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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Background: Allergic asthma is characterized by a Th2 response induced by dendritic cells (DCs) that present inhaled allergens. Although the mechanisms by which they instruct Th2 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 in T cells is essential for allergic airway inflammation (AAI). 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 double-deficient mice had eosinophilic airway inflammation and a Th2 cell activation phenotype that was not different from that 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 Th2-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;:)

Key words: Allergic asthma, Notch signaling, Gata-3, Th2 immunity, Th1 immunity, house dust mite, Jagged, recombination signal–binding protein for immunoglobulin Jκ region, cytokines

Allergic asthma is a Th2 cell–mediated disease characterized by chronic airway inflammation, airway hyperreactivity, and episodes of bronchoconstriction. Inflammatory dendritic cells (DCs) are necessary for induction of Th2 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 naïve T cells.2 On antigenic stimulation by DCs, Th2 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 Th2 transcriptional regulator Gata-3.3 Th2 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 Th2 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 Th12 response, remains unclear.

One of the pathways that has been implicated in the initiation of Th2 cell differentiation is the Notch signaling pathway. It has been demonstrated that Notch signaling has the capacity to induce Th2 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 Th2 responses to parasite antigens, as was shown in mice deficient for the RBPJκ region or the Notch 1 and Notch 2 receptors and in mice expressing dominant negative MAML.8 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 Th2 cytokine production after immunization with ovalbumin (OVA) in an asthma model.9 Several lines of research support that the Notch ligands Delta-like ligand (DLL) and Jagged instruct Th1 and Th2 cell differentiation, respectively.9 Surface DLL expression was shown to promote Th1 cell generation and to reduce Th2 responses,
whereas Jagged-expressing antigen-presenting cells stimulated T_{H2} effector generation. 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, and on stimulation with Trypanosoma brucei–derived antigens, as well as through TNF, Dermatophagoides pteronyssinus group 7 allergen (Der p 7), and low-dose LPS. Jagged 1 was shown to be crucial in the induction of a T_{H2} response in a model of airway hypersensitivity using OVA-pulsed, in vitro–cultured, GM-CSF bone marrow–derived dendritic cells (BMDCs). Although evidence was provided that Jagged 2 is dispensable for the induction of T_{H2} cells in vivo, Jagged 2 was shown to have the capacity to induce T_{H2} cell differentiation in vitro. 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.

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 or boosting antigen sensitivity through promotion of costimulatory signals in T cells.

Therefore in this report we aimed to determine whether Notch signaling is critical for HDF-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 naïve 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.

**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, 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^{+}/F4/80^{+}/CD115^{+} GM–monocyte-derived dendritic cells (MoDCs), CD11c^{+} MHCII^{hi}/F4/80^{+}/CD115^{+} GM-DCs, and CD11c^{+} MHCII^{hi}/F4/80^{+}/CD115^{+} GM-macrophages (Fig 1, A) based on the study by Helft et al. On HDM stimulation, Jag1 mRNA was upregulated on GM-MoDCs and GM-DCs, whereas LPS stimulation induced upregulation of DLL4 mRNA on GM-MoDCs and GM-macrophages. Expression of Jag2 and DLL1 was not altered on GM-CSF BMDCs on stimulation (Fig 1, A). Thus Jag1 mRNA is substantially upregulated on in vitro GM-CSF BMDCs after HDM stimulation.

Jagged is crucial during the sensitization phase in a model that uses GM-CSF BMDCs to induce AAI

To delete Jag1 and Jag2 specifically in DCs, we used Jag1^{fl/fl} and Jag2^{fl/fl} mice, in which the Jag loci contain loxP sites, as well as CD11c-Cre transgenic mice, expressing Cre recombinase under the control of the DC-specific CD11c promoter. Efficiency of CD11c-Cre–mediated deletion was confirmed in CD11c-Cre transgenic ROSA\_EYFP mice with Cre-mediated excision of a loxP-flanked transcriptional STOP sequence. GM-CSF BMDCs were cultured from CD11c-Cre×ROSA\_EYFP and WT×ROSA\_EYFP mice with GM-CSF. Analysis of enhanced yellow fluorescent protein (EYFP) expression by means of flow cytometry indicated that GM-CSF BMDC subsets manifested Cre-mediated deletion in 70% to 74% of the cells (see Fig E1, A, in this article’s Online Repository at www.jacionline.org).

