

A New aDENNDum to Genetics of Childhood Asthma

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Childhood asthma is typically associated with a polarized Th2 response to inhaled allergens and is influenced by genetics. Yang et al. show that the asthma susceptibility gene *DENND1B* controls cytokine production in Th2 lymphocytes by controlling the rate of TCR internalization and routing to endosomes, providing a molecular basis for how *DENND1B* contributes to asthma pathogenesis.

Asthma is a complex and heterogeneous disease of the small airways characterized by inflammation, mucus overproduction, and smooth muscle contraction leading to airway narrowing. In the clinic, two forms of asthma have been traditionally defined: allergic asthma (often childhood-onset asthma) and non-allergic asthma (often adult-onset asthma, associated with nasal polyposis). In the former, eosinophilic airway inflammation and bronchial hyperactivity (BHR) are driven by adaptive type 2 T helper (T_H2) cells that are stimulated by dendritic cells (DCs) to produce IL-5, IL-13, and IL-4 (Figure 1A). In the latter, while some cases are driven by innate lymphocytes type 2 (ILC2) that produce IL-5 and IL-13, others are driven by T_H17 cells that cause neutrophilic inflammation (Lambrecht and Hammad, 2015). Asthma is heavily influenced by both environment and genetics. In this issue of *Cell*, Yang et al. reveal that the asthma susceptibility gene *DENND1B* controls cytokine production of antigen-stimulated T_H2 cells by modulating the duration of T cell receptor (TCR) stimulation and significantly further our understanding of the pathophysiology of childhood asthma (Yang et al., 2016).

Recent genome-wide associations studies (GWAS) and their meta-analyses identified four loci carrying asthma susceptibility genes: the 2q12 locus (*IL1RL1* and *IL18R1*), 5q22 (*TSLP*), 9q24 (*IL33*), and 17q21 (*ORMDL3* and *GSDML*). The first three associations highlight the importance of epithelial-cell-derived

cytokines or their receptors (TSLP and IL-33) to promote differentiation and activation of DCs, ILC2s, and T_H2 cells (Hammad and Lambrecht, 2015). In contrast, variation at the 17q21 locus is specifically associated with risk for childhood-onset asthma and might be involved in the synthesis of proinflammatory sphingolipids or unfolded protein response regulation. In addition, a recent GWAS for persistent asthma uncovered several single-nucleotide polymorphisms (SNPs) that significantly associated with asthma at a novel locus on 1q31. This association was also replicated in an independent series of childhood-onset asthma patients of European ancestry and of African ancestry. These studies implicate *DENND1B* as a susceptibility gene for asthma, and interestingly, some polymorphisms in this gene are also related to body mass index in asthmatic children (Marat and McPherson, 2010; Melén et al., 2013; Sleiman et al., 2010). Moreover, *DENND1B* gene variants have been linked with elevated exhaled nitric oxide levels in healthy neonates, a phenotype associated with wheezing illnesses early in life and with a family history of allergy or allergic disorders (Chawes et al., 2015). While these studies link *DENND1B* with human pathology, how *DENND1B* contributes to childhood asthma remained unknown. Yang et al. now shed light into this question by revealing a role for *DENND1B* in fine-tuning T_H2 cell signaling and effector functions (Yang et al., 2016). To dissect *DENND1B* function, the authors generate *Dennd1b*^{-/-} mice, which exhibit hyper-

allergic responses following antigen challenge. *Dennd1b*^{-/-} T_H2 , but not other T_H cells, exhibit delayed receptor-induced TCR internalization and increased production of T_H2 effector cytokines. The *DENND1A-1C* family has been implicated in diverse cellular functions, including receptor-mediated endocytosis, synaptic vesicle endocytosis, polarized secretion of proteins, and autophagy. In particular, *DENND1B* has been shown to act as a guanine nucleotide exchange factor (GEF) for the early endosomal small GTPase Rab35 and to bind clathrin and the clathrin adaptor protein 2 (AP-2). Here, Yang et al. show that *DENND1B* also binds AP-2 and Rab35 in T_H2 cells. Consequently, T_H2 cells deficient in AP-2 or in Rab35 also exhibit delayed downregulation of surface TCRs and enhanced TCR-mediated effector functions. Moreover, human T_H2 cells carrying asthma-associated *DENND1B* variants phenocopy *Dennd1b*^{-/-} T_H2 cells. The specificity of *DENND1B* in the regulation of the early phase of TCR internalization in T_H2 cells, but not in T_H17 or T_H1 cells, provides an intriguing mechanistic basis for the genetic association of *DENND1B* gene variants specifically with early childhood asthma (Chawes et al., 2015; Sleiman et al., 2010).

