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The *CARD11-BCL10-MALT1 (CBM)* signalosome complex: Stepping into the limelight of human primary immunodeficiency

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CLINICAL IMPLICATIONS: Mutations in the *CARD11-BCL10-MALT1 (CBM)* signalosome complex must be considered in the differential diagnosis of patients with the clinical presentation of combined immunodeficiency.

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Abstract

Next-generation DNA sequencing has accelerated the genetic characterization of many human primary immunodeficiency diseases (PIDs). These discoveries can be lifesaving for the affected patients, and also provide the unique opportunity to study the impact of specific genes on human immune function. In the past 18 months, a number of independent groups have begun to define novel PIDs caused by defects in the CARD11–BCL10–MALT1 (CBM) signalosome complex. The CBM complex forms an essential molecular link between the triggering of cell surface antigen receptors and NF- κ B activation. Germline mutations affecting the CBM complex are now recognized as the cause of novel combined immunodeficiency phenotypes which all share abnormal NF- κ B activation and dysregulated B cell development as defining features. For this *Current Perspectives* we have engaged experts in both basic biology and clinical immunology to capture the worldwide experience in recognizing and managing patients with PIDs caused by CBM complex mutations.

Keywords

CARD11–BCL10–MALT1 (CBM) signalosome complex; Primary immunodeficiency diseases; Combined immunodeficiency; Congenital B cell lymphocytosis; Paracaspase; Next generation sequencing; NF- κ B; CARMA1

INTRODUCTION

Primary immunodeficiency diseases (PIDs) are a group of heritable genetic disorders in which parts of the human immune system are missing or are dysfunctional¹. PIDs interfere with essential protective immune functions, greatly enhancing susceptibility to infections, autoimmunity, inflammatory organ damage, and malignancy.

PIDs, often referred to as ‘experiments of nature’, have had a critical role in expanding our understanding of the immune system and in the development of new treatments that have applications far beyond immunodeficiency diseases. Key discoveries in fundamental biology have also emerged from the identification of PID-causing genes. Examples of these transformative discoveries arising from the study of rare PIDs include immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) caused by mutations of *FOXP3*^{2, 3}, severe autoimmunity caused by mutations in the tolerance regulator gene *AIRE*^{4, 5}, and severe combined immune deficiency (SCID) caused by *ORAI1* mutations which defined a new class of calcium channels⁶.

The increasing accessibility of next-generation DNA sequencing has accelerated the genetic characterization of many human PIDs⁷. In the past 18 months, a number of independent groups have begun to define novel PIDs caused by defects in the CARD11–BCL10–MALT1 (CBM) complex^{8–12}. For this *Current Perspectives* we have engaged experts in both basic biology and clinical immunology to capture the worldwide experience in recognizing and managing patients with PIDs caused by CBM mutations.

CARD11–BCL10–MALT1 (CBM) SIGNALOSOME COMPLEX & NF-κB ACTIVATION IN LYMPHOID IMMUNE CELLS

The transcription factor NF-κB is a chief regulator of lymphocyte activation, survival, and proliferation. The clinical relevance of NF-κB signaling is highlighted by PIDs caused by disabling mutations in the pathway, as well as by the association between aberrant constitutive activation of NF-κB and inflammatory, autoimmune and neoplastic disorders^{13–15}. Over the past several decades, assembly of the CARD11–BCL10–MALT1 (CBM) signalosome complex has emerged as an essential step in regulating NF-κB in lymphoid immune cells^{16–18}.

Initial insights gained through the study of lymphoma have profoundly informed our current appreciation of the CBM complex. In the 1990s, B cell lymphomas affecting the mucosa-associated lymphoid tissue (MALT lymphomas) were noted to be associated with several recurring chromosomal translocations, including t(1;14)(p22;q32) and t(14;18)(q32;q21). These translocations bring the *BCL10* and *MALT1* genes, respectively, under the control of the IgH enhancer of chromosome 14, leading to dysregulated expression of BCL10 or MALT1 (which is also known as a paracaspase)¹⁷. An additional translocation, t(11;18)(q21;q21), is also frequently found in MALT lymphomas¹⁷. This translocation creates a gain of function fusion protein, IAP2-MALT1, consisting of the carboxy terminus of MALT1 linked to the amino terminus of cellular inhibitor of apoptosis 2. IAP2-MALT1 can drive constitutive activation of the canonical NF-κB pathway, promoting cell growth and survival¹⁹. At the biochemical level, BCL10 and MALT1 were found to directly interact with one another and to synergistically activate the NF-κB pathway upon ectopic expression¹⁹. At the same time, another protein, CARD11 (also called CARMA1) was found to interact with BCL10 and to promote NF-κB activation²⁰. Finally, *in vivo* data from the analysis of genetically engineered mice with targeted disruptions of *Bcl10*, *Malt1* or *Card11* revealed that all three proteins are essential for adaptive immunity and specifically required to mediate NF-κB activation after B cell and T cell antigen receptor (AgR) stimulation^{16, 18, 21, 22}.

