell anergy, considering a constitutively active form of IKK β (caIKK β) renders murine T cells hyporesponsive, with diminished responses to bacterial infection (26). In contrast, Goodnow and colleagues showed that ectopic expression of GOF CARD11 mutants could rescue self-reactive, activated mature B cells from antigen-induced cell death and instead promote proliferation, plasmablast generation and autoantibody secretion (27). This apparent discrepancy with the humoral immune deficits noted for BENTA patient B cells raises several interesting questions. How do germline GOF CARD11 mutations alter B cell development and enforcement of self-tolerance? Are problems with B cell differentiation in BENTA patients reflective of intrinsic B cell defects, impaired T cell help, or both? Mouse models featuring germline-encoded GOF CARD11 mutations are currently under development, and should help to address these issues. Interestingly, caIKK β expression alone induced spontaneous proliferation of mature murine B cells without

protecting them from self-antigen-induced death or triggering plasmablast differentiation (18, 27). These findings necessitate further studies to determine if dysregulation of other CARD11-dependent signaling pathways (e.g. JNK, mTOR) alters the function of BENTA patient lymphocytes.

In summation, our initial studies of BENTA disease suggest that GOF CARD11 mutants affect B and T cells differently, despite extensive similarities in their AgR signaling machinery. The mechanisms by which GOF CARD11 signaling in BENTA patients promotes proliferation and survival of B cells versus anergy in T cells remain poorly understood. Nevertheless, we can conclude that overactive CARD11 signaling paradoxically contributes to a state of mild combined immunodeficiency; suggesting the AgR signal must be properly “tuned” for effective adaptive immunity. Thus new compounds for modulating CARD11-dependent signaling without blocking it completely, such as MALT1 protease inhibitors, might be attractive treatment options for these patients in the future (28, 29). Currently, most BENTA patients are carefully monitored for monoclonal B cell outgrowths and chronic infections with minimal clinical intervention.

APDS / PASLI Disease

Another recently described immunodeficiency known as Activated PI-3K δ Syndrome (APDS, a.k.a. p110 δ -activating mutation causing senescent T cells, lymphadenopathy and immunodeficiency, PASLI) is caused by GOF mutations in a signaling molecule critical for adaptive immunity: phosphoinositide-3-kinase (PI-3K) (30–32). Class I PI-3K is a heterodimeric lipid kinase that plays a prominent role in immune cell development, differentiation and function. In leukocytes, PI-3K molecules are composed of a p110 δ catalytic subunit and a p85 α regulatory subunit that regulates the stability, membrane localization and activity of the enzyme (33). Ligation of various immune receptors (including AgRs and costimulatory molecules) stimulates receptor tyrosine kinases that activate PI-3K. Activated PI-3K then phosphorylates phosphatidyl inositol 4,5-bisphosphate (PIP2) to generate phosphatidyl inositol-3,4,5-trisphosphate (PIP3), which in turn recruits pleckstrin homology domain-containing target proteins such as Akt and PDK1 to the plasma membrane (34). In this manner, PI-3K can activate mechanistic target of rapamycin (mTOR), the critical checkpoint kinase that governs metabolic programming in lymphocytes. Additionally, activated Akt mediates the phosphorylation and degradation of FOXO transcription factors (e.g. FOXO3) that regulate T cell expansion and memory formation (35, 36).

The first GOF mutation in *PIK3CD* (encoding p110 δ) was reported in one patient diagnosed with primary B-cell immunodeficiency (31). The recent discovery of additional kindreds with three distinct p110 δ GOF mutations has provided a more complete picture of this PID, including molecular insights into disease pathogenesis (30, 32). APDS/PASLI patients typically present with recurrent childhood ear and sinopulmonary infections, progressive airway damage, chronic herpesvirus viremia, and general lymphoproliferation manifesting in splenomegaly, lymphadenopathy, and/or mucosal lymphocytic nodules. Lymphomas are found in some patients. Imbalances in circulating B cell populations, including elevated transitional B cells but significantly lower frequencies of class-switched/memory B cells, are

likely linked to skewed serum immunoglobulin concentrations (high IgM, low IgG2/IgG4/IgA). This phenotype resembles defects in Ig class switch recombination noted for murine B cells deficient in PTEN, which directly opposes PI-3K function by converting PIP3 into PIP2 (37). Consistent with impaired humoral immunity, recurrent ear and respiratory infections correlate with poor responses to bacterial vaccines such as *Streptococcus pneumoniae* and *Haemophilus influenzae* type B.

