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Elevated IgE from attenuated CARD11 signaling: lessons from atopic mice and humans

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Abstract

CARD11 encodes a large scaffold protein responsible for integrating antigen-receptor engagement with downstream signaling to NF-kB and other outputs in lymphocytes. Over the past 10 years, several human-inborn errors of immunity have been linked to pathogenic CARD11 mutations. Most recently, severe atopic patients were discovered that carried heterozygous dominant-negative CARD11 mutations. Here, we review the mechanistic connections between attenuated CARD11 signaling, elevated IgE, and atopy, comparing and contrasting key insights from both human patients and murine models. Continued investigation of abnormal CARD11 signaling in both contexts should inform novel therapeutic strategies to combat allergic pathogenesis.

Introduction

Atopy refers to the predisposition or tendency toward developing symptoms of allergic inflammation. This inflammation can include type-I immediate hypersensitivity, elicited by IgE antibodies recognizing benign environmental antigens and associated with mast cell degranulation, as well as chronic inflammation caused by type-II cytokines such as IL-4, IL-5, and IL-13 and related effector mechanisms such as eosinophilic infiltration and local mucosal inflammation. Serum IgE titers are typically very low under normal conditions, but in some cases, inappropriate overproduction of IgE may predispose individuals to allergic hypersensitivities. In other cases, systemic (possibly nonspecific) IgE elevation can simply reflect local allergic inflammation due to type-II cytokines. Importantly, IgE elevations can occur in nonatopic settings when not directed toward allergens — such as parasitic infection. In recent years, several monogenic ‘primary atopic disorders’ (PADs) have been described in patients that present with severe forms of allergic inflammation [1•]. At the molecular and cellular level, pathogenesis typically involves a complex milieu of skewed

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type-2 CD4 helper T-cell (Th2) responses involving excess secretion of IL-4, IL-13 and IL-5, eosinophilia, elevated IgE, and abnormal mast cell degranulation. At the genetic level, we now appreciate that genetic lesions affecting epithelial barrier function, cytokine signaling, mast cell, and CD4 T-cell activation can all give rise to PADs in humans, with or without additional syndromic comorbidities [2]. Altered CD4 T-cell function is a common cause, with many PADs ascribed to gene mutations that specifically attenuate T-cell receptor (TCR) signaling (including CD28 costimulation), disrupt cytoskeletal remodeling, impede glycosylation and metabolic reprogramming, and alter cytokine signals in CD4 T cells, resulting in enhanced Th2 differentiation and B-cell class switching to IgE [2,3].

This brief review focuses on atopic immune dysregulation linked to hypomorphic mutations in *CARD11,* which encodes a large scaffold protein (also known as CARD-containing MAGUK protein 1) expressed in lymphocytes. In resting lymphocytes, CARD11 is maintained in a 'closed' conformation by a complex inhibitory domain, inactivating multiple domains involved in protein–protein interactions [4-6] (Figure 1a). Upon T- or B-cell receptor engagement, CARD11 'opens' to recruit BCL10 and MALT1 to form the 'CBM complex', an elaborate signalosome comprised of BCL10 filaments nucleated by CARD11 oligomers and decorated with MALT1 (an active protease), the E3-ligase TRAF6, and HOIP (a component of the linear ubiquitin assembly complex), among other proteins [4,7•,8•] (Figure 1a). Complex polyubiquitination allows for recruitment of additional signaling partners, including IκB kinase (IKK) [9], culminating in IκB degradation and release of canonical NF-κB heterodimers into the nucleus for target gene transcription. Although NF-κB stimulation is the most familiar outcome, the CBM complex is also required for TCR-induced activation of c-Jun N-terminal kinase 2 (JNK2) and mechanistic target of rapamycin complex 1 (mTORC1) [10,11], highlighting CARD11 as a critical arbiter of T-cell activation in mediating downstream gene transcription and anabolic metabolism.