Antigen receptor-mediated NF-κB signaling is initiated by the activation of the Src family of protein tyrosine kinases, which phosphorylate immunoreceptor tyrosine-based activation motifs (ITAMs) within AgR signaling chains, driving the recruitment and activation of the SYK family kinases, SYK or ZAP-70^{23, 24} (for a schematic overview see Fig. 1). Subsequently, the adaptor proteins—LAT and SLP-76 in T cells, or BLNK in B cells—associate with the activated AgR complex and recruit further mediators such as the TEC kinases, ITK (T cells) and BTK (B cells), for the activation of phospholipase C (PLC)-γ1 and -γ2, respectively²⁵. These events trigger a cascade of downstream events, such as the

formation of inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) as second messengers, that lead to the release of intracellular calcium and the activation of the serine/threonine protein kinases, PKC- θ in T cells and PKC- β in B cells, and their recruitment to the AgR complex^{26, 27}. The membrane-associated guanylate-kinase (MAGUK)-like protein, CARD11, is recruited to the immunological synapse where it is phosphorylated in the linker region by PKC- θ in T cells and PKC- β in B cells^{28–30}. Additional kinases, including PDK1, Akt, TAK1, CK1 α and IKK, can also associate with and phosphorylate CARD11³¹. Phosphorylation of the linker region of CARD11 is believed to induce a conformational change within the CARD11 molecule, relieving autoinhibition and allowing the recruitment of BCL10 to CARD11. BCL10 itself is constitutively associated with MALT1, and hence the trimeric protein complex composed of CARD11, BCL10 and MALT1 is formed³². CARD11 and BCL10 interact via their N-terminal CARD domains, whereas the Ser/Thr-rich C-terminal portion of BCL10 associates with the Ig-like domains of MALT1³³. CARD11 may also directly interact with the paracaspase domain of MALT1³⁴ (Fig. 2). Recent data suggest that CARD11 induces BCL10 to oligomerize into helical filamentous structures, which form a platform for the downstream signaling events of the CBM complex³³. While CARD11 is only expressed in the hematopoietic system and appears to be specific for signaling through the TCR and BCR, BCL10 and MALT1 are much more broadly expressed. Consequently, other CARD proteins replace CARD11 and interact with BCL10 and MALT1 to form a CBM complex in other cells, inducing NF- κ B activation in receptor signaling pathways. For example, CARD10 (also known as CARMA3) contributes to NF- κ B activation by cell surface G protein-coupled receptors and receptor tyrosine-kinase pathways, and CARD9 is involved in some innate immune responses and the C type lectin receptor pathway (as reviewed in^{17, 35}). Currently, the exact mechanism by which BCL10 and MALT1 regulate IKK-mediated NF- κ B activation is not completely understood. However, MALT1 can act as a scaffold protein for the induction of downstream signaling events (Fig. 1). The association of MALT1 with TRAF6-containing ubiquitin ligase complexes results in the addition of K63-linked ubiquitin chains to a multitude of proteins, among others TRAF6, BCL10, MALT1, and the IKK regulator, NEMO³⁶. Linear ubiquitin chains are also conjugated to NEMO via the linear ubiquitin chain assembly complex (LUBAC), which was recently shown to interact with the CBM complex in B cell lymphomas^{37, 38}. Both types of ubiquitination events are essential for the recruitment and activation of the IKK complex, which can then phosphorylate the NF- κ B inhibitor, I κ B α ³⁹. In resting cells, I κ B α is mainly bound to NF- κ B dimers, keeping those transcription factors inactive in the cytoplasm. Upon phosphorylation by the IKK complex, I κ B α is modified by K48-linked ubiquitin chains and subsequently degraded by the proteasome, resulting in the translocation of NF- κ B dimers from the cytoplasm to the nucleus where they can mediate transcription of a large set of immunity-relevant target genes⁴⁰.

