Jagged-1 Reduces Th2 Inflammation and Memory Cell Expansion in Allergic Airway Disease

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ABSTRACT

Notch ligands present during interactions between T cells and dendritic cells (DCs) dictate cell phenotype through a myriad of effects including the induction of T cell regulation, survival, and cytokine response. The presence of Notch ligands on DCs varies with the context of the inflammatory response; Jagged-1 is constitutively expressed, whereas Delta-like 1 and Delta-like 4 are induced in response to pathogen exposure. Although Delta-like and Jagged ligands send different signals through the same Notch receptor, the role of these two ligands in peripheral T cell immunity is not clear. The goal of our studies was to determine the role of Jagged-1 in the pathogen-free inflammation induced by OVA during allergic airway disease in mice. Our studies show that a deletion in DC-expressed Jagged-1 causes a significant increase in cytokine production, resulting in increased mucus production and increased eosinophilia in the lungs of mice sensitized and challenged with OVA. We also observed that a reduction of Jagged-1 expression is correlated with increased expression of the Notch 1 receptor on the surface of $CD4^+$ T cells in both the lung and lymph node. Through transfer studies using OT-II transgenic T cells, we demonstrate that Jagged-1 represses the expansion of $CD44^+CD62L^+CCR7^+$ memory cells and promotes the expansion of $CD44^+CD62L^-$ effector cells, but it has no effect on the expansion of naive cells during allergic airway disease. These data suggest that Jagged-1 may have different roles in Ag-specific T cell responses, depending on the maturity of the stimulated T cell. *ImmunoHorizons*, 2023, 7: 168–176.

INTRODUCTION

The Notch system consists of four receptors and five ligands that interact to determine cell fate and cause cellular differentiation. In T cells, Notch signaling has been linked to every stage of T cell development from thymic maturation to cytokine production to memory cell metabolism (1). Notch ligand expression on APCs is known to influence T cell phenotype. Of the five canonical ligands that can induce Notch signaling in T cells, interactions between T cells and APCs have focused on the Delta-like ligands 1 or 4 (DLL1 and DLL4) and Jagged-1 (JAG1). APCs increase their expression of both Delta-like ligands in response to infection, with DLL1 induction occurring through the type 1 IFN pathways in macrophages (2) and DLL4 induction occurring through MyD88 in dendritic cells (DCs) (3, 4). In either case, expression of the ligand is associated with a reduction in Th2 cytokine production during allergic airway disease in mice (5, 6). In contrast to the Delta-like ligands, JAG1 is constitutively expressed on DCs and macrophages (7, 8) and is not regulated by pathogen-associated signaling pathways. JAG1 is generally considered to reduce T cell activation and inflammation both in vitro (9) and in vivo (7, 10) in the context of allergic airway disease.

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Abbreviations used in this article: DC, dendritic cell; DLL1, Delta-like ligand 1; DLL4, Delta-like ligand 4; i.t., intratracheal(ly); JAG1, Jagged-1; MFI, median fluorescence intensity; qPCR, quantitative PCR.

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During lymphocyte development, DLL1 and DLL4 cause the differentiation of lymphocyte precursors to T cells, whereas JAG1 shifts development toward B cells (11, 12). Although the mechanism of these changes in cell fate remains speculative, exposure to Delta-like ligands increases the expression of at least one Notch target gene (*Hes1*) in thymic precursors compared with JAG1 (11), suggesting that the two ligands send different signals in this context. Another factor contributing to differences in cell fate may be the changing pattern of Notch receptor expression in the precursor cells as they mature, which correlates with altered expression of Notch target genes (13–15).

Previous work from our group suggests that Notch receptor expression changes as $CD4^+$ T cells move through the stages of maturation during Ag sensitization and challenge. We observed that memory T cells ($CD4^+CD44^{hi}CD62L^+CCR7^+$) increase Notch receptor expression compared with effector cells ($CD4^+$ $CD44^{hi}CD62L^{lo}$) in the same animal during a Th2-driven immune response (16). In the current study, we demonstrate that a deletion of JAG1 on DCs results in increased Th2 inflammation, accompanied by increased production of cytokines, including IL-4 and IL-5. We observed that blockade of JAG1 during OVA challenge does not increase cytokine production from T cells, but instead increases the proliferation of Ag-specific memory T cells. We speculate that the increased number of these cells is what augments the Th2 inflammatory response in the absence of JAG1.

MATERIALS AND METHODS

Mice

Jagged$^{f/f}$ mice were a gift of Dr. Julian Lewis on behalf of Cancer Research UK (17). These mice were crossed to a C57BL/6 background 10 times to ensure a consistent genetic phenotype. Mice were then crossed to the *Cd11c$^{Cre\text{-}1Reiz}$* mouse from The Jackson Laboratory (stock no. 008068) (18) to make *Cd11c^{Cre+}Jagged$^{f/f}$* mice. In some experiments, these mice were further crossed to the *Foxp3^{eGFP}* mouse (19), also purchased from The Jackson Laboratory (stock no. 006769), to make a *Cd11c^{Cre+}Jagged$^{f/f}$ Foxp3^{eGFP}* strain. *OT-II RAG$^{-/-}$* mice were purchased from Taconic (stock no. 11490) and maintained as a breeding colony at the University of Michigan. CD45.1/*Ptprc^{a}* mice were purchased from Jackson laboratories (stock no. 002014) and maintained as a breeding colony at the University of Michigan.

Studies performed at the University of Michigan were approved by the University of Michigan Institutional Animal Care and Use Committee under PRO00006469, in accordance with the guidelines and regulations set forth by that regulatory body. Studies performed at the University of Florida were approved under Institutional Animal Care and Use Committee no. 202111551.