Why is CARD11 associated with atopy? Previous studies have shaped the general paradigm that attenuation (but not outright ablation) of TCR signal strength can result in preferential Th2 differentiation [12,13]. Indeed, defects in NF-κB, JNK, or mTORC1 can promote Th2 skewing in mice [14-17]. Several lines of genetic evidence suggest a specific connection between attenuated CBM complex function and IgE-associated allergic disease. Genome-wide association studies (GWAS) identified *CARD11* as risk locus for atopic dermatitis (AD) in the Japanese population [18]. Patients with both immunodeficiency and homozygous loss-of-function (LOF) *MALT1* mutations (but not BCL10) can also present with elevated IgE levels, eosinophilia, and eczematous rashes with variable penetrance [19,20•]; indeed, aging Malt1-deficient mice can also develop AD [21]. GWAS also revealed the *MALT1* locus as the peak association locus for peanut allergy, including a strong IgE response to peanut allergens [22••]. Furthermore, Goodnow et al. originally described 'unmodulated' (unm) mice that harbor a hypomorphic *CARD11* mutation and display lymphocyte-activation defects and dermatitis with increased serum IgE [23]. More recently, we discovered and characterized a cohort of patients with severe atopy and germline LOF mutations in *CARD11,* which we now refer to as "CARD11-associated atopy with dominant interference of NF-κB signaling (CADINS)" disease [24••-26••]. In contrast to combined immunodeficiency patients carrying homozygous LOF/null CARD11 mutations that require hematopoietic stem cell transplantation, CADINS patients harbor

heterozygous LOF mutations that dominantly interfere with wild-type (WT) CARD11 function, reducing downstream CBM-dependent signaling outputs to varying extents without blocking them entirely. Emerging evidence is defining CADINS as a primary immune regulation disorder (PIRD) that can present a broad spectrum of clinical phenotypes with incomplete penetrance, although severe allergic manifestations (e.g. AD, asthma, and food allergies) are noted in ~90% of patients, making PAD an appropriate classification [27,28]. Studies in both mouse models and CADINS patients have underscored common phenotypic differences between complete versus partial loss of CBM signaling, with the latter driving heightened IgE production. As elaborated below, we have gleaned many mechanistic insights from studying the consequences of LOF/dominant-negative (DN) mutations in mice and humans, with notable discrepancies that demand further investigation.

Card11-associated atopy in mice

Mouse models have offered valuable opportunities for investigating how CARD11 mutations elicit primary immunodeficiency accompanied by elevated IgE and other atopic features. Unmodulated mice (unm) homozygous for the hypomorphic Card11 L298Q mutation, develop high IgE and atopic dermatitis in an age-dependent manner, accompanied by reduced Treg numbers and spontaneous skewing of T cells to a Th2 fate [23••,29]. The adoptive transfer of WT regulatory T cells (Tregs) into unm mice effectively reduced the high Th2 numbers and the incidence of dermatitis. These findings informed a model in which reduced Treg function in unm mice leads to Th2 skewing and abnormal Th2 function that promotes AD on the one hand and on the other, the elaboration of Th2 cytokines that promote class switching in B cells and the development of high IgE [29•,30]. High IgE levels are not required for AD development in unm mice, since breeding a CD79a null allele into the unm mice, which causes B-cell deficiency, eliminated IgE production but did not affect the incidence of dermatitis [29•]. This is in contrast to the effect of breeding in a TCRα null allele and αβ T-cell deficiency, which eliminated both dermatitis and high IgE [29•]. Importantly, the atopic phenotypes of unm mice are absent in Card11$^{-/-}$ mice, which show profound defects in mature lymphocyte activation and adaptive immunity [31-33]. Hence, the total lack of functional Card11 protein in B and T cells completely prevents any activation and differentiation of effector cells that are necessary for atopy. Notably, the L298Q mutation in unm mice only elicits elevated IgE and AD phenotypes in the homozygous state. When considering the range of Card11 activity across the spectrum of Card11$^{WT/WT}$, Card11$^{unm/WT}$, Card11$^{unm/unm}$, and Card11$^{-/-}$ mice, only the homozygous CARD11$^{unm/unm}$ mice exhibit a level of hypomorphic CARD11 signaling activity that can couple diminished Treg-mediated regulation with reduced, but sufficient T- and B-cell activation that results in dysregulated differentiation and atopy [23••]. Loco and king mice, which carry non-coding and coding mutations in *Card11,* respectively, that reduce Card11 protein expression, also exhibit reduced thymic and peripheral Treg numbers and develop dermatitis as they age, but only when homozygous [34,35]. Importantly, the low numbers of peripherally induced Tregs in loco mice could be expanded and shown to be suppressive, suggesting Card11 is required for the development of normal numbers of thymic and peripheral Tregs, but ultimately dispensible for Treg function [34].