Within the CBM signaling complex, the paracaspase MALT1 serves also as a caspase-like protease that shares structural homology with the family of caspase-like proteins known as metacaspases found in yeast, plants, and parasites⁴¹. Like metacaspases, MALT1 cleaves substrates after arginine residues, indicating that the enzymatic cleavage activity is quite distinct from caspases, which in general require an aspartate at the P1 position. Although initial attempts to show a caspase-like activity of MALT1 were unsuccessful, mutations in

the predicted active-site cysteine 464 residue impaired optimal activation of NF- κ B, suggesting an important biological role for MALT1-mediated proteolysis⁴¹. It was not until 2008 that the first paracaspase substrates were identified. The list of substrates is still growing and includes BCL10, A20, CYLD, RelB, and Regnase1^{17, 42–47}. MALT1 can cleave BCL10 to regulate cell adhesion to fibronectin⁴². The ubiquitin editing protein, A20, normally functions to remove K63-linked ubiquitin chains from NF- κ B activators such as TRAF6, NEMO, and MALT1 to provide a negative feedback loop within the NF- κ B pathway¹⁴³. By cleaving and inactivating A20, MALT1 contributes to the amplification and prolongation of the NF- κ B signal⁴³. MALT1 can also cleave the de-ubiquitinating enzyme, CYLD, and thereby positively regulate JNK signaling⁴⁴. Similarly, MALT1 cleaves the NF- κ B subunit, RelB, to enforce canonical NF- κ B signaling⁴⁸; and it cleaves the RNA-binding protein, Regnase-1, freeing T cells from Regnase-1-mediated suppression that regulates the mRNA stability of multiple immune effector genes⁴⁷.

CBM COMPLEX MUTATIONS AS A NOVEL CAUSE OF HUMAN COMBINED IMMUNODEFICIENCY

Combined immunodeficiency disorders (CID) are a spectrum of human diseases affecting cellular and humoral immune responses which typically predispose patients to opportunistic infections^{1, 49, 50}. The most severe form of CID presents as severe combined immunodeficiency (SCID) in infancy with pneumonitis, chronic diarrhea, and failure to thrive and is often characterized by absence of functional T lymphocytes with or without B-cell deficiency. Recently, beside hypomorphic variants of classic SCID-associated genes (e.g. variants of recombination-activating genes 1 & 2), mutations in an increasing number of new genes have been identified leading to a delayed onset of CID often associated with disturbed T cell homeostasis and function, rather than the absence of T cells^{1, 51}. These patients often have a more variable clinical phenotype and have been a challenging group in terms of both diagnosis and therapeutic approach⁴⁹. Recent discoveries add CBM mutations to the growing list of genetic defects that must be considered in the differential diagnosis of combined immunodeficiency.

The NF- κ B family of transcription factors governs key proliferation, anti-apoptosis, and immune function genes. Not surprisingly, overactive NF- κ B is often associated with oncogenic signaling, and particularly B cell malignancy. Gain-of-function somatic mutations (i.e. alterations in DNA that occur after conception that are neither inherited nor passed to offspring) in the CBM complex and related NF- κ B pathway signaling molecules have now been convincingly linked to the development of B cell malignancies (extensively reviewed in⁵²). Therefore, our focus will be on the recent discovery of germline mutations (i.e. heritable alterations in DNA in germ cells that can be passed to subsequent generations) affecting the CBM complex to cause human PIDs (Tables 1&2). These novel PIDs have a phenotype that is quite distinct from other known PIDs that impact NF- κ B axis, such as mutations in *IKBKG* (or NEMO)⁵³, *NFKBIA*⁵⁴, and *IKBKB*⁵⁵.

1. *CARD11* mutations

a) Loss-of-function mutations (OMIM #615206)—Loss-of-function mutations in *CARD11* were the first to be discovered in the CBM complex^{10, 11}. The original reports independently described two children of Palestinian and Central European descent, presenting with hypogammaglobulinemia and *Pneumocystis jirovecii* pneumonia (PjP) at 13 and 6 months of life, respectively, preceded by recurrent respiratory tract infections in the Palestinian patient. The family history of the Palestinian patient was notable for consanguineous parents and two siblings who had died at 3 and 15 months due to severe respiratory distress of unknown origin, likely PjP, although no specific diagnosis was established. Since the original reports, we are aware of one more child with *CARD11* deficiency (unpublished data B. Neven). This male patient of French descent presented at the age of 6 months with PjP. He suffered additionally from a severe eczematous dermatitis and nail dystrophy. No other comorbidities, and especially no autoimmunity or lymphoproliferation, have been reported in *CARD11* deficiency.

The *CARD11* mutations in Patients 1 & 2 were identified by whole exome sequencing, while *CARD11* was sequenced in Patient 3 as a candidate gene based on clinical and immunological phenotype. The mutations and their molecular characterization are summarized in Table 2.