Next, we analyzed Jagged mRNA expression in DCs from CD11c-Cre transgenic Jag1^{fl/fl} Jag2^{fl/fl} mice (Jg1Jg2^{CD11c/ACD11c}) and Jag1Jg2^{+/+} control mice (Fig 1, B). We found reduced expression of Jag1 and Jag2 compared with that seen in WT DCs in all GM-CSF BMDC subsets. Finally, recombination of Jag1 and Jag2 was confirmed on genomic DNA of GM-CSF BMDCs from Jg1Jg2^{CD11c/ACD11c} mice compared with Jg1Jg2^{+/+} mice (see Fig E1, B).

To confirm that Jagged expression on DCs is essential for AAI induction by means of intratracheal transfer of allergen-pulsed GM-CSF BMDCs, we sensitized WT mice with HDM-pulsed total GM-CSF BMDCs from Jg1Jg2^{ACD11c/ACD11c} or Jg1Jg2^{+/+} mice and challenged the mice with HDM (Fig 2, A). HDM-stimulated Jg1Jg2^{+/+} GM-CSF BMDCs induced AAI, as evidenced by a significant increase in numbers of eosinophils, macrophages, neutrophils, B cells, T cells, and DCs in bronchoalveolar lavage (BAL) fluid compared with numbers in control mice (Fig 2, B). Accordingly, numbers of IL-4^{+}, IL-5^{+}, IL-13^{+}, IFN-γ^{+}, and IL-17A^{+} T cells in BAL fluid (Fig 2, C) or Gata-3^{+} T_{H2} cells in mediastinal lymph nodes (MedLNs; Fig 2, D and E) were reduced when mice were sensitized with Jg1Jg2^{ACD11c/ACD11c} GM-CSF BMDCs compared with control DCs. Numbers of Rorγt^{+} T_{H17} cells or Foxp3^{+} regulatory T cells in MedLNs were not different between the 2 groups of mice, and T box–containing protein expression was not detected (data not shown).
The defective capacity of $Jg1Jg2^{CD11c/-}$ GM-CSF BMDCs to induce $T_{H2}$ polarization in vivo was likely not due to cell-intrinsic defects because these DCs expressed similar levels of costimulatory molecules (see Fig E2, B), DLL1, and DLL4 (see Fig E2, C) and produced similar amounts of proinflammatory cytokines (see Fig E2, D), as did control DCs on in vitro activation with a variety of stimuli.

Finally, to investigate whether expression of Jagged 1 and Jagged 2 is perhaps also required during the challenge phase of AAI induction, $Jg1Jg2^{CD11c/-}$ or $Jg1Jg2^{-/-}$ mice were sensitized with WT GM-CSF BMDCs and challenged with HDM. We found...
comparable AAI induction in Jg1 Jg2ΔCD11cΔCD11c and Jg1 Jg2+/− mice (not shown), indicating that for AAI induction, Jagged expression is only required on GM-CSF BMDCs during the sensitization phase and not during HDM challenge.

Taken together, these findings confirm that expression of Jagged 1 and Jagged 2 is crucial during the sensitization phase in a model in which GM-CSF BMDCs are used to induce HDM-driven AAI.