OVA sensitization and challenge

We sensitized mice to OVA by i.p. and s.c. injections consisting of 100 μl at each site with a 1:1 mixture of incomplete Freund's adjuvant (Sigma-Aldrich, no. F5506) and a 1 mg/ml solution of endotoxin-free OVA (InvivoGen, no. vac-pova). Two weeks after immunization, we anesthetized mice and administered four once-daily intratracheal (i.t.) instillations given during a consecutive 4-d period. Instillations consisted of 30 μl of a 1 mg/ml solution of low-endotoxin OVA dissolved in sterile saline. Mice were euthanized at the specified time point after the last instillation.

Cell culture

For in vitro culture of T cells, murine T cells were isolated using a MACS naive T cell isolation kit II (Miltenyi Biotec) and then stimulated using 1.0×10^5 cells/well in a 96-well flat-bottom plate in complete RPMI 1640 (10% FCS, 1% nonessential amino acid, 1% sodium pyruvate, 1% penicillin/streptomycin, 1% L-glutamine). Prior to the addition of the cells, each well used for culture was incubated with 3 μg/ml anti-CD3 (BioLegend clone 145-2C11) and 3 μg/ml anti-CD28 (BioLegend clone 37.51) diluted in sterile PBS for 3 h to coat the plate. Recombinant JAG1 (no. Q63722) was purchased from R&D Systems and coated on plates at the same time as anti-CD3/anti-CD28.

Flow cytometry

A single-cell suspension of lung cells was obtained by mincing whole mouse lungs in RPMI 1640 and then digesting each lung in 5 ml of RPMI 1640 + 10% FCS with 125 μg/ml Liberase (Sigma-Aldrich, no. 5401119001) and 10 U/ml DNAse I (Sigma-Aldrich, no. D5025) for 45 min in a shaking incubator at 37°C. After incubation, tissue fragments were passed through a 5-ml syringe with an 18G needle 15–20 times. Cells were centrifuged at 400 × *g* for 5 min and then washed twice in flow cytometry buffer (Dulbecco's PBS + 2% FCS + 1 mM EDTA) and resuspended in 3 ml of the same buffer. Tissue debris was removed by passing the solution through a 100-μm cell culture filter (Corning, no. 352360 or similar). One hundred microliters of the final 3-ml suspension was used for flow cytometry analysis.

For lymph node and spleen flow cytometry, a single-cell suspension was obtained by mincing the tissue and gently pushing fragments through a 100-μm filter using the plunger from a 3-ml syringe. The filter was washed extensively with flow cytometry buffer after the tissue was dispersed to remove all of the cells. Cell suspensions were centrifuged and resuspended in 3 ml of flow cytometry buffer for the spleen, or 0.2 ml of flow cytometry buffer for a single lymph node. One hundred microliters of the suspension was used for flow cytometry analysis.

Cell suspensions were first stained for viability using a fixable viability stain (Thermo Fisher Scientific, no. L34597) and then stained with Ab cocktails in 100 μl of flow cytometry buffer at room temperature with mild shaking (50–80 rpm) on an orbital shaker for 20 min. Dilutions of Abs ranged from 0.2 to 2 μl per 100 μl of buffer based on titration. Cells were fixed with formalin or run live through a BD LSR II flow cytometer and analyzed with FlowJo 10. Clones and dilutions of Abs were as follows: anti-CD25 clone 3C7 used at 1:200, anti-CD4 clone RM4-5 used at 1:200, anti-CD11c clone N418 used at 1:150, anti-CD11b clone M1/70 used at 1:200, anti-CD103 clone 2E7 used at 1:200, anti-Ly6C clone HK1.4 used at 1:200, anti-JAG1 clone HMJ1-29 used

at 1:100, anti-CD44 clone IM7 used at 1:200, anti-CD62L clone MEL-14 used at 1:200, anti-CD45.1 clone A20 used at 1:200, anti-CD45.2 clone 104 used at 1:200, anti-Notch 1 clone HMN1-12 used at 1:200, anti-Notch 2 clone MHN2-35 used at 1:200, anti-CCR7 clone 4B12 used at 1:200, and anti-CD16/32 clone 2.4G2 used at 1:200. All Abs were purchased from BioLegend.

When flow cytometry sorting was used to isolate cell populations, Ab depletion cocktails were used for pre-enrichment. Pre-enrichment for APCs prior to sorting was done with biotinylated anti-CD3 (clone 145-2C11) and anti-CD19 (clone 6D5), both from BioLegend. Cell suspensions were incubated with Abs for 10 min at room temperature at a dilution of 1:200. Each spleen was resuspended in 600 μl of flow cytometry buffer. After incubation, cells were washed once in 2 ml of flow cytometry buffer and then incubated with anti-biotin beads (Miltenyi Biotec) according to the manufacturer's instructions. Samples were then run through a MACS LS column to deplete the Ab-labeled cells. For all flow cytometry assays, a forward scatter/side scatter gate was first used to distinguish cells from debris. Cells were then gated on a viable population of single cells before further analysis.

Real-time PCR

RNA was isolated using TRIzol via spin columns (Zymo Research, no. R2051), quantified using a NanoDrop spectrophotometer, and 100 ng of RNA was reverse transcribed using iScript (Bio-Rad, no. 1708891). Exon-spanning predeveloped assays for each of our targets were purchased from Thermo Fisher Scientific/Applied Biosystems: *Notch1* (Mm00627185_m1), *Jag1* (Mm00496904_m1), and *Muc5ac* (Mm00473758_m1). Assays were run on an Applied Biosystems TaqMan 7500 machine for 40 cycles using TaqMan master mix.