Two of the three patients experienced progressive hypogammaglobulinemia and one presented with agammaglobulinemia at the age of 6 months. Importantly, standard immunological characterization demonstrated normal total T and B cell numbers (3/3 patients), normal naïve T cell numbers (3/3 patients), and normal T cell receptor excision circle (TREC) numbers (tested in only one patient). Therefore, clinicians should be aware that this phenotype may escape detection by both classic diagnostic tests focused on lymphocyte subsets and TREC-based newborn screening for SCID. The major consistent phenotypic abnormalities related to *CARD11* mutations were the absence of regulatory T cells, as previously observed in *Card11*^{-/-} mice⁵⁶, and a severe block in peripheral B cell differentiation. In *CARD11* deficiency, memory B cells failed to develop and a large proportion of the naïve B cells displayed a transitional CD10⁺ CD38^{hi} phenotype. Discordant findings seen only in single patients included monocytopenia and high IgE serum levels that normalized over time.

Detailed functional analysis revealed a complete loss of AgR- and phorbol 12-myristate 13-acetate (PMA)-induced canonical NF-κB activation in B and T cells of both published patients. *CARD11* deficiency was associated with a severe proliferative defect in T cells following anti-CD3/CD28 in all three patients, while the response to phytohaemagglutinin (PHA) was preserved in two of them. Both published patients had reduced Th1 and Th17 cytokine production, while Th2 cytokines were not consistently reduced. Given normal NF-κB signaling downstream of CD40 and preserved plasmablast differentiation after CD40 and IL-21 stimulation *in vitro*, the severe antibody deficiency cannot be completely explained by the B cell intrinsic defect. A more complex disturbance of the differentiation of plasma cells *in vivo* related to disrupted T-B interactions may have contributed to the progressively severe hypogammaglobulinemia experienced by all patients.

CARD11-deficient patients have a profound combined immunodeficiency as highlighted by their early life susceptibility to PjP. Therefore, the principal treatment goal should be rapid, definitive immune reconstitution with allogeneic hematopoietic stem cell transplantation. As a bridge to transplantation, patients should receive immunoglobulin replacement and PjP prophylaxis. After conditioning involving busulfan (myeloablative dose), fludarabine, and anti-thymocyte globulin (ATG), patients 1 and 3 have reconstituted and no subsequent complications have been reported. The skin disease of patient 3 went into long term remission. Patient 2 had pulmonary disease and received a reduced toxicity conditioning regimen consisting of treosulfan, fludarabine, and alemtuzumab. This patient achieved mixed chimerism after several transfusions of donor lymphocytes. No clinical complications have been observed. At this time the patients are 14, 30, and 15 months post-transplant, respectively.

In summary, loss-of-function *CARD11* mutations typically present with progressive hypogammaglobulinemia within the first year of life. PjP is the leading infectious threat in these patients. Unfortunately classic diagnostic approaches and TREC-based newborn screening may fail to identify the profound combined immunodeficiency in these patients, which might potentially delay performing the essential hematopoietic stem cell transplantation.

b) Gain-of-function mutations (OMIM #606445)—Recently, germline heterozygous gain-of-function mutations in *CARD11* have been linked to a novel congenital B cell lymphoproliferative disorder referred to as BENTA (B cell Expansion with NF- κ B and T cell Anergy)¹². BENTA patients develop massive B cell lymphocytosis within the first year of life with associated splenomegaly and lymphadenopathy. The unremarkable appearance of small, resting lymphocytes in the blood generally rules out a diagnosis of overt leukemia, and the mild anemia and thrombocytopenia noted in some patients have been attributed to splenic sequestration. Despite excessive B cell accumulation, manifestations of autoimmunity are largely absent. Mild immunodeficiency is characteristic of BENTA disease, perhaps relating to intrinsic B and T cell signaling abnormalities. Specific infections have included recurrent sinopulmonary infections and viral infections (molluscum contagiosum, BK virus, Epstein-Barr virus).