**FIG 2.** Jagged 1 and Jagged 2 are crucial during the sensitization phase when using GM-CSF BMDCs to induce AAI. A, Sensitization and challenge scheme of HDM-driven AAI in mice using cultured total BMDCs. B, Numbers of macrophages (FSCintSSChighAutofluorescent, CD11c+Siglec-F+), eosinophils (FSCintSSChighSiglec-F+), neutrophils (Ly-6G+), B cells (CD19+), T cells (CD3+), and DCs (CD11c+ MHC class II+) in BAL fluid in mice treated with either PBS-pulsed or HDM-pulsed BMDCs from Jg1 Jg2ΔCD11cΔCD11c or Jg1 Jg2+/− mice. FSC, Forward scatter; SSC, side scatter. C, Numbers of IL-4+, IL-5+, IL-13+, IFN-γ+, and IL-17A+ CD3+ CD4+ T cells in BAL fluid in mice treated with either PBS-pulsed or HDM-pulsed BMDCs. D, Flow cytometric profile of Gata-3/Rorγt expression in CD3+CD4+ T cells in mice treated with HDM-pulsed BMDCs from Jg1 Jg2ΔCD11cΔCD11c or Jg1 Jg2+/− mice. E, Total numbers of Gata-3+, Rorγt+, and Foxp3+CD25+ CD3+CD4+ T cells in MedLNs from mice treated with PBS- or HDM-pulsed BMDCs from Jg1 Jg2ΔCD11cΔCD11c or Jg1 Jg2+/− mice, as indicated. Data are shown as means ± SEMs of 4 (PBS) or 6 (HDM) mice per group in 1 experiment. *P < .05 and **P < .01, Mann-Whitney U test.

Jagged 1 is highly upregulated on in vivo migratory CD11b+ conventional DCs on HDM exposure

To analyze the role of Jagged expression in a more physiologic HDM-driven airway inflammation model, we first aimed to establish which in vivo DC subsets express crucial Notch ligands during HDM exposure. In this context CD11b+ conventional dendritic cells (cDCs) were shown to be the main DC subset involved in induction of Th2 cells in the draining lymph nodes,
whereas MoDCs play a crucial role during the challenge phase. We sorted resident MoDCs, migratory MoDCs, resident CD11b+cDCs, and migratory CD11b+cDCs from MedLNs of WT mice intranasally treated with HDM or PBS for 72 hours. In migratory CD11b+cDCs, both Jagged 1 and DLL4 were expressed at baseline and significantly upregulated on exposure to HDM, whereas Jagged 2 and DLL1 were not detected (Fig 3, A).

Resident MoDCs, migratory MoDCs, and resident CD11b+cDCs expressed very low levels of Jag1 mRNA, and expression of other Notch ligands was not detected (data not shown). Jag1 and Jag2 are effectively deleted in DCs from Jg1Jg2 CD11c/D×CD11c mice

To check the efficacy of CD11c-Cre–mediated in vivo gene deletion, we analyzed DCs from CD11c-Cre×ROSA EYFP and control mice. EYFP was expressed in 88% to 97% of CD11c+MHC class IIhigh DCs in lungs, BAL fluid, MedLNs, and spleens and was unaltered when mice were challenged with 50 μg of HDM 72 hours before analysis (Fig 3, B, and see Table E2 in this article’s Online Repository at www.jacionline.org for a detailed analysis of EYFP expression in DC subsets and other immune cells). In accordance with the EYFP data, Jag1 and Jag2 mRNA expression was not detected in migratory CD11b+cDCs sorted from MLNs from Jg1Jg2 CD11c/D×CD11c mice (data not shown).

Together, these data show that Jagged 1, but not Jagged 2, is substantially upregulated on migratory CD11b+cDCs on stimulation with HDM. In addition, DCs from Jg1Jg2 CD11c/D×CD11c mice show almost complete in vivo deletion of both Jagged 1 and Jagged 2.