Measurement of cytokines in supernatants

Cytokines in cell culture supernatants were measured by a Multiplex assay from Bio-Rad on a Luminex machine following the manufacturer's instructions.

Statistical analysis

All statistical analysis was performed using GraphPad Prism software.

RESULTS

Generation of Jagged1^{ff}Cd11c^{Cre} mice

To determine the role of DC-expressed JAG1, we generated *Jagged1^{ff}Cd11c^{Cre+}* mice. We confirmed a reduction in JAG1$^+$ CD11c$^+$CD11b$^+$ cells, but not other CD11c$^+$ subsets, in the lungs of unsensitized *Jagged1^{ff}Cd11c^{Cre+}* mice using flow cytometry (Fig. 1A, 1B). We also observed a decrease in the JAG1$^+$CD11c$^+$ CD11b$^+$ population in the spleens of *Jagged1^{ff}Cd11c^{Cre+}* mice (Fig. 1C). Once mice were challenged with OVA we observed

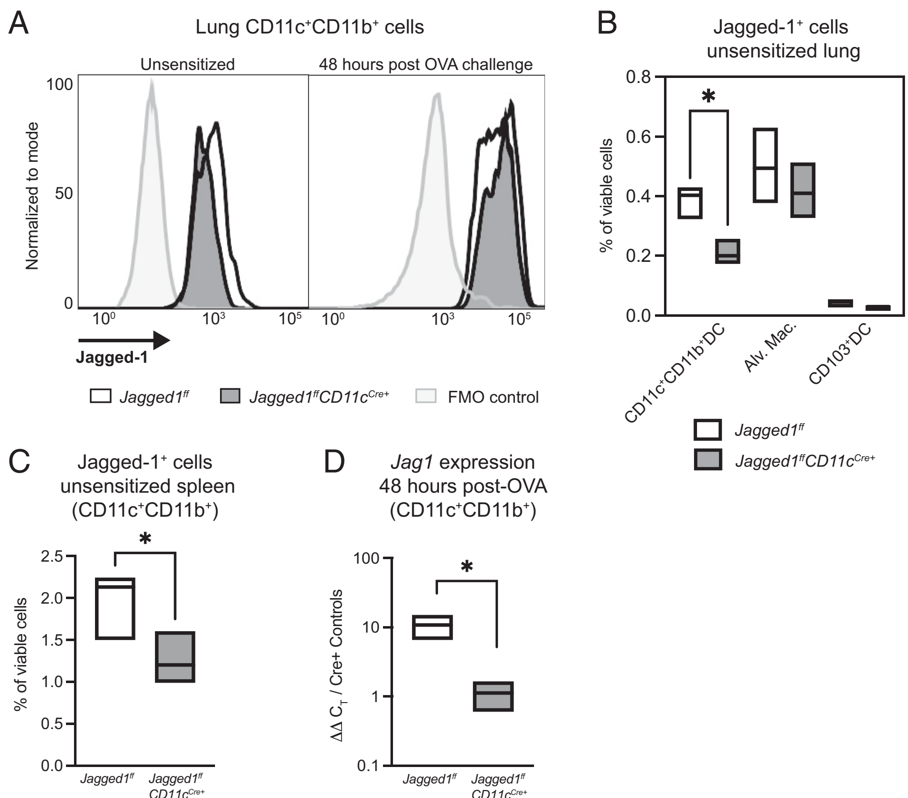

FIGURE 1. Deletion of *Jag1* in DCs.
(**A**) Histograms of JAG1 staining in lung CD11c$^+$ CD11b$^+$ cells using samples from unsensitized mice and mice that had been sensitized and challenged with OVA. (**B**) Quantification of (A) for the indicated cell subsets in the lungs of unsensitized mice. $^*p = 0.04$, $n = 3$ mice per group. (**C**) Proportion of JAG1$^+$ cells in the spleens of *Jagged-1^{ff}CD11c^{Cre+}* animals or controls prior to sensitization with OVA. $^*p = 0.04$, $n = 3$ mice per group. (**D**) Expression of exon 2 of *Jag1* expressed as a fold increase over *Jagged-1^{ff}CD11c^{Cre+}* in CD11c$^+$CD11b$^+$ cells sorted from the lung 48 h after OVA challenge. $^*p = 0.1$, $n = 3$ mice per group. Significance in this figure was determined by an unpaired *t* test.

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that JAG1 staining was not significantly different between *Jagged1*^{ff}*Cd11c*$^{Cre+}$ mice and littermate controls. To confirm that the reduction in JAG1 expression was maintained on CD11c^{+} CD11b^{+} cells in *Jagged1*^{ff}*Cd11c*$^{Cre+}$ mice sensitized and challenged with OVA, we FACS sorted the CD11c^{+}CD11b^{+} population from the lungs of these mice at 48 h after the last i.t. challenge. We measured *Jag1* expression in these cells by quantitative PCR (qPCR) using primers specific for the deleted exon (20) and observed a 10-fold increase in *Jag1* expression in *Jagged1*^{ff} control mice when compared with *Jagged1*^{ff}*Cd11c*$^{Cre+}$ littermates (Fig. 1D). These data confirm that JAG1 expression is reduced in CD11c^{+}CD11b^{+} cells in the lungs of *Jagged1*^{ff} *Cd11c*$^{Cre+}$ mice during OVA challenge.

Our initial assessment of these mice did not reveal any abnormalities in thymic progenitor cells, splenic T cells, and splenic and lung myeloid cells at baseline when compared with *Jagged1*^{ff} littermate controls. These mice aged normally and were not diagnosed with any health concerns during their tenure in our housing facility.