The discovery of CARD11 mutations in CADINS disease that are LOF but can cause disease when heterozygous led to the revelation that certain CARD11 LOF mutations are dominant negative (DN) (i.e. capable of reducing signaling from the co-expressed WT CARD11 protein). Indeed, there is currently little evidence of CARD11 haploinsufficiency in humans or mice carrying a heterozygous null allele (see next section). Recently, mice expressing the Card11 R30W mutation originally identified in CADINS patients have documented the ability of certain orthologous Card11 mutations to elicit in mice some phenotypic features of CADINS when heterozygous [36••], with some interesting differences with unm mice. Card11$^{R30W/WT}$ mice exhibit a pronounced reduction in Tregs and develop high IgE in an age-dependent manner with 50% penetrance, but do not exhibit obvious Th2 skewing and do not develop AD. Thus, the phenotype of CARD11$^{R30W/WT}$ mice decouples high IgE from Th2 skewing and AD incidence, raising some interesting possibilities. First, it is possible that environmental exposures are key to the difference in Th2 skewing and AD incidence between unm and R30W mice, and that particular antigenic exposures are required for Th2 dysregulation and atopic disease and were not present in the environment in which R30W mice were bred and analyzed. Second, it is possible that the strength of the DN activity of the R30W mutation is key in determining phenotype. CARD11 LOF mutations exhibit a continuous range of DN activity when expressed in the presence of WT, and when incorporated into mixed WT:mutant CARD11 oligomers, can prevent at least two steps in the CARD11 signaling cycle [37]. It is possible that in mice, the R30W mutant acts as such a strong DN in T cells that there is insufficient residual Card11 signaling activity present to support the Th2 skewing and effector function that are necessary for AD induction.

CADINS disease: CARD11 lessons from atopic human patients

Our ongoing studies of CADINS disease have yielded valuable insights on how attenuated CARD11 signaling can ultimately manifest as atopy in most human patients. Similar to aforementioned mice, the frequency of atopic disease manifestations (~89%) and IgE overproduction (~75%) in patients harboring bona fide LOF/DN mutations is high but incomplete, which speaks to variable penetrance and expressivity. AD is the predominant atopic presentation (73%), with asthma, food allergies, rhinitis, and eosinophilic esophagitis observed in smaller subsets of patients with or without AD [25••,27]. Other clinical sequelae can include cutaneous viral and respiratory infections (~68%), auto-immune complications (e.g. alopecia, inflammatory bowel disease) (~20%), neutropenia (~15%), oral ulcers (~15%), and lymphoma (< 10%). Even as we discover more patients carrying novel LOF/DN CARD11 variants, definitive genotype–phenotype correlations remain nebulous, likely reflecting modifier gene mutations and environmental factors that influence syndromic features noted for other PIRDs. Evidence for true DN activity is stronger for some mutations (e.g. those in the CARD domain) than others. The healthy carrier parents of CID patients with biallelic LOF mutations suggest no disease association with CARD11 haploinsufficiency. However, it remains possible that the presence of a monoallelic LOF mutation with very weak or absent DN capacity might predispose patients to CADINS-like symptoms when combined with other genetic mutations or environmental triggers. Further

studies are required to potentially connect pure LOF heterozygous mutations with specific phenotypes.