Mice lacking Jagged expression on DCs have AAI similar to that seen in WT animals

Next, we used an acute AAI model by sensitizing and challenging Jg1Jg2 CD11c/D×CD11c and Jg1Jg2+/+ mice with HDM. Four days after the last challenge, mice were analyzed (Fig 4, A). Surprisingly, after HDM exposure, both

![Diagram](image_url)

**FIG 3.** Jagged 1 is upregulated on migratory CD11b+ cDCs on HDM exposure, and CD11c-Cre is effective in in vivo DCs. **A,** Gating strategy of ex vivo–sorted DC subsets from C57BL/6 mice intranasally treated with 50 μg of HDM or PBS (top). mRNA expression of the indicated Notch ligands, as determined by using qRT-PCR, in DAPI+ MHC class II+CD11b+CD103+CD64 (migratory) DCs from MedLNs after 72 hours of in vivo stimulation (bottom). Six mice were pooled per sample. Data are shown as means ± SEMs of 3 samples per group in 1 experiment. **B,** EYFP expression in CD11c+ MHC class II+DCs in the indicated tissues from WT×ROSA EYFP and CD11c-Cre×ROSA EYFP mice after 72 hours of in vivo stimulation with 50 μg of HDM or PBS. Data are shown as histogram overlays of EYFP expression in the indicated mice. Samples were concatenated, and data are shown as means ± SDs of 4 mice (CD11c-Cre×ROSA EYFP) or 2 to 3 mice (WT×ROSA EYFP) per group in 1 experiment.
FIG 4. Jagged 1 and Jagged 2 expression on DCs is dispensable for the development of AAI in vivo.
A, Scheme of HDM-mediated AAI induction in mice. B, Total numbers of indicated cell populations in BAL fluid from PBS- or HDM-treated Jg1/Jg2<sup>−/−</sup> or Jg1/Jg2<sup>−/−</sup> mice. C and D, Intracellular flow cytometric analysis of cytokine production by CD3<sup>+</sup>CD4<sup>+</sup> T cells in BAL fluid from the indicated mice.
Jg1Jg2<sup>2CD11c<sup>−/−</sup></sup> and Jg1Jg2<sup>+/+</sup> mice had similar AAI inflammation characterized by increased numbers of macrophages, eosinophils, neutrophils, B cells, and T cells in BAL fluid compared with those in PBS-sensitized mice (Fig 4, B). Jg1Jg2<sup>2CD11c<sup>−/−</sup></sup> and Jg1Jg2<sup>+/+</sup> mice showed similar increases in IL-4<sup>−/-</sup>, IL-5<sup>−/-</sup>, IL-13<sup>−/-</sup>, and IL-9<sup>−/-</sup> expressing CD4<sup>+</sup> T cells, and numbers of IFN-γ<sup>+</sup> or IL-17A helper T cells were similar (Fig 4, C and D). Accordingly, restimulated MedLN cells from Jg1Jg2<sup>2CD11c<sup>−/−</sup></sup> and Jg1Jg2<sup>+/+</sup> mice showed no difference in HDM-induced IL-5 production (Fig 4, E). In addition, numbers of Gata-3<sup>1</sup> mice were not different between the 2 groups (Fig 4, F). The box–containing protein–positive T cells were not detected in these experiments (data not shown). Although total serum IgE levels were higher in Jg1Jg2<sup>2CD11c<sup>−/−</sup></sup> mice compared with those in Jg1Jg2<sup>+/+</sup> mice, HDM-specific IgE and IgG<sub>1</sub> levels in serum were similar in the 2 HDM-treated mouse groups (Fig 4, G). When we analyzed single-gene conditional knockouts, we found, as expected, that Jg1Jg2<sup>2CD11c<sup>−/−</sup></sup> and Jg1Jg2<sup>+/+</sup> mice had AAI similar to that seen in WT littermates on HDM exposure (Fig 4, H).