JAG1 regulates pulmonary allergic responses

To determine whether depletion of JAG1 on CD11c^{+}CD11b^{+} cells altered T cell immune responses, we sensitized mice with endotoxin-free OVA for 2 wk and then challenged them with four daily i.t. instillations of OVA to localize the inflammation to the lung. The resulting inflammation was biased toward a Th2 response, characterized by histology with mucus secretion in the airways and a leukocytic infiltrate containing a high number of eosinophils (Fig. 2D). We observed a significant increase in Th2 cytokines, including IL-4 and IL-5, in cultures of lung-draining lymph node cells from *Jagged1*^{ff}*Cd11c*$^{Cre+}$ mice compared with their *Jagged*^{ff} littermate controls (Fig. 2A). These higher levels of cytokines produced in lymph node cultures corresponded to an increase in the expression of the *Muc5ac* gene, associated with mucus production, in the lungs of *Jagged1*^{ff}*Cd11c*$^{Cre+}$ mice as measured by qPCR (Fig. 2B). We also observed a significant increase in the number of eosinophils in the lung (Siglec-F^{+}MHC class II^{-}CD11b^{+}) as measured by flow cytometry 48 h after the last i.t. challenge (Fig. 2C).

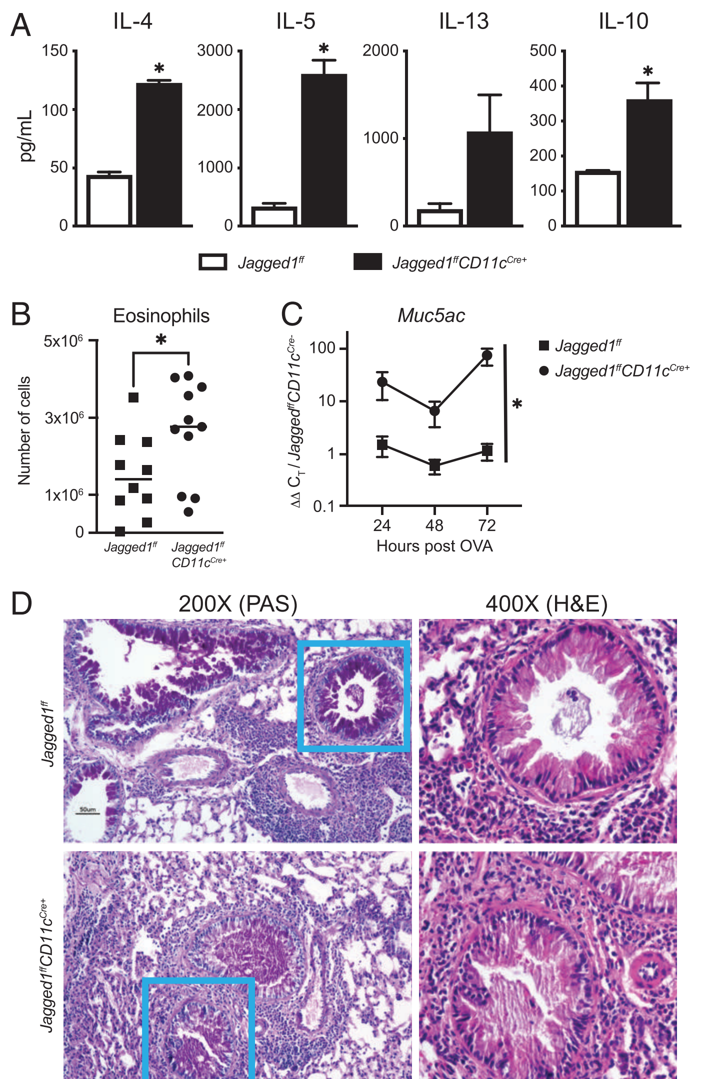

FIGURE 2. *Jag1* deletion increases Th2 responses.

(**A**) Levels of cytokines in cell culture supernatants of lung-draining lymph nodes stimulated with OVA for 48 h. $*p \leq 0.01$ as determined by an unpaired *t* test. (**B**) Expression of *Muc5ac* in lungs of mice sensitized and challenged with OVA as determined by qPCR. $*p = 0.03$ as determined by two-way ANOVA, with genotype accounting for 14% of the variance in the experiment. (**C**) Number of eosinophils in mice from (B) using whole lung at 48 h after OVA challenge as determined by flow cytometry for Siglec-F^{+}CD11b^{+}MHC class II^{-} cells. $*p = 0.04$ using an unpaired *t* test. (**D**) Micrographs of lung tissue formalin-fixed, paraffin-embedded sections stained with periodic acid–Schiff (PAS; original magnification, ×200) and H&E (original magnification, ×400) highlighting mucus production and leukocyte infiltration in this model.

JAG1 regulates Notch 1 expression

Engagement of a Notch receptor by a canonical cell-bound ligand causes transendocytosis of the receptor (21–23). Based on this research, we tested the hypothesis that a reduction in JAG1 expression by APCs would increase the amount of Notch 1 present on the cell surface of T cells in the lung and lymph node of OVA-challenged mice for Notch 1. We observed a significant increase in the median fluorescence intensity (MFI) of Notch 1 on all CD4$^+$ T cell subsets in the draining lymph node of *Jagged1$^{f/f}$Cd11c^{Cre+}* mice (Fig. 3D). We also observed an increase in the MFI of Notch 1 in CD4$^+$CD44^{hi}CD62L^{hi} cells in the lungs of these mice (Fig. 3E–G). We then tested whether JAG1 could decrease Notch 1 expression in vitro using naive T cells stimulated with anti-CD3/anti-CD28 in the presence of rJAG1 protein. We observed a significant decrease in Notch 1 receptor gene expression using qPCR in CD4$^+$ T cells at 24 h poststimulation with 5 nM rJAG1 (Fig. 3A). We confirmed that Notch 1 was decreased on the cell surface in the presence of rJAG1 using flow cytometry (Fig. 3B). Although cell surface–associated Notch 1 was decreased at the 10 nM concentration of ligand, we did not observe a decrease in viability in these cultures, indicating that the recombinant protein was not toxic to CD4$^+$ T cells (Fig. 3C).