Most patients also present with progressive CD4⁺ T cell lymphopenia. This phenotype actually contrasts with mice carrying a T cell-specific deletion of PTEN, which succumb to CD4⁺ T cell hyperplasia and lymphomas (38). Conversely, APDS/PASLI is also distinguished by an excessive accumulation of terminally differentiated, senescent CD8⁺ effector T cells, but a marked deficiency in naïve T cells. Severe herpesvirus infections including Epstein-Barr virus (EBV), Cytomegalovirus (CMV) and Varicella Zoster Virus (VZV) are detected in several patients, indicating reduced T cell-dependent control of persistent viral replication.

Structure-function analyses indicated that the germline GOF missense mutations in p110δ (E1021K, N334K, E525K) mirrored homologous, somatic GOF mutations in the related PI-3K isoform p110α (39–41). GOF p110α mutations are known to increase PI-3K activity in cancer cells by enhancing its association with membranes (42, 43). Indeed, biochemical analyses demonstrated that APDS/PASLI-associated p110δ mutations result in stronger plasma membrane association, elevated PIP3 levels, Akt hyperphosphorylation and reduced FOXO1 expression in resting and activated T cells. Importantly, elevated PI-3K activity also resulted in increased mTOR signaling and aerobic glycolysis (32). Although enhanced glycolysis is required for the expansion of effector T cells, downmodulation of mTOR signaling and conversion back to catabolic metabolism (e.g. fatty acid oxidation, mitochondrial respiration) is essential for prolonged survival and entry into the memory pool (44). PI-3K-mTOR hyperactivity in APDS/PASLI patient CD8⁺ T cells therefore results in chronic activation and increased susceptibility to activation-induced cell death (30). This culminates in an overabundance of senescent effector cells and short-lived effector memory T cells that exhibit poor recall responses *in vitro*, contributing to defective anti-viral immunity *in vivo*. Interestingly, similar immunologic abnormalities are also observed in patients with GOF mutations in *PIK3RI* (encoding the p85α subunit), which alter binding to p110δ and produce a dominant hyperactivation of PI-3K (45, 46). The APDS/PASLI moniker therefore applies to these patients as well.

In defining this mechanism of pathogenesis in patients with GOF p110δ and p85α mutations, we now appreciate how PI-3K hyperactivity contributes to T cell immunodeficiency, extending beyond previous findings in mouse models. More importantly, this knowledge informs viable therapeutic approaches for restoring T cell homeostasis and function in these patients. For example, administration of the mTOR inhibitor rapamycin in one patient was found to reduce hepatosplenomegaly and lymphadenopathy, increase naïve T cell frequencies, and restore T cell proliferation and IL-2 secretion (32). Moreover, selective p110δ inhibitors IC87114 and GS-1101 can reduce the activity of GOF mutant p110δ protein, presenting another option for correcting T cell

immunity and combating lymphomagenesis in APDS/PASLI (30). Indeed, a heightened predisposition to lymphoid malignancy is now firmly established in these patients (47).

Our comparison of BENTA and APDS/PASLI disease herein reveals many shared clinical and immunological anomalies that ultimately result in a similar state of combined immunodeficiency (Table 1), characterized by recurrent ear/respiratory infections, chronic EBV viremia, and increased risk of B cell lymphoma. A common theme in molecular pathogenesis is also evident, whereby overactive signaling through either CARD11 or PI-3K results in skewing of B and T cell subsets, selective antibody deficiencies, and impaired anti-viral/anti-tumor-directed T cell responses. Given the recent finding that CARD11 is required for AgR-dependent mTOR activation in T cells (8), it will be interesting to determine whether constitutive mTOR activity also contributes to T cell hyporesponsiveness in BENTA disease. These findings underscore the importance of appropriate “tuning” of AgR-driven signaling pathways; too little or too much signaling can interfere with shared checkpoints in lymphocyte development and differentiation. Moreover, the study of BENTA, APDS/PASLI, and related disorders reiterate the need for highly selective inhibitors and/or partial antagonists that can “reset” AgR signaling outputs to normal levels without blocking them entirely, rendering lymphocytes completely unresponsive. Indeed, homozygous LOF mutations in *CARD11* or *PIK3R1* manifest in more severe forms of combined or B cell-specific immunodeficiency, respectively (48–50).