Nevertheless, better definition of the quantitative and qualitative impact of specific DN mutations on CBM-dependent signaling outcomes remains imperative for understanding pathogenesis. Our collective work to date suggests that (a) all CARD11 DN mutations appear to disrupt canonical NF-κB signaling, (b) DN variants are most frequently found in the CARD, LATCH, and coiled-coil domains nested in the N-terminal half of CARD11 (Figure 1b), where they can disrupt distinct 'opening' and 'cofactor association' steps in the CARD11 signaling process [37,38], and (c) most (but not all) DN mutations also perturb JNK and mTORC1 activation [25••,37,38]. Although AD-like skin inflammation and mild Th2 deviation was observed in RelB-deficient mice in the context of cutaneous vaccinia virus infection [39], atopic disease has not been observed to date in patients with monogenic immune disorders caused by LOF mutations in NF-κB components (e.g. *REL* genes, *NFKB1, NFKB2*), raising the possibility that other pathways are required for the development of atopy [40]. Reduced mTORC1 activation is particularly intriguing, given that the CBM complex governs TCR-induced uptake of the essential amino acid glutamine through the surface transporter ASCT2, which facilitates mTORC1 activation upon T-cell stimulation [41,42]. T cells from *Asct2*-knockout mice display impaired glutamine uptake, reduced IFN-γ secretion, and enhanced IL-4 production, while attenuation of mTORC1 impairs Treg function, Th1 and Th17 differentiation, but supports Th2 differentiation [43]. Indeed, CADINS patient T cells exhibit similar defects in ASCT2 upregulation and glutamine import that influence Th2 skewing. Remarkably, we found that culturing patient T cells in excess glutamine partially restored TCR-dependent proliferation and IFN-γ secretion in patient T cells, but only if NF-κB-activating cytokines (e.g. TNF, IL-6) were also included [26••]. This important observation implies that defects in *both* NF-κB and mTORC1 activation drive atopic immunopathology, although recovering both signals is arguably insufficient to completely correct Th function. JNK deficiency leads to a strong Th2 diathesis in mice [15,17], and one patient from a recently reported family with human JNK1 haploinsufficiency had AD, however, none exhibited other Th2 phenotypes [44]. Our preliminary findings suggest that impaired JNK signaling in human T cells may also contribute to Th2 skewing (data not shown).

While several CADINS-associated signaling defects were recapitulated in Card11$^{R30W/WT}$ mice, discrepancies are apparent in mice and humans carrying hypomorphic CARD11 mutations. Most importantly, the frequency and suppressive function of Tregs appears normal in CADINS patients, although elevated GATA3 expression was observed in this subset [26••]. Moreover, very few CADINS patients present with IPEX-like symptoms (e.g. failure to thrive, diabetes mellitus, and severe enteropathy). These results suggest that atopy in CADINS may not result from an overt Treg defect, although more work (on more patients) is required to interrogate the phenotype and function of patient Tregs more comprehensively. Indeed, typical inflammatory manifestations associated with a classic 'Tregopathy' are likely offset by concomitant defects in effector T-cell subsets in CADINS patients. Beyond Treg phenotypes, quantitative differences in serum IgE levels, T- and B-cell activation markers, proliferation, and cytokine secretion in response to specific antigens are also apparent even in related CADINS patients sharing the same DN variant (e.g. R30W).

This phenotypic variation does not necessarily parallel all findings in orthologous mice [24••,25••,36••].

Future directions

As with most monogenic inborn errors of immunity, work in both murine models and human patients provides a powerful one–two punch to deciphering how CARD11 DN mutations predispose to enhanced Th2 responses, increased IgE production, and atopic disease. More mouse models are sorely needed, including additional mutations with weak versus strong DN effects on multiple Card11-dependent signaling outputs (including pathway-selective effects) [37], as well as conditional alleles that can isolate cell-type-specific contributions to pathogenesis. This approach would also allow for interrogation of abnormal CARD11 function in non-hematopoietic cell types. Interestingly, CARD11 is frequently mutated with elevated expression in cutaneous squamous cell carcinomas, although CARD11 expression in normal skin is low [45]. Nevertheless, it is possible that the impact of LOF/DN CARD11 expression in epithelial cells could influence AD development via perturbed barrier function, akin to the impact of LOF mutations in the homologous CARD14 protein expressed exclusively in keratinocytes [46•]. In turn, these mouse models can be utilized to manipulate environmental variables (e.g. microbiome, diet, etc.) or perform preclinical testing of potential therapeutics to assess their phenotypic impacts *in vivo*. Indeed, our preliminary data suggest that oral glutamine supplementation in unm mice reduces IgE production and AD incidence (data not shown), consistent with beneficial effects noted for CADINS patient T cells cultured in excess glutamine [26••]. We must also continue to catalog and characterize impactful human CARD11 mutations across the entire polypeptide sequence, including private/rare and more common variants derived from PAD patients and broader cohorts of atopic patients, respectively. This should help clarify potential genotype–phenotype correlations, including what distinguishes CARD11 LOF/DN mutations that do *not* elicit hyper-IgE/atopy. Simultaneously, we must continue to study critical extrinsic regulators of CBM complex activity (e.g. A20, CYLD) and MALT1 itself, contemplating how impactful mutations in these genes might contribute to immune dysregulation and atopic propensity [47-49]. For instance, several lines of murine evidence point to NF-κB defects as possible contributors to the atopic diathesis [39,50,51], even though LOF mutations of NF-κB components are not associated with allergic disease in humans. All such studies must expand beyond T cells to ascertain relevant intrinsic abnormalities in B and NK cells, for which we have only scratched the surface [36••]. These efforts can be significantly bolstered by sophisticated *in vitro* multiplex assays of variant effect, which have been successfully applied to simultaneously interrogate the functional impact of thousands of variants in a given gene (including CARD11) through saturation genome editing technology [52,53••]. Combined with detailed mechanistic investigations at the biochemical and cellular level, this collective approach will further elucidate the astonishing, multi-pronged potency of CBM complex signaling and its connection to aberrant type-2 immunity, revealing novel treatment strategies in the process.