Notch receptor expression is increased on Ag-specific T cells

To determine whether cytokine production in Ag-specific CD4$^+$ T cells was directly linked to an absence of JAG1, we transferred CD45.2$^+$ OT-II cells into CD45.1 mice and sensitized the mice with OVA for 6 wk to develop a memory response. We then stimulated splenocytes from these mice with either the OT-II OVA peptide (24) or whole OVA protein in vitro in the presence and absence of an anti-JAG1 Ab and performed intracellular staining to assess IL-4 production. We observed that the Ag-specific CD45.2$^+$ cells produced more IL-4 in response to stimulation with both peptide and whole protein than CD44^{hi} CD4$^+$CD45.1$^+$ T cells, likely due to differences in receptor repertoire, which favor a response from CD45.2$^+$ cells as they all express a TCR specific for OVA (Fig. 4B). There were no

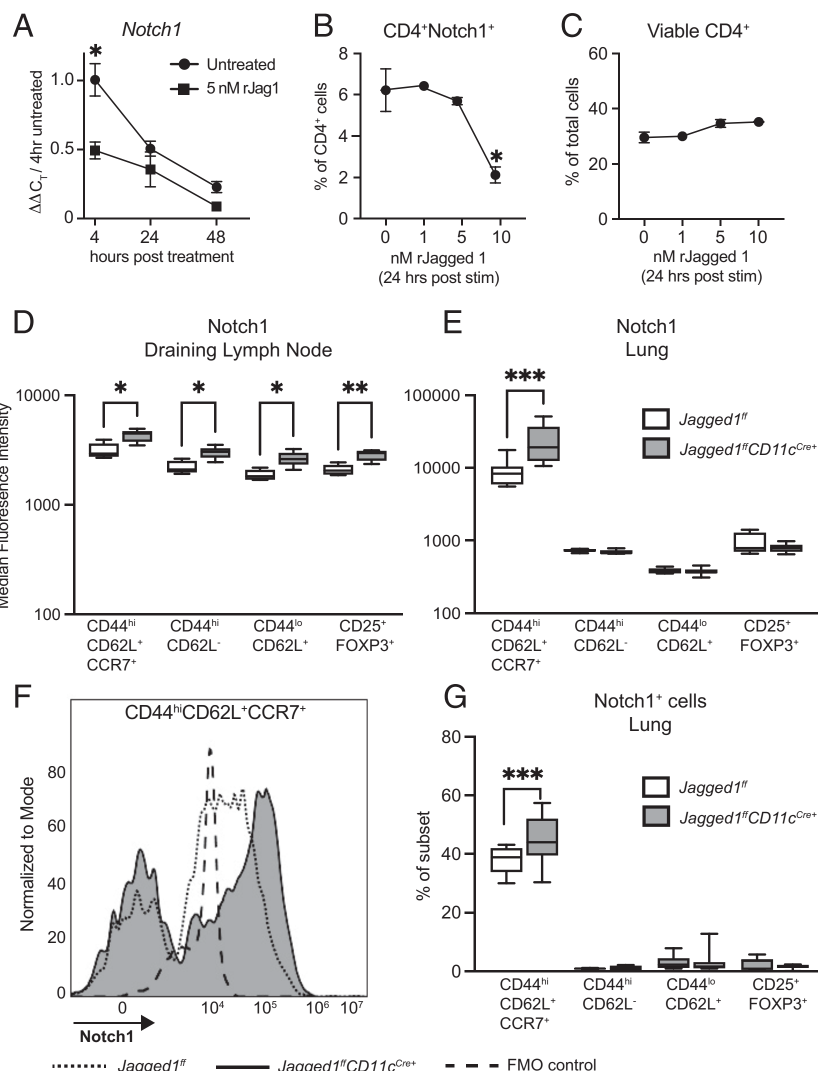

FIGURE 3. JAG1 alters Notch 1 receptor expression.
(**A**) qPCR for the *Notch1* receptor gene demonstrates a significant reduction in gene expression at 4 h after stimulation with anti-CD3/anti-CD28 in the presence of 5 nM rJAG1. *$p < 0.0001$ as determined by two-way ANOVA with Bonferroni's posttest; $n = 4$ samples per group. (**B**) Significant reduction in surface receptor Notch 1, as determined by flow cytometry, after stimulation of CD4$^+$ T cells with anti-CD3/anti-CD28 for 24 h in the presence of 10 nM rJAG1, with no change in T cell viability (**C**) under the same conditions. *$p < 0.0001$ using a one-way ANOVA with a posttest. (**D** and **E**) MFI of Notch 1 in CD4$^+$ T cell subsets in the lung and lung-draining lymph nodes at 48 h after OVA challenge. *$p < 0.01$, **$p < 0.001$, ***$p < 0.0001$ using an unpaired *t* test. (**F**) Histogram of data quantified in (E), focused on the memory cell subset. (**G**) Quantification of the percentage of each CD4$^+$ T cell subset that was expressing Notch receptor at 48 h after OVA challenge. ***$p <$ 0.0001 as determined by an unpaired *t* test. For (D), (E), and (G), $n = 6$ mice per group.