This “Goldilocks” principle for immune signal regulation is applicable to other PIDs associated with GOF mutations in key signaling molecules. For example, heterozygous in-frame deletions in the autoinhibitory domain of phospholipase C γ 2 (PLC γ 2) result in PLC γ 2-associated antibody deficiency and immune dysregulation (PLAID), characterized by cold induced urticaria, recurrent pulmonary infections, granulomatous rash, and hypogammaglobulinemia (51). Increased circulating transitional B cells with low levels of class switched memory B cells are also observed in PLAID patients. GOF mutations in signal transducer and activator of transcription 1 (STAT) proteins STAT1 and STAT3 result in increased susceptibility to chronic mucocutaneous candidiasis (CMC) and mycobacterial infections, respectively, although PIDs associated with STAT mutations are more commonly associated with autoimmunity (2, 3). Considering CMC results from impaired Th17 immunity linked to either GOF STAT1 or LOF STAT3 alleles, proper cytokine signaling responses likely require a balance of competitive STAT1 versus STAT3 signaling.

Conclusion

Although mouse models offer valuable insights into immune system function, the study of monogenic PIDs has exposed significant differences in associated disease phenotypes in humans. Advanced genome sequencing technology will undoubtedly increase the number of PIDs linked to GOF mutations in signaling molecules (3). To understand and manage these disorders effectively, the challenge remains to define pathogenesis at the molecular level. Continued research of these disorders may be guided by the emerging principle highlighted here: balanced signal regulation through the AgR and other immune receptors is essential for proper maintenance of immune homeostasis and effector function. Although we recognize shared clinical phenotypes among several GOF monogenic PIDs, determining the distinct

mechanisms behind these signaling abnormalities will still be critical for tailored therapeutic interventions.

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Key Points

- Gain or loss of function mutations in key immune signaling molecules can result in immunodeficiency
- This review highlights PIDs such as BENTA and APDS/PASLI associated with gain of function mutations that result in constitutive activation of important signaling pathways in lymphocytes
- BENTA and APDS/PASLI patients share common clinical and immunological features such as recurrent pulmonary infections, lymphoproliferation, antibody deficiencies and increased susceptibility to microbial infections
- Increased predisposition to lymphoid malignancy is established in these patients
- Understanding of the molecular mechanisms behind these and related PIDs will hopefully yield targeted therapeutics to treat disease by “retuning” abnormal lymphocyte signaling

Table 1
Comparison of key clinical/immunological features of BENTA and APDS/PASLI disease.

		BENTA	APDS / PASLI
Shared features	Clinical	<ul style="list-style-type: none">• Recurrent ear/sinopulmonary infections• Chronic herpesvirus infections (e.g. EBV)• Increased risk of B cell malignancy• Splenomegaly, lymphadenopathy	
	Immunological	<ul style="list-style-type: none">• Elevated transitional B cells• Low class-switched / memory B cells• Low serum IgA, IgG*• Poor responses to certain vaccines (e.g. bacterial capsule)• T cell hyporesponsiveness	
Distinctions	Gene (product)	BENTA <i>CARD11</i>	APDS / PASLI <i>PIK3CD</i> (p110δ); <i>PIK3RI</i> (p85a)
	Clinical	<ul style="list-style-type: none">• Other viral infections (molluscum contagiosum)	<ul style="list-style-type: none">• Progressive airway damage• Mucosal lymphoid nodules• Autoimmune cytopenias, arthritis*
	Immunological	<ul style="list-style-type: none">• Increased circulating mature B cells (declines with age)• Low serum IgM	<ul style="list-style-type: none">• High serum IgM• CD4⁺ T lymphopenia• Skewed CD8⁺ T subsets (↓ naïve, ↑ EM/EMRA)• Accumulation of CD8⁺CD57⁺ senescent T cells