The duration of signaling by activated receptor complexes shapes the biological fate of many ligand-receptor interactions. In resting T cells, TCRs are pre-clustered within the plasma membrane and are dynamically regulated through a continuous process of endocytosis and

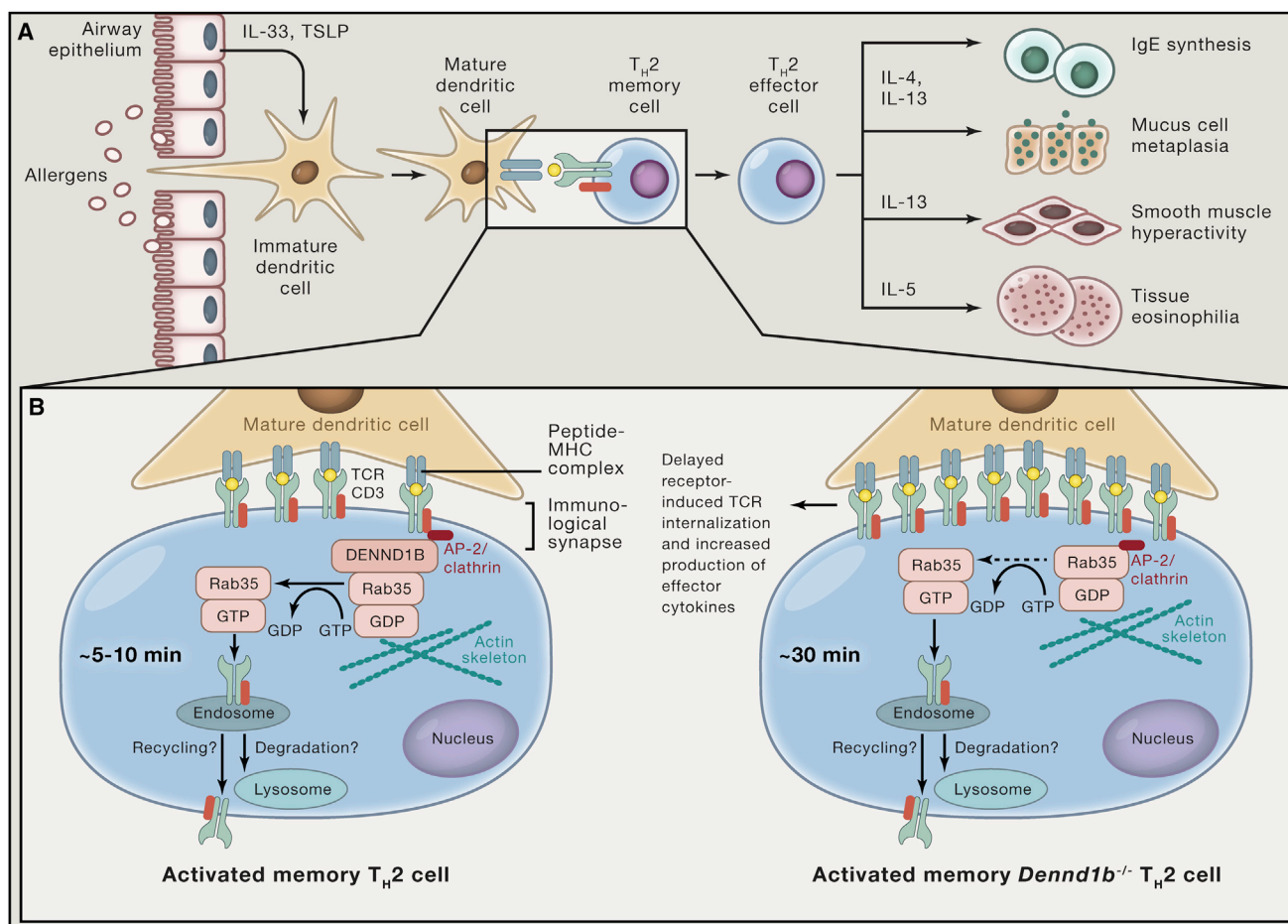


Figure 1. DENND1B Regulates TCR Internalization to Fine-Tune T_H2 Cell Signaling and Effector Functions

(A) Allergic eosinophilic airway inflammation. Eosinophilic airway inflammation and BHR are driven by adaptive T_H2 cells that are stimulated by dendritic cells to produce IL-5, IL-13, and IL-4—the latter driving IgE synthesis.