Immunological phenotyping of BENTA patients confirms that ~50–80% of peripheral blood mononuclear cells are CD19⁺CD20⁺CD5^{int} B cells (~4000–9000 cells/ μ l; normal = 390–1400 cells/ μ l) representing mainly polyclonal, IgD^{hi} naïve mature B cells, with a significant elevation of CD10⁺CD24^{hi}CD38^{hi} transitional B cells, whereas absolute T cell counts fall within or just above normal ranges. Congruently, histological analysis reveals marked follicular hyperplasia with striking accumulation of IgD⁺ B cells in mantle zones. Several phenotypic features suggest B cell differentiation may be partially impaired in BENTA patients, including: (a) very low percentages of circulating memory and class-switched B cells (although absolute counts may be within normal range); (b) poor immunoglobulin secretion and plasmablast differentiation *in vitro*; (c) incomplete and transient humoral responses to T-cell independent, polysaccharide-based vaccines, reminiscent of Specific Antibody Deficiency (SAD)⁵⁷. A few patients also fail to mount protective antibody titers to other vaccines, including measles and VZV. Most patients exhibit low serum IgM, whereas

total IgG and IgA levels typically fall at the low end of normal range. Additionally, both CD4⁺ and CD8⁺ T cells from BENTA patients are hyporesponsive *ex vivo* unless robust co-stimulation is provided. Poor proliferation and IL-2 secretion upon mitogenic stimulation suggests patient T cells are mildly anergic, which may contribute to defects in T cell help and/or vulnerability to certain viral infections. These hallmarks surprisingly suggest that gain-of-function *CARD11* mutations create a unique state of combined immunodeficiency in BENTA, albeit far less severe than that experienced by patients with loss-of-function mutations.

To date six BENTA patients have been identified, all harboring germline gain-of-function mutations in *CARD11*. These heterozygous missense mutations typically reside within the coiled-coil (CC) or LATCH domain of CARD11 protein, with one mutation found in the CARD domain. Somatic mutations typically restricted to the coiled-coil (CC) region of CARD11 have been described in several forms of diffuse large B-cell lymphoma (DLBCL) that exhibit elevated expression of NF- κ B-dependent genes^{58–61}. These mutations are thought to decouple TCR/BCR-triggered phosphorylation of the regulatory linker domain from CC-dependent CBM complex assembly and oligomerization, resulting in spontaneous CBM signalosome formation and constitutive NF- κ B activation^{62, 63}.

BENTA disease is currently managed with minimal therapeutic intervention and close monitoring for infections and any signs of oligoclonal or monoclonal B cell expansion. Indeed it is likely that BENTA patients face an increased predisposition to B cell malignancy (with one patient developing B-cell chronic lymphocytic leukemia (B-CLL) around age 44 years), although their *CARD11* mutations alone do not appear to be capable of outright B cell transformation. Interestingly, peripheral B cell counts rose sharply in two patients who underwent splenectomy (ALC ~ 100000 cells/ μ l, normal ALC = 1000–5000 cells/ μ l), suggesting this approach will not reduce B cell burden. The utility of B cell depleting agents like rituximab or other general immunosuppressive drugs remains to be determined. Newer drugs under investigation for the treatment of certain DLBCL, including lenalidomide and MALT1 protease inhibitors, may represent options to specifically target the activity of CBM signaling pathway.

2. *MALT1* mutations

To date, autosomal recessive loss-of-function mutations in *MALT1* have been identified in 3 patients with combined immunodeficiency causing a clinical syndrome of recurrent sinopulmonary infections, inflammatory gastrointestinal disease, periodontal disease, dermatitis, and failure to thrive associated with abnormal cellular and humoral immunity (OMIM #615468).

The first two cases were female and male siblings born to first cousin parents of Lebanese origin⁸. Both patients experienced recurrent bacterial pulmonary infections from an early age resulting in bronchiectasis. They also developed mastoiditis, chronic aphthous ulcers, cheilitis, and gingivitis. Endoscopic examinations revealed widespread gastrointestinal inflammation. Their growth was delayed, but neurological development was normal. Both patients died, at 7 and 13.5 years of age, from respiratory failure secondary to recurrent

infections. The only documented immune restorative therapy administered was prophylactic intravenous immunoglobulins.

Immunological studies revealed normal absolute lymphocyte counts, as well as normal percentages of CD3⁺, CD4⁺ and CD8⁺ T cells, CD4⁺CD45RA⁺ and CD4⁺CD45RO⁺ T cells, and CD19⁺ B cells. Lymphocytes had impaired proliferation to common mitogens. Serum immunoglobulins levels were normal, but there was no production of isohemagglutinins, or anti-tetanus and anti-pneumococcal antibodies despite vaccination. Genetic studies combining microarray analysis with whole genome sequencing revealed a homozygous missense mutation in *MALT1* (c. 266G>T) that resulted in an amino acid change from serine to isoleucine at position 89 in the MALT1 death domain rendering the mutant protein susceptible to degradation. The patients were able to make normal levels of *MALT1* mRNA but immunoblotting of primary T cell lysates failed to detect any MALT1 protein. The lack of functional MALT1 protein was confirmed by the severe impairment of IκBα degradation and IL-2 production in primary T cells after stimulation, and the inability of the patients' *MALT1* mutation to correct defective NF-κB activation and IL-2 production in Malt1-deficient mouse T cells.