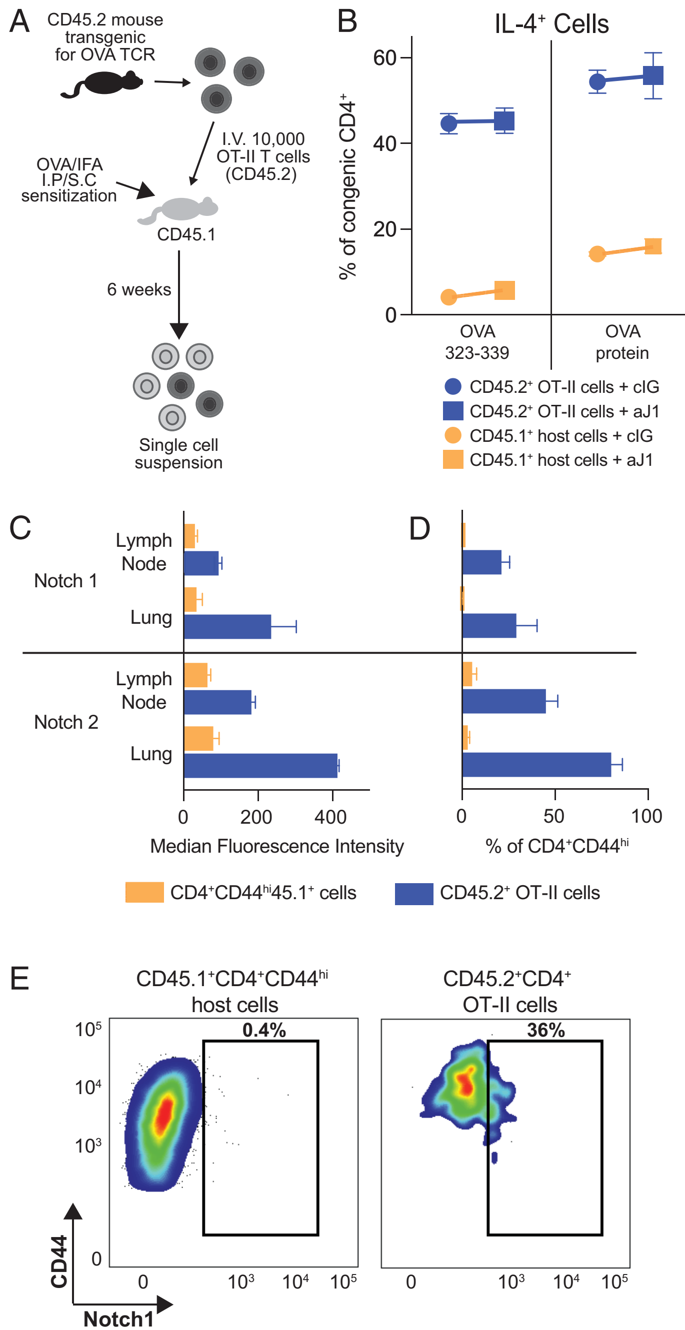

FIGURE 4. Expression of Notch receptors on OT-II$^+$ cells.
(**A**) Schematic of the transfer model used. (**B**) Intracellular staining for IL-4 after stimulation of splenocytes from mice sensitized with OVA after in vitro stimulation with either whole OVA or the OVA peptide specific for the OT-II receptor in the presence of anti-JAG1 or isotype control. (**C**) MFI of Notch 1 and Notch 2 on CD45.1$^+$CD4$^+$CD44^{hi} host cells and CD45.2$^+$CD4$^+$ OT-II cells in the lung and lung-draining lymph node. (**D**) Percentage of CD45.1$^+$ CD4$^+$CD44^{hi} host cells and CD45.2$^+$ OT-II cells expressing Notch 1 and Notch 2 in the spleen. (**E**) Flow cytometry plots of Notch 1 receptor expression on CD4$^+$ CD44^{hi} splenocytes after sensitization with OVA for 6 wk.

significant differences in the production of IL-4 as a result of JAG1 blockade in these cultures.

The high percentage of CD4$^+$CD44^{hi}CD62L^{hi} cells with Notch receptors on the cell surface, as depicted in Fig. 2, prompted us to examine Notch receptor expression as a function of Ag specificity during OVA sensitization and challenge. To test this, we transferred 1.0 × 10^4 CD4$^+$ T cells from CD45.2 *RAG*$^{-/-}$ OT-II mice, which contain a transgenic TCR specific for OVA peptide, into congenic CD45.1 mice. We sensitized these mice with OVA for 2 wk and challenged them as previously described. At 48 h after the last i.t. challenge, we assessed CD4$^+$ cells in the draining lymph node and lungs for expression of Notch 1 and Notch 2 receptors by flow cytometry. We observed that a greater proportion of CD4$^+$CD45.2$^+$ cells, which contain the OT-II transgene, expressed Notch receptors than did CD4$^+$ CD44^{hi}CD45.1$^+$ host cells (Fig. 4D, 4E). The MFI of Notch receptor expression was increased on CD45.2$^+$ Ag-specific cells (Fig. 4C).