To verify that DC migration and responsiveness were comparable between Jg1Jg2<sup>2CD11c<sup>−/−</sup></sup> and Jg1Jg2<sup>+/+</sup> mice, the DC response to HDM was analyzed 24 hours after intranasal administration of either PBS, 10 μg of HDM, or 50 μg of HDM (see Fig E3, A, in this article’s Online Repository at www.jacionline.org). We did not detect differences in the numbers of cells of individual DC subsets (see Fig E3, B and C) or in the expression of costimulatory molecules on total DCs (see Fig E3, D) or separate DC subsets (data not shown) in the MedLNs or lungs between Jg1Jg2<sup>2CD11c<sup>−/−</sup></sup> and Jg1Jg2<sup>+/+</sup> mice. We noticed a small but significant increase in DLL4 expression on DCs in the MedLNs of Jg1Jg2<sup>2CD11c<sup>−/−</sup></sup> mice compared with Jg1Jg2<sup>+/+</sup> mice.

Taken together, our analysis demonstrates that in the HDM-driven asthma model there is no evidence for a role for Jagged 1 or Jagged 2 expression on DCs.

**Conditional Jagged 1 and Jagged 2 knockout mice have normal T<sub>H1</sub>1 responses in vivo**

Although T<sub>H1</sub>1 responses still developed in the HDM model in mice with Jagged-deficient DCs, it remained possible that these mice had a shift in T<sub>H1</sub>1/T<sub>H1</sub>2 balance. However, when we analyzed *in vitro* recall responses to OVA, there was no difference in T-cell activation, T<sub>H1</sub>1 cells, or T<sub>H2</sub>2 cells (see Fig E4, A-D, in this article’s Online Repository at www.jacionline.org) or IL-4<sup>+</sup> and IFN-γ<sup>+</sup> T cells (not shown) between *in vitro* OVA-restimulated lymph node cells from Jg1Jg2<sup>2CD11c<sup>−/−</sup></sup> and Jg1Jg2<sup>+/+</sup> mice. Likewise, no differences were found in T cell–dependent B-cell responses because total or high affinity tri-nitrophenol keyhole limpet hemagglutinin–specific IgM<sup>-</sup> and IgG<sub>1</sub>-driven IgG<sub>1</sub> levels were similar in Jg1Jg2<sup>2CD11c<sup>−/−</sup></sup> and Jg1Jg2<sup>+/+</sup> mice (see Fig E4, F and G). Therefore the absence of Jagged expression on DCs does not affect the T<sub>H1</sub>1/T<sub>H1</sub>2 balance in vivo.

**Canonical Notch signaling through RBPJ<sup>k</sup> in CD4<sup>+</sup> T cells is required for AAI development**

Mice with T-cell–specific conditional deletion of the downstream transcription factor RBPJ<sup>k</sup> were studied to establish whether Notch signaling in T cells is critical for induction of T<sub>H1</sub>2 differentiation. We exposed CD4<sup>+</sup> mice transgenic for RBPJ<sup>k</sup> and non–CD4<sup>+</sup>–expressing RBPJ<sup>k</sup> littermates (terming RBPJ<sup>k</sup>+/−) to our HDM-driven AAI model (Fig 5, A). Strikingly, in the absence of RBPJ<sup>k</sup> in T cells, mice displayed a significant decrease in numbers of macrophages, eosinophils, neutrophils, B cells, T cells, and DCs in BAL fluid compared with WT littermates (Fig 5, B). Also, the numbers and percentages of IL-4<sup>−/-</sup>, IL-5<sup>−/-</sup>, and IL-13<sup>−/-</sup> T cells were lower in RBPJ<sup>k</sup>+/− mice, whereas we found similar numbers and increased percentages of IFN-γ<sup>+</sup> and IL-17A<sup>+</sup> T cells in BAL fluid, MedLNs, and lungs (Fig 5, C and D, and data not shown). Moreover, the ratio of cytokine-producing T cells shifted from a predominant T<sub>H2</sub>2 phenotype to a more equal T<sub>H1</sub>1/T<sub>H1</sub>2/T<sub>H17</sub> phenotype in the absence of RBPJ<sup>k</sup> in T cells (Fig 5, E). In addition, induction of Gata-3 was particularly impaired in RBPJ<sup>k</sup>+/− mice in CD4<sup>+</sup> cells in BAL fluid, MedLNs, and lungs (Fig 5, F and G, and data not shown). Furthermore, serum IgE levels (Fig 5, H) and airway resistance to methacholine were significantly lower in RBPJ<sup>k</sup>+/− mice compared with values in RBPJ<sup>k</sup>−/− mice (Fig 5, I).

