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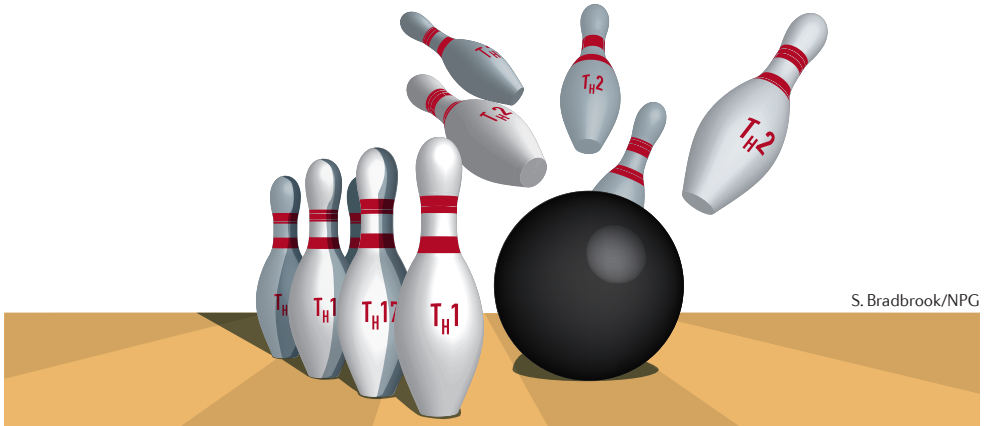

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T CELL SIGNALLING

TCR takedown in T_H2 cells

Mutations in DENN domain-containing protein 1B (*DENND1B*) are associated with the development of childhood asthma and other immune disorders, but the reasons for this are not clear. Chan and colleagues now report that DENND1B is required for the internalization of the T cell receptor (TCR) in T helper 2 (T_H2) cells, but not in other helper T cells.

DENND1B is a guanine nucleotide exchange factor involved in the activation of the small GTPase RAB35, which is a regulator of endocytosis. To examine how mutations in *DENND1B* might contribute to disease, the authors generated *Dennd1b*$^{-/-}$ mice. Compared with controls, young *Dennd1b*$^{-/-}$ mice had similar immune cell numbers in the spleen, lymph nodes, thymus and bone marrow. However, by 7 months of age, *Dennd1b*$^{-/-}$ mice had increased numbers of effector T cells in the spleen and lymph nodes. This was not related to any aberration in dendritic cell function, and naive *Dennd1b*$^{-/-}$ T cells did not show any defects in TCR-mediated activation or in T_H cell differentiation. However, in response to *in vitro* activation, *Dennd1b*$^{-/-}$ T_H2 cells showed markedly increased production of interleukin-4 (IL-4), IL-5 and IL-13. By contrast, *Dennd1b*$^{-/-}$ T_H1 and T_H17 cells showed similar cytokine production to control T cells.

> “DENND1B is required for the internalization of the TCR in T_H2 cells, but not in other helper T cells”

Examination of TCR signalling events showed that TCR cross-linking in *Dennd1b*$^{-/-}$ T_H2 cells leads to increased and sustained phosphorylation of the TCR signalling components CD3ζ, ZAP70, LCK, SLP76, PLCγ1 and VAV, and increased downstream activation of ERK and NF-κB signalling pathways. These differences were not due to higher expression levels of signalling components in the *Dennd1b*$^{-/-}$ T_H2 cells. Instead, the authors found that these cells showed a delay in TCR downregulation from the cell surface, suggesting that defective internalization and degradation of the TCR in *Dennd1b*$^{-/-}$ T_H2 cells augments their effector functions. In support of this idea, *Dennd1b*$^{-/-}$ mice showed an increase in antigen-specific allergic responses in a model of intranasal immunization. A similar phenotype was seen when mice with a T cell-specific deletion of *Dennd1b*$^{-/-}$ were immunized intranasally, suggesting that the overt allergic response is caused by a failure in T_H2 cell regulation.

The authors next compared T_H2 cells from human donors carrying the minor A (rs2786098 single nucleotide polymorphism) allele of *DENND1B* — which is associated with protection against asthma — and/or the major C allele. *In vitro*-differentiated T_H2 cells from *DENND1B*$^{C/C}$ donors produced higher levels of IL-4 and IL-13 following TCR stimulation and showed delayed downregulation of surface TCRs compared with T_H2 cells from *DENND1B*$^{A/C}$ or *DENND1B*$^{A/A}$ donors. Notably, T_H1 cell responses were similar between all three genotypes. Expression analyses indicated that mRNA and protein levels of DENNDB1 are increased in T cells from *DENND1B*$^{A/A}$ and *DENND1B*$^{A/C}$ donors compared with *DENND1B*$^{C/C}$ donors. Therefore, the *DENND1B*^{A} allele seems to limit T_H2-type responses by increasing the expression of DENND1B.

Further experiments using mouse T cells showed that DENND1B associates with CD3ε, RAB35 and the clathrin adaptor AP2 in all resting T_H cells. However, TCR activation only led to increased association of DENND1B with CD3ε and RAB35 in T_H2 cells. T_H2 cells in which RAB35 or AP2 had been knocked down or that expressed a mutant DENND1B that could not interact with AP2 and clathrin also showed defective TCR internalization and increased cytokine responses. These data confirm that the interaction of DENND1B with RAB35 and AP2 is necessary for its regulatory activity in T_H2 cells.

In summary, this study identifies a previous unappreciated role for DENND1B in regulating TCR internalization in T_H2 cells, but not in other T_H cells subsets. The authors suggest that each helper T cell subset may have lineage-specific mechanisms of TCR regulation that are linked to their unique biological functions.

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