Jagged alters memory and effector T cell expansion in vivo

The difference in Notch receptor expression between memory and effector cell subsets, as shown in Fig. 3, led us to the hypothesis that the effects of Notch signaling may depend on the stage of the T cell life cycle. To test this, we again transferred CD45.2$^+$ OT-II cells into a CD45.1$^+$ mouse as described. After a 6-wk sensitization phase to allow for the generation of memory and effector cell populations specific to OVA, we isolated CD45.2$^+$ memory (CD44^{hi}CD62L$^+$CCR7$^+$) and effector (CD44^{hi} CD62L$^-$) T cell subsets from the spleen by FACS and transferred them into separate CD45.1$^+$ hosts that had previously been sensitized with OVA for 2 wk. We performed four consecutive i.t. challenges with OVA in the presence and absence of an anti-JAG1 Ab (Fig. 5A). The use of the Ab, which has been shown to increase Th2 responses in vivo (16), allowed us to take advantage of congenic tracking technology. At 48 h after the last i.t. challenge with OVA, we quantified the number of CD45.2$^+$ cells in the lung and draining lymph node of recipient mice. We observed that a blockade of JAG1 caused a significant reduction in the number of CD4$^+$CD45.2$^+$ OT-II cells derived from the transfer of CD44^{hi}CD62^{hi} effector cells at both sites. In contrast to the effector cell progeny, the population of cells derived from the CD4$^+$CD44^{hi}CD62L$^+$CCR7$^+$ cell transfer expanded in the presence of anti-JAG1 treatment (Fig. 5B, 5C).

To determine whether previous Ag exposure altered the function of Notch in T cells, we transferred naive CD45.2$^+$ OT-II cells directly into unsensitized CD45.1 mice and then challenged them with OVA through the i.t. route in the presence and absence of anti-JAG1 blockade (Fig. 5D). We did not observe any differences in T cell expansion in this system, indicating that the sensitization phase of the model was important for generating the altered response to Notch ligand exposure (Fig. 5E).

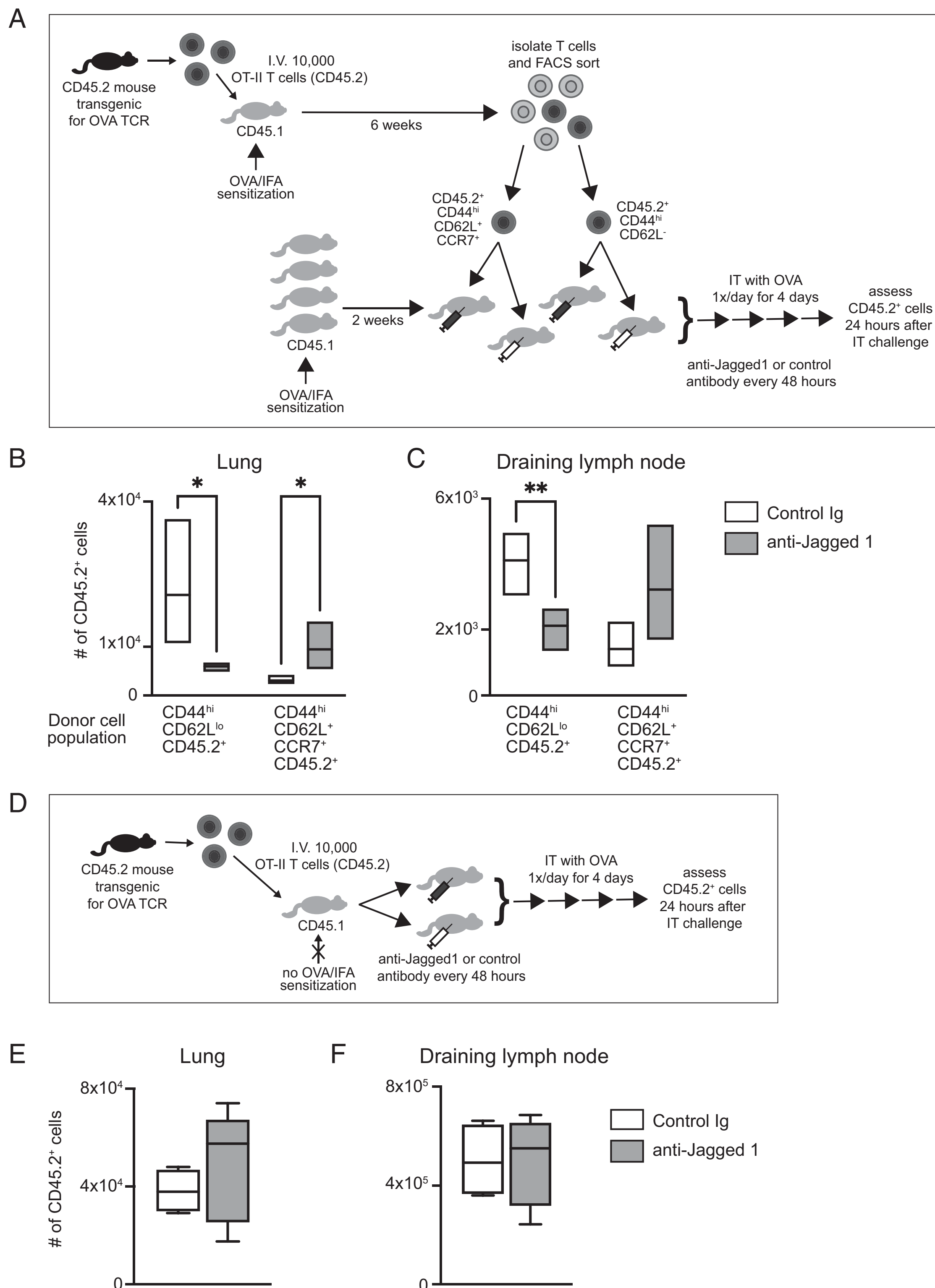

FIGURE 5. Effect of JAG1 blockade on expansion of CD4$^+$ T cell subsets.