In summary, these results demonstrate that canonical RBPJ<sup>k</sup>-mediated Notch signaling in CD4<sup>+</sup> T cells is crucial for the induction of AAI and airway hyperreactivity in vivo.

**DISCUSSION**

Notch signaling in T cells is crucial to induce a T<sub>H1</sub>2 response. This was shown earlier in mouse models using parasite antigens<sup>4,7</sup> and in asthma models using OVA.<sup>3</sup> In line with these reports, we found that mice with T-cell–specific RBPJ<sup>k</sup> deficiency did not mount a T<sub>H1</sub>2 response in an HDM-induced mouse AAI model. However, the role of the Notch ligands Jagged 1 and Jagged 2 in T<sub>H1</sub>2 induction remains more elusive. Here we show that on HDM exposure, Jagged 1 is specifically upregulated on migratory CD11b<sup>+</sup> cDCs in MedLNs, but expression of Jagged 1 and Jagged 2 on DCs is dispensable for the induction of HDM-induced AAI in vivo.

Although we found a substantial increase of Jagged 1 expression on HDM stimulation both *in vivo* and *in vitro*, Jagged...
**A**

Day: 0 10 11 12 13 14 18

Sensitisation with 10 μg HDM or PBS

Challenges with 10 μg HDM or PBS x5

Analysis

- PBS
- RBPJκ+/
- RBPJκΔCD4/ΔCD4

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**C**

- RBPJκ+/
- RBPJκΔCD4/ΔCD4

**D**

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**E**

- RBPJκ+/
- RBPJκΔCD4/ΔCD4

**F**

- RBPJκ+/
- RBPJκΔCD4/ΔCD4

**G**

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**H**

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**I**

Metacholine (μg/ml) vs. R (cm H20 ∙ s/ml)

- PBS
- RBPJκ+/
- RBPJκΔCD4/ΔCD4

IFN-γ, IL-4, IL-5, IL-13, IL-17A, IgE, Gata3, Foxp3, T-bet, Rorγt, CD49b, Macrophages, Eosinophils, Neutrophils, B cells, T cells, DCs, PBS, HDM.
Notch can perform both roles, enhance general T-cell activation, and T H2-instructive signal. We speculate that heterogeneous cell population, consisting of both cDC-like cells and monocyte-derived macrophages, is incapable of inducing AAI in vivo in an OVA-based model. Thus the requirement for Jagged expression on GM-CSF BMDCs for their capacity to induce AAI does not appear to be dependent on the nature of the allergen (HDM or OVA) but is likely related to the use of GM-CSF BMDCs to sensitize the mice. In particular, it was recently shown that GM-CSF BMDCs comprise a heterogeneous cell population, consisting of both cDC-like cells and monocyte-derived macrophages. These findings indicate that data obtained by using in vivo transfer of GM-CSF BMDCs should be interpreted with care.

Although there is no doubt that Notch is required to induce proper effector T-cell responses, it is currently under debate whether Notch ligands have an instructive role in helper T-cell differentiation or whether Notch signaling acts as an amplifier of helper T-cell responses. The results obtained after instillation of Jagged-deficient DCs would appear to support a general role for Notch in promoting helper T-cell responses. In contrast, in RBPJ-k-deficient mice treated with HDN, we clearly observed a selective defect in Th2 cell responses, whereas numbers of Th1 and Th17 cells were similar to those in WT mice, arguing for a role for Notch as a Th2-instructive signal. We speculate that Notch can perform both roles, enhance general T-cell activation, and function as a more specific promoter of Th2 responses, depending on the repertoire of signals mobilized. Thus when HDN-treated DCs are used to prime the response, the repertoire of additional T-cell–activating signals might be limited. In that case T-cell activation would become more dependent on Notch activation. When, on the other hand, HDN is inhaled, many cell types (innate lymphocytes, epithelial cells, and tissue-resident myeloid cells) will contribute to the generation of activating signals that might override the requirement for Notch in T-cell priming. In this latter scenario only the Th2-promoting function of Notch would be critical.