(B) DENND1B regulates TCR internalization in T_H2 cells. Panel shows the proposed mechanism by Yang et al. to explain the role for DENND1B in TCR internalization through its interactions with AP-2/clathrin and its GEF activity, likely via Rab35. Under normal physiological conditions, TCRs in resting T cells traffic through a continuous process of endocytosis and recycling via the endosomes. After engagement with antigen, TCRs molecules in activated T cells are targeted to the immunological synapse through polarized recycling but are subsequently targeted to endosomes for degradation or recycling to other cellular compartments within 5–10 min. In DENND1B-deficient T cells, TCR internalization is significantly slowed down, leading to prolonged TCR signaling that could bias toward stronger effector T_H2 cytokine (IL-4, IL-5, IL-13) production.

recycling. Activation of T cells results in the formation of an immunological synapse characterized by a central supramolecular activation cluster enriched for TCRs (Figure 1B). Modulation of surface TCRs by receptor internalization, decreased endosome recycling, and increased degradation through lysosomal and proteasomal pathways control the duration of TCR signaling, which has been shown to profoundly affect Th polarization. Whereas short TCR stimulus is sufficient for induction of T_H1 responses in T_H cells, prolonged TCR stimulation was shown to be required for T_H2 responses (Bradley et al., 2002; Iezzi et al.,

1999). While many animal and in vitro human studies demonstrated the importance of duration of MHC-mediated antigen presentation to CD4 T cells in directing the outcome of Th responses, no studies had ever associated prolonged TCR stimulation with a potential risk factor for asthma development. Using Cre-loxP-mediated cell-selective targeting of DENND1B, Yang et al. clearly identify defects of DENND1B function in CD4 T cells as the potential culprit of asthma (Yang et al., 2016). However, other effects of DENND1B also merit additional investigation, as other cell types involved in asthma pathogenesis, such as a sub-

group of DCs, and natural killer cells also express DENND1B. Moreover, DENND1B is significantly upregulated in effector memory T cells as compared with naive T cells (Marat and McPherson, 2010). It will also be important to investigate how other proteins encoded by asthma susceptibility genes affect DENND1B function. It is known that triggering of the IL-33R (T1/ST2) on memory T_H2 cells promotes T_H2 cytokine secretion, and further studies on the involvement of reduced TCR internalization and DENND1B involvement are certainly warranted.

In the immune system, DENND1B polymorphisms have also been associated

with risk for Crohn's disease. The current paper by Yang et al. makes a link between *DENND1B* polymorphisms and increased eosinophilic inflammation, thereby offering a potential explanation of why *DENND1B* might also be related to this disease of the intestinal mucosa. Indeed, recent studies identified an important contribution of eosinophils and T_H2 immunity to the pathogenesis of Crohn's. Therefore, it will be important to study the mechanistic involvement of *DENND1B* in this disease. More generally, there are 18 human DENN domain-containing proteins and their alternative spliced variants. Missense mutations, chromosomal translocations, and altered levels of expression have been described in association with neurological disorders, ocular disorders, and cancers (Marat et al., 2011). The experimental data provided by Yang et al. offer a framework by which other DENN-domain-containing proteins may regulate yet-to-be identified receptor systems to maintain homeostasis that, when dysregulated, contributes to disease. Moreover, this paradigm further

illustrates how alterations in duration and/or subcellular localization of signaling may alter biological consequences to contribute to disease pathogenesis.

In conclusion, Yang et al. help to unravel some of the mysteries surrounding the contribution of *DENND1B* to asthma pathogenesis. However, some important questions are still open, such as the implication of *DENND1B* deficiency in other challenge models in vivo (e.g., worm or viral infections) and whether *DENND1B* and Rab35-mediated internalization of the TCR mainly affects degradation or recycling away from the immunological synapse. However, the most interesting question that remains is why the *DENND1B* pathway is specifically regulating T_H2 effector cytokine production, but not T_H1 or T_H17 effector cytokine production. The identification of preferentially expressed adaptors or post-translational modifications of *DENND1B* in T_H2 cells may further reveal how T_H2 -polarizing conditions could regulate these yet-to-be identified mechanisms to confer T_H2 specificity.

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Street View of the Cognitive Map

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To understand the origins of spatial navigational signals, Acharya et al. record the activity of hippocampal neurons in rats running in open two-dimensional environments in both the real world and in virtual reality. They find that a subset of hippocampal neurons have directional tuning that persists in virtual reality, where vestibular cues are absent.

The hippocampus is a brain structure crucial for both memory and spatial navigation. For the past four decades, the dominant theory for the role of the hippocampus in spatial navigation has been that certain hippocampal neurons, called “place cells,” are active selectively when animals or people occupy certain loca-

tions in space and act as the building blocks for a cognitive map (O'Keefe and Dostrovsky, 1971; O'Keefe and Nadel, 1978). Although this theory has been hugely successful—John O'Keefe was awarded the 2014 Nobel Prize in Physiology or Medicine for this work, along with May-Britt and Edvard Moser—three

challenges to this model have lurked in the shadows, all of which feature in a new study from Acharya et al. (2016) in this issue of *Cell*.

First, the cognitive map theory rests upon the idea that place cells encode spatial location and little else. However, various data have mounted to suggest