At the same time, an independent group identified a case of human MALT1 deficiency in a 15-year old female born to first cousin parents of Kurdish descent⁹. She experienced significant growth delay with short stature, low weight, and delayed bone age. Pathologic fractures may prove to be an additional feature of human MALT1 deficiency, as she had very low bone mineral density and fractured her femur and both tibiae after low-impact injuries. She too had frequent viral and bacterial respiratory infections that contributed to the development of chronic inflammatory lung disease, bronchiectasis and nail clubbing. Severe inflammatory gastrointestinal disease necessitated a Nissan fundoplication, repeated esophageal stricture dilatation and elemental formula feeding via jejunostomy. She also suffered from widespread excoriated and lichenified dermatitis complicated by methicillin-resistant *S. aureus* (MRSA) and herpes simplex virus (HSV) superinfection, chronic cheilitis, and gingivitis (indeed a case report describing the patient's dental challenges was published in 2005 before a specific molecular diagnosis had been made⁶⁴).

Absolute lymphocyte count was normal. In contrast to the other two MALT1-deficient patients, the patient had severe B cell lymphopenia with a developmental arrest characterized by reduced transitional, but elevated percentage of naïve IgD⁺IgM⁺CD27⁻ B cells, near absent IgD⁺IgM⁺CD27⁺ marginal zone B cells, and reduced IgD⁻IgM⁻CD27⁺ switched memory B cells. However, serum immunoglobulins were normal (except for with a chronically elevated IgE) with protective antibody titers post-vaccination, as well as isohemagglutinins.

Whole exome sequencing revealed a homozygous missense mutation in *MALT1* (c. 1739G>C) that converts a tryptophan to serine at position 580, resulting in normal *MALT1* mRNA expression but very low expression of MALT1 protein. Functional impairment was demonstrated by absence of paracaspase activity, disruption of the constitutive association between MALT1 and BCL10, as well as absent IκBα degradation and p65/RelA phosphorylation in primary T cells after PMA/ionomycin stimulation. Importantly, artificial

expression of normal MALT1 protein in the patient's primary T cells rescued their ability to activate NF- κ B.

At this time due to the very limited number published cases of human MALT1 deficiency, it is not possible to provide definitive guidance on treatment options. However, based on MALT1 biology, immune function should be able to be restored in MALT1 deficient patients following allogeneic hematopoietic stem cell transplantation.

3. Unifying Clinical Features of Loss-of-Function CBM Complex Mutations

From a clinical perspective, features that should raise suspicion for loss-of-function mutations affecting the CBM complex include combined immunodeficiency with normal T cell numbers, abnormal T cell proliferation, and failure to activate NF- κ B following stimulation with PMA. Although more patients with CBM complex mutations need to be identified before firm conclusions can be drawn, defective B cell development with a transitional B cell block is also likely to be a defining feature as this is a unifying phenotype of the *Malt1*^{-/-}, *Card11*^{-/-}, and *Bcl10*^{-/-} mice.^{16, 18, 21, 65}

Given the normal T cell numbers, TREC-based newborn screening may well fail to identify patients with mutations in the CBM complex. Therefore, when there is a clinical suspicion of a CBM mutation and standard testing reveals a failure of TCR stimulation-induced proliferation, more specific tests should be pursued including assessment of PMA-induced NF- κ B activation and genetic testing.

Intriguing differences between the clinical presentation of human CARD11- and MALT1-deficiency suggest that the CBM members may have individual nuanced and independent functions, perhaps related to the fact that CARD11 expression is restricted to hematopoietic cells while MALT1 is more broadly expressed. Nevertheless, many questions remain unanswered — Why is PjP so prominent in CARD11- but not MALT1-deficiency? Why is gastrointestinal inflammation a defining aspect of *MALT1* mutations? Ultimately these insights into human biology will be answered through the diagnosis and detailed immunological characterization of more patients with mutations affecting the CBM complex.

CONCLUSIONS

The NF- κ B family of transcription factors plays a crucial role in immune cell activation, survival, and proliferation. Aberrant NF- κ B activity is associated with a range of human diseases, including cancer, immunodeficiency, and autoimmunity. The CBM signalosome complex links AgR triggering to NF- κ B activation. Empowered by next generation sequencing technology, very recently a number of independent groups have confirmed that germline mutations affecting the CBM complex are the cause of novel combined immunodeficiency phenotypes which all share abnormal NF- κ B activation and dysregulated B cell development as defining features. Informed by these recent discoveries, it is anticipated that many additional patients with CBM mutations will be diagnosed over time.