It has previously been suggested that the Notch ligands DLL and Jagged instruct Th1 and Th2 responses, respectively. However, we found that mice with a conditional deletion for Jagged 1 and Jagged 2 on DCs had Th2 responses to HDM to a similar extent as their WT littermates. These findings indicate either (1) a critical role for other Jagged-expressing cells, implying an instructive role for Notch signaling, or (2) redundancy between various Notch ligands (Jagged 1, Jagged 2, DLL1, and DLL4) on DCs during induction of Th2 responses, which would argue for a role for Notch as an unbiased amplifier.

One explanation for the induction of a Th2 response in the absence of Jagged 1 and Jagged 2 on DCs could be that there is a redundancy of other Jagged-expressing cells. It is not likely that Jagged expression on alveolar macrophages is required for Th2 priming. First, although macrophages can take up HDN, they have been reported to lack the capacity to induce T-cell proliferation. Second, our finding of greater than 94% EYFP expression in alveolar macrophages from CD11c-Cre×ROSA EYFP mice (see Fig E2, B) would indicate that these cells are Jagged deficient in the Jg1Jg2ACD11cACDH12/12 mice also. Another candidate would be B cells, which have been implicated in induction of Th2-mediated AAI. Also, B cells are important in the development and maintenance of follicular helper T cells, which play an important role in AAI by secreting IL-4 and IL-21. However, in fluorescence-activated-cell-sorted activated and nonactivated B cells from HDN-treated and control mice, Jagged 1 was not detected, and levels of Jagged 2 were very low (I. Tindemans, unpublished findings), which is inconsistent with a role for Jagged expression on B cells in Th2 cell induction.

On stimulation with HDN, we found that DLL4 expression was increased on migratory CD11b+ cDCs in vivo (Fig 1, B). In the absence of Jagged 1 and Jagged 2 on DCs, DLL4 expression was increased (see Fig E4, D), raising the possibility that DLL4 compensates for the absence of Jagged 1 and Jagged 2. DLL4 signaling was originally thought to be associated with Th1 response induction. Indeed, DLL4 is upregulated on DCs in response to Th1 stimuli, including bacterial LPS, respiratory syncytial virus (RSV), and dengue virus. However, later studies showed that it is also induced by certain Th2 stimuli, including cockroach allergen, low-dose LPS, and RSV-mediated allergic asthma exacerbations. Furthermore, a regulatory role for DLL4 was demonstrated in Th2 responses to cockroach allergen and when DLL4-pretreated BMDCs stimulated with OVA were adoptively transferred to induce AAI. On the other hand, Th2 responses were decreased when DLL4 was neutralized in vivo in a mouse model for RSV-mediated allergic asthma exacerbations. Therefore it is unclear whether DLL4 compensates for the absence of Jagged molecules on DCs or whether DLL4 has a regulatory role in this setting. Further studies targeting both Jagged 1 and DLL4 Notch ligands are required to resolve this question.

In summary, we showed that Notch signaling is crucial for induction of HDN-mediated eosinophilia, Th2 responses, and...
airway hyperreactivity in vivo, indicating that Notch on T cells could be a potential therapeutic target in patients with allergic asthma. In addition, our data indicate that there is redundancy, either between various Jagged-expressing cells or between Jagged and DLL on DCs. Therefore further studies are required to identify which cells and which ligands provide the Notch signals that are essential for Th2 induction in patients with allergic asthma.

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**Clinical implications:** The Notch signaling pathway in T cells is critical for development of HDM-driven AIA in mice, indicating it could be a potential therapeutic target in asthmatic patients.

**REFERENCES**