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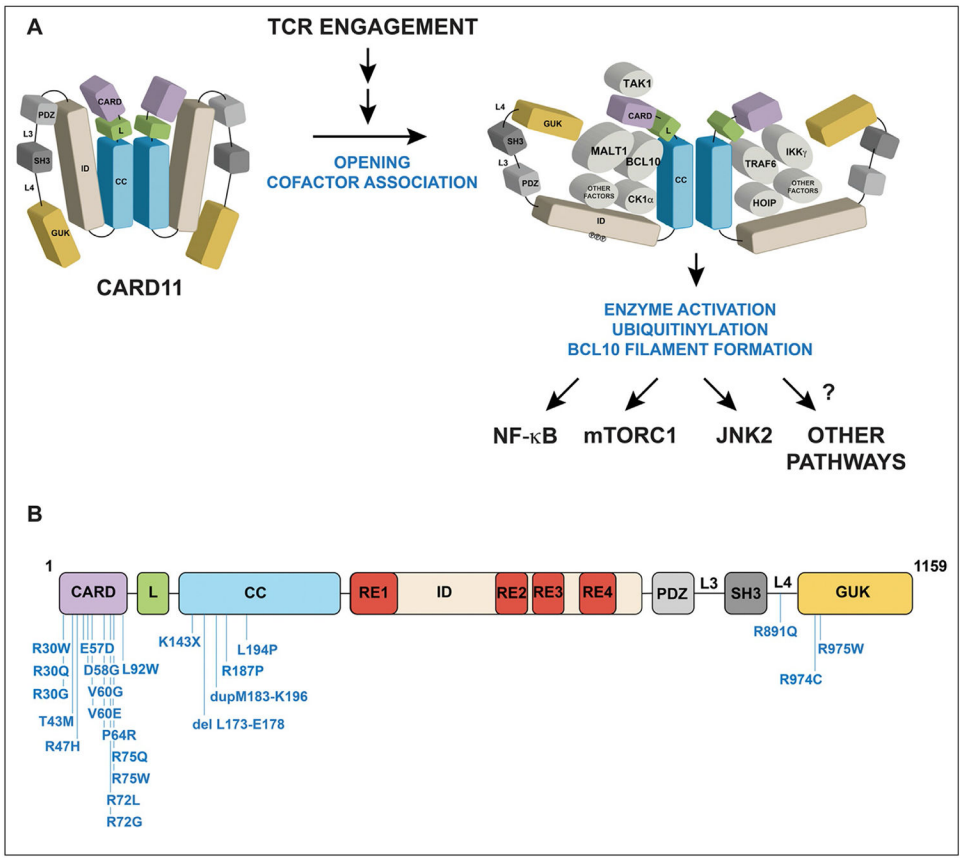

Figure 1.

CADINS mutations in CARD11, an obligate signaling hub in TCR signaling. **(a)** During TCR signaling, CARD11 converts from a closed inactive state to an open scaffold that recruits multiple proteins into a signaling complex, leading to the activation of branching downstream pathways. **(b)** Location of identified DN CADINS mutations in the CARD11 domain structure.