(**A**) Schematic of the experimental model used in (**B**) and (**C**). (B and C) Quantification of Ag-experienced CD4$^+$CD45.2$^+$ OT-II cells from lung and lung-draining lymph node after i.v. transfer into OVA-sensitized mice that were subsequently challenged i.t. with OVA in the presence or absence of anti-JAG1 blockade. *$p \leq 0.05$ as determined by unpaired *t* test, $n = 4$–5 mice per group. (**D**) Diagram of the model used in (**E**) and (**F**). (E and F) Quantification of the number of CD4$^+$CD45.2$^+$ OT-II cells in the lung and lymph node of CD45.1 mice that received a transfer of naive CD45.2$^+$ OT-II T cells and were subsequently challenged i.t. with OVA in the presence or absence of anti-JAG1 blockade.

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DISCUSSION

Depletion of JAG1

Our work demonstrates that *Jag1* can successfully be depleted from CD11c^{+}CD11b^{+} DCs in both the spleen and the lung. Using flow cytometry in naive mice, we observed that our *Cd11c*$^{Cre+}$ strain depleted JAG1 on CD11c^{+}CD11b^{+} DCs but not macrophages, a result consistent with previous studies using this strain (18). We also observed that JAG1 on the surface of CD103^{+} CD11c^{+} DCs was the lowest of any of the myeloid cell subsets analyzed in naive mice (Fig. 1B). In OVA-sensitized mice we observed a significant reduction in the expression of the JAG1 gene by qPCR in *Jagged1*^{ff}*Cd11c*$^{Cre+}$ mice. In mice sensitized and challenged with OVA, genetic deletion of *Jag1* resulted in higher expression of *Muc5ac*, a gene associated with mucus production, and an increase in eosinophils in the lung at 48 h after the last i.t. challenge. The increase in *Muc5ac* and eosinophils was accompanied by an increase in the production of Th2 cytokines, including IL-4 and IL-5, from lung-draining lymph nodes (Fig. 2). These results replicate other studies where *Jag1* has been depleted or overexpressed in the context of an Th2 inflammatory response (10, 25).

JAG1–Notch interactions on T cells

Consistent with the hypothesis that interactions between a Notch receptor and a cognate ligand cause internalization of the receptor, we observed that T cell exposure to JAG1 reduced the amount of Notch 1 staining on the cell surface in vitro (Fig. 3) (22, 23). However, our data also demonstrate that rJAG1 can reduce the transcription of the *Notch1* gene, suggesting that there are multiple levels of regulation for this receptor upon interaction with JAG1. In vivo data using *Jagged1*^{ff}*Cd11c*$^{Cre+}$ mice also demonstrate that JAG1 is a regulator of Notch 1 receptor expression on the cell surface (Fig. 3D–G). Taken together, these data suggest that exposure to specific Notch ligands during Ag presentation may reduce the level of Notch receptor surface expression by the T cell, therefore influencing cell fate.

Because the level of Notch 1 on the surface of Ag-experienced CD4^{+} cells was increased over naive CD4^{+} cells, we reasoned that educated, Ag-specific OT-II cells would also have more surface-associated Notch receptors than the total pool of activated T cells. Our data in Fig. 4 confirm this hypothesis. However, we did not observe any change in cytokine production from Ag-specific cells when JAG1 was blocked during T cell stimulation. This suggests that, despite an overall increase in Th2 cytokines in our model, JAG1 does not directly impact cytokine production from individual Ag-specific T cells in this system.

To assess whether the increase in Th2-driven pathology observed in the absence of JAG1 was linked to alterations in T cell populations, we performed a series of transfer experiments to test the expansion of memory and effector T cell subsets in a model of OVA-driven inflammation. These experiments, inspired by our previous work with *Schistosoma mansoni* (16), were designed to determine whether Ag-specific memory and effector cells (26) have distinct proliferative responses to Notch ligand stimulation in the context of Th2 inflammation. In our model of OVA-driven inflammation, JAG1 blockade inhibits effector CD4^{+} T cell expansion and enhances memory CD4^{+} T cell expansion in a TCR-independent manner. Our data suggest that the increased Th2 immune response in *Jagged1*^{ff}*Cd11c*$^{Cre+}$ mice may be linked to the expansion of memory cells in these animals. This expanded population may produce more cytokines than the mix of memory and effector cells that normally contributes to a Th2 response.

JAG1 in the expansion of T cell subsets

Notch signaling in memory T cells has been demonstrated to enhance glucose uptake and cell survival in autoimmune disease (27). However, our data demonstrate that the interactions between Ag-specific memory T cells and APCs are more complex than simple stimulation through the Notch receptor. There are many factors to consider in this interaction: the T cell subset involved, the type of immune reaction, and the type of Notch ligand expressed on APCs during the inflammatory process. Bacterial and viral infections, known to induce DLL1/4, may have a different effect on Ag-specific memory and effector T cell expansion than JAG1. Our previous studies suggest that DLL4 blockade may increase effector cell expansion, which is in opposition to our findings with JAG1 (16). It is well documented that DLL4 and JAG1 signaling cause different cell fates in lymphocyte progenitors (12, 28) and peripheral T cells (29). Our findings suggest that memory and effector cell fate, as dictated by Notch, may also be context specific.

The expression of the Notch receptor itself may also act as a regulator of Notch signaling in the context of T cell activation. We observed consistent Notch 1 expression on CD4^{+} T cell subsets in the draining lymph node of OVA-challenged mice, but a dramatic reduction in Notch 1 receptor expression on naive and effector subsets in the lungs of these same mice (Fig. 3). In the spleen, Notch receptor expression on Ag-activated cells is lower than what was observed in the lung-draining lymph node (Figs. 3, 4). The difference in expression between spleen and lymph node may be caused by the recent administration of OVA Ag prior to analysis of lymph node cells.

It is difficult to justify why expansion of memory cells occurs in the absence of JAG1, whereas the population of effector cells with the same TCR would contract under these conditