# Notch signaling in T cells is essential for allergic airway inflammation, but expression of the Notch ligands Jagged 1 and Jagged 2 on dendritic cells is dispensable

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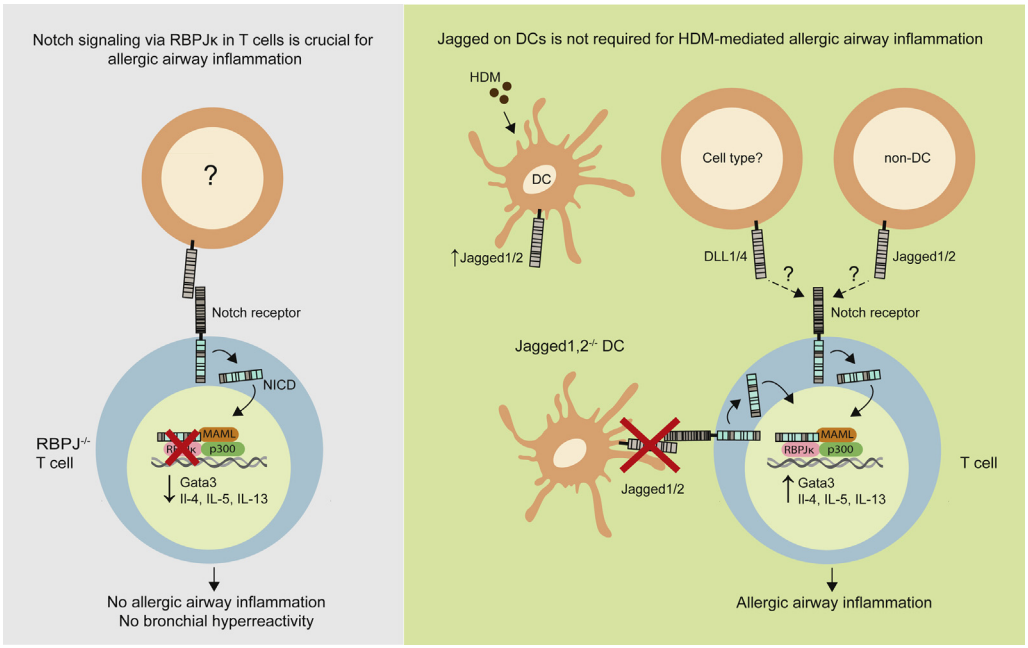
## GRAPHICAL ABSTRACT




**Background: Allergic asthma is characterized by a $T_H2$ response induced by dendritic cells (DCs) that present inhaled allergen. Although the mechanisms by which they instruct $T_H2$ differentiation are still poorly understood, expression of the Notch ligand Jagged on DCs has been implicated in this process.**

**Objective: We sought to establish whether Notch signaling induced by DCs is critical for house dust mite (HDM)–driven allergic airway inflammation (AAI) *in vivo*.**

**Methods: The induction of Notch ligand expression on DC subsets by HDM was quantified by using quantitative real-time PCR. We used an HDM-driven asthma mouse model to compare the capacity of Jagged 1 and Jagged 2 single- and double-deficient DCs to induce AAI. In addition, we studied AAI in mice with a T cell–specific deletion of recombination signal–binding protein for immunoglobulin Jκ region (RBPJκ), a downstream effector of Notch signaling.**

**Results: HDM exposure promoted expression of Jagged 1, but not Jagged 2, on DCs. In agreement with published findings, *in vitro*–differentiated and HDM-pulsed Jagged 1 and Jagged 2 double-deficient DCs lacked the capacity to induce AAI. However, after *in vivo* intranasal sensitization and challenge with HDM, DC-specific Jagged 1 or Jagged 2 single- or double-deficient mice had eosinophilic airway inflammation and a $T_H2$ cell activation phenotype that was not different from that**

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**in control littermates. In contrast, RBPJκ-deficient mice did not experience AAI and airway hyperreactivity.**
**Conclusion: Our results show that the Notch signaling pathway in T cells is crucial for the induction of $T_H2$-mediated AAI in an HDM-driven asthma model but that expression of Jagged 1 or Jagged 2 on DCs is not required. (J Allergy Clin Immunol 2017;140:1079-89.)**

***Key words:*** *Allergic asthma, Notch signaling, Gata-3, $T_H2$ immunity, $T_H1$ immunity, house dust mite, Jagged, recombination signal–binding protein for immunoglobulin Jκ region, cytokines*

Allergic asthma is a $T_H2$ cell–mediated disease characterized by chronic airway inflammation, airway hyperreactivity, and episodes of bronchoconstriction. Inflammatory dendritic cells (DCs) are necessary for induction of $T_H2$ immunity to inhaled house dust mite (HDM) allergen in mice, as was shown in CD11c–diphtheria toxin receptor mice in which DCs were specifically depleted by diphtheria toxin exposure.[1] Lung-resident DCs continuously sample the airway lumen for the presence of allergens, such as HDM, and once activated, these cells mature and migrate to the draining lymph nodes to activate naive T cells.[2] On antigenic stimulation by DCs, $T_H2$ cell differentiation is initiated whereby the polarizing cytokine IL-4, which induces phosphorylation and activation of signal transducer and activator of transcription 6, enhances expression of the key $T_H2$ transcriptional regulator Gata-3.[3] $T_H2$ cells are potent producers of cytokines that induce IgE synthesis (IL-4), recruit eosinophils (IL-5), and cause smooth muscle hyperreactivity and goblet cell hyperplasia (IL-13).