Beyond the benefits individual patients and their families experience following a specific molecular diagnosis, there is a rich history of key discoveries in fundamental biology emerging from the identification of PID-causing genes. Today there is considerable interest in developing MALT1 inhibitors for treatment of lymphomas ‘addicted’ to NF- κ B signaling through the CBM complex⁶⁶, although the *in vivo* protease function of MALT1 is still not well characterized. However, because inhibition of the CBM complex and MALT1 protease activity also impair optimal NF- κ B activation and interleukin-2 production in T cells^{8, 9, 42}, inhibitors that selectively target CBM complex activity may be promising candidates for clinical use in many immune diseases, such as allergic inflammation, autoimmunity, and rejection of transplanted tissues. Ultimately, a deeper understanding of the clinical and immunological impact of human mutations in the CBM signalosome will be invaluable in guiding the development of CBM complex inhibitors for broad therapeutic applications.

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Abbreviations

AgR	Antigen receptor
Akt	Serine/threonine-specific protein kinase also known as Protein kinase B
BCL10	B-cell CLL/lymphoma 10
B-CLL	B-cell chronic lymphocytic leukemia
BCR	B-cell receptor
BENTA	B cell expansion with NF- κ B and T cell anergy
BLNK	B-cell linker
BTK	Bruton agammaglobulinemia tyrosine kinase
CARD11	Caspase recruitment domain family, member 11
CARMA1	CARD-containing MAGUK protein 1
CBM	CARD11-BCL10-MALT1
CC	Coiled-coil
CK1α	Casein kinase 1 alpha
DAG	Diacylglycerol
DLBCL	Diffuse large B-cell lymphoma

HSV	Herpes simplex virus
IAP2	Inhibitor of apoptosis 2
IgH	Immunoglobulin heavy locus
IKK	Inhibitor of kappa B (IκB) kinase
IL-2	Interleukin 2
IP3	Inositol-1, 4, 5-trisphosphate
ITK	IL2-inducible T-cell kinase
IκBα	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
JNK	c-Jun N-terminal kinase
LAT	Linker for activation of T cells
MAGUK	Membrane-associated guanylate-kinase
MALT lymphomas	Mucosa-associated lymphoid tissue lymphomas
MALT1	Mucosa associated lymphoid tissue lymphoma translocation gene 1
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
NEMO	NF-kappa-B essential modulator
NF-κB	Nuclear factor of kappa light polypeptide gene enhancer in B-cells
PDK1	Pyruvate dehydrogenase kinase, isozyme 1
PHA	Phytohaemagglutinin
PID	Primary immunodeficiency disease
PKC-β	Protein kinase C, beta type
PKC-θ	Protein kinase C, theta type
PLC	Phospholipase C
PMA	Phorbol 12-myristate 13-acetate
SAD	Specific Antibody Disorder
SCID	Severe combined immunodeficiency
SLP-76	Lymphocyte cytosolic protein 2
SYK	Spleen tyrosine kinase
TAK1	Transforming growth factor beta-activated kinase 1
TCR	T-cell receptor
TEC	Tec protein tyrosine kinase
TRAF6	TNF receptor-associated factor 6

VZV

Varicella zoster virus

ZAP-70

Zeta-chain-associated protein kinase 70

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FIGURE 1.

Schematic overview highlighting the central role of the CBM complex linking antigen receptor activation to NF-κB activation. Insert outlines the protein structure of the CBM components.

FIGURE 2.

Schematic overview of the protein domain structure and interactions between CBM complex members.

TABLE 1

Clinical and immunological phenotypes, and clinical outcomes of autosomal recessive *CARD11* and *MALT1* mutations.