Therefore initiation of $T_H2$ cell differentiation through the IL-4/signal transducer and activator of transcription 6 axis is suggestive of an autocrine loop that leads to expansion of IL-4–producing T cells. However, the primary origin of IL-4, which induces the $T_H2$ response, remains unclear.

One of the pathways that has been implicated in the initiation of $T_H2$ cell differentiation is the Notch signaling pathway. It has been demonstrated that Notch signaling has the capacity to induce $T_H2$ cell differentiation by (1) directly activating the upstream *Gata3* gene promoter and (2) regulating *Il4* gene transcription through activation of a 3′ enhancer.[4-6] Both of these are dependent on a nuclear complex that includes recombination signal–binding protein for the immunoglobulin Jκ (RBPJκ) region and the coactivator mastermind-like 1 (MAML1). Notch signaling in $CD4^+$ T cells is required for physiologic $T_H2$ responses to parasite antigens, as was shown in mice deficient for the RBPJκ region or the Notch 1 and Notch 2 receptors[4] and in mice expressing dominant negative MAML.[7] Moreover, pharmacologic inhibition of γ-secretase, the enzyme that liberates the intracellular Notch domain from the plasma membrane, allowing it to function as a transcription factor in the nucleus, led to decreased $T_H2$ cytokine production after immunization with ovalbumin (OVA) in an asthma model.[8]

Several lines of research support that the Notch ligands Delta-like ligand (DLL) and Jagged instruct $T_H1$ and $T_H2$ cell differentiation, respectively.[9] Surface DLL expression was shown to promote $T_H1$ cell generation and to reduce $T_H2$ responses, whereas Jagged-expressing antigen-presenting cells stimulated $T_H2$ effector generation.[6] Jagged 1 can be upregulated on DCs by stimuli that promote $T_H2$ cell responses, such as through thymic stromal lymphopoietin, which is produced by diesel exhaust particle–treated human bronchial epithelial cells,[10] and on stimulation with *Trypanosoma brucei*–derived antigens, as well as TNF, *Dermatophagoides pteronyssinus* group 7 allergen (Der p 7), and low-dose LPS.[11-13] Jagged 1 was shown to be crucial in the induction of a $T_H2$ response in a model of airway hyperresponsiveness using OVA-pulsed, *in vitro*–cultured, GM-CSF bone marrow–derived dendritic cells (BMDCs).[14] Although evidence was provided that Jagged 2 is dispensable for the induction of $T_H2$ cells *in vivo*,[15,16] Jagged 2 was shown to have the capacity to induce $T_H2$ cell differentiation *in vitro*.[15] Correspondingly, DLL1 and DLL4 ligands are induced on DCs by stimuli that elicit $T_H1$ responses and have the capacity to induce $T_H1$ differentiation *in vitro*.[17,18]

In contrast to this model, it has been hypothesized that Notch signaling acts as a general amplifier of helper T-cell responses rather than an instructive director of specific cell fates. This could be through either enhancing proliferation, cytokine production, and antiapoptotic signals[19-21] or boosting antigen sensitivity through promotion of costimulatory signals in T cells.[22,23]

Therefore in this report we aimed to determine whether Notch signaling is critical for HDM-driven allergic airway inflammation (AAI) *in vivo*. In particular, we questioned whether Jagged 1 and Jagged 2 on DCs are required for the induction of polarization of naive T cells into $T_H2$ cells. We found that expression of Jagged 1 or Jagged 2 on DCs is not required, whereas T cells do need Notch signals, specifically to differentiate into $T_H2$ cells.

*Abbreviations used*
AAI: Allergic airway inflammation
BAL: Bronchoalveolar lavage
BMDC: Bone marrow–derived dendritic cell
cDC: Conventional dendritic cell
DC: Dendritic cell
DLL: Delta-like ligand
EYFP: Enhanced yellow fluorescent protein
HDM: House dust mite
MedLN: Mediastinal lymph node
MoDC: Monocyte-derived dendritic cell
OVA: Ovalbumin
qRT-PCR: Quantitative real-time PCR
RBPJκ: Recombination signal–binding protein for immunoglobulin Jκ region
RSV: Respiratory syncytial virus
WT: Wild-type

## METHODS

For detailed methods, including mice used, experimental protocols and statistical analysis, see the Methods section in this article's Online Repository at www.jacionline.org.

## RESULTS

### Jagged 1 is upregulated on *in vitro* GM-CSF BMDCs on exposure to HDM

Because several research groups have shown a role for Jagged in the orchestration of T-cell responses by using GM-CSF BMDCs,[6,11,14-16] we first investigated the expression of Notch ligands on BMDCs on stimulation with the pro-$T_H2$ stimulus HDM and the pro-$T_H1$ stimulus LPS. GM-CSF BMDCs were cultured from wild-type (WT) mice and sorted at day 9 into $CD11c^+$MHC class $II^{int}F4/80^-CD115^+$ GM–monocyte-derived