	Autosomal Recessive <i>CARD11</i> Mutations	Autosomal Recessive <i>MALT1</i> Mutations
CLINICAL PHENOTYPE		
Infections	<ul style="list-style-type: none"> ■ <i>P. jirovecii</i> pneumonia ■ Recurrent sinopulmonary bacterial infections 	<ul style="list-style-type: none"> ■ Recurrent sinopulmonary infections resulting in bronchiectasis. Organisms included: <i>S. pneumoniae</i>, <i>H. influenzae</i>, <i>K. pneumoniae</i>, <i>S. aureus</i>, <i>P. aeruginosa</i>, <i>C. albicans</i>, cytomegalovirus
Autoimmunity/inflammatory disease	<ul style="list-style-type: none"> ■ Severe eczematous dermatitis reported in one patient 	<ul style="list-style-type: none"> ■ Widespread inflammatory gastrointestinal disease with predominantly T-cell lymphocytic infiltration ■ Severe eczematous dermatitis
Lymphoproliferation	<ul style="list-style-type: none"> ■ None 	<ul style="list-style-type: none"> ■ None
Other notable features	<ul style="list-style-type: none"> ■ None reported to date 	<ul style="list-style-type: none"> ■ Low bone mineral density with recurrent pathological fractures. ■ Profound failure-to-thrive affecting both height and weight
IMMUNOLOGICAL PHENOTYPE		
Cellular immunity	<ul style="list-style-type: none"> ■ Normal T cell numbers with abnormal proliferation following anti-CD3/CD28 stimulation, normal naïve T cell numbers, normal TREC copy numbers, and normal TCR repertoire. ■ Normal B cell and KREC copy numbers but disordered B cell development with a block at the late transitional B cell stage and lack of mature B cells ■ Defective NF-κB activation following BCR/TCR and PMA stimulation ■ Reduced numbers of Tregs and T_H17 cells ■ Normal numbers of NK cells 	<ul style="list-style-type: none"> ■ Normal T-cell numbers but reduced T-cell proliferation. ■ Variable B cell numbers with developmental arrest at the transitional and mature naïve stage and the absence of marginal zone B cells ■ Defective NF-κB activation following BCR/TCR and PMA stimulation ■ Normal numbers of Tregs and TH17 cells ■ Normal numbers of NK cells
Humoral immunity	<ul style="list-style-type: none"> ■ Progressively worsening panhypogammaglobulinemia with inability to produce specific antibodies 	<ul style="list-style-type: none"> ■ Normal immunoglobulin levels ■ Variable ability to produce specific antibody against protein and polysaccharide antigens
CLINICAL OUTCOME		
	<ul style="list-style-type: none"> ■ Fatal in first 2 years of life without specific treatment. ■ Immune competence restored with hematopoietic stem cell transplantation. 	<ul style="list-style-type: none"> ■ Fatal in the first 2 decades of life without specific treatment.

This table is based on both published and unpublished data from the authors.

TABLE 2

Summary of human germline mutations in *CARD11* and *MALT1*

Loss-of-function <i>CARD11</i> mutations causing combined immunodeficiency				
Patient	Race/Ethnicity	Mutation	Impact on Protein	Reference
1	Palestinian	Homozygous 1377-bp genomic deletion, including the entire sequence for exon 21 of <i>CARD11</i>	Absent	Stepensky <i>et al.</i> ¹⁰
2	German of Central European ancestry	Homozygous premature stop codon mutation (c.2833C>T) p.Q945*	Truncated protein without GUK domain	Greil <i>et al.</i> ¹¹
3	French Caucasian	Compound heterozygous (c.1091G>A) p.R364H and (c.2671C>T) p.891X	Analysis ongoing	Unpublished (B. Neven, A. Durandy, A. Fischer)

Gain-of-function <i>CARD11</i> mutations causing B cell expansion with NF-κB and T cell anergy (BENTA)				
Patient	Race/Ethnicity	Mutation	Impact on Protein	Reference
1–3	Caucasian	Heterozygous missense mutation (c.401A>G) p.E134G	Spontaneous aggregation & signaling	Snow <i>et al.</i> ¹²
4	Chinese	Heterozygous missense mutation (c.367G>A) p.G123S	Spontaneous aggregation & signaling	Snow <i>et al.</i> ¹²
5	Caucasian	Heterozygous missense mutation (c.146G>A) p.C49Y	Spontaneous aggregation & signaling	Unpublished (D. Buchbinder, A. Snow)
6	Caucasian	Heterozygous missense mutation (c.368G>A) p.G123D	Spontaneous aggregation & signaling	Unpublished (J. Moscow, A. Snow, J. Khan)

Loss-of-function <i>MALT1</i> mutations causing combined immunodeficiency				
Patient	Race/Ethnicity	Mutation	Impact on Protein	Reference
1 & 2	Lebanese	Homozygous missense mutation (c.266G>T) p.S89I	Absent	Jabara <i>et al.</i> ⁸
3	Kurdish Canadian	Homozygous missense mutation (c.1739G>C) p.W580S	Very low level expression with disruption of paracaspase and scaffold functions	McKinnon <i>et al.</i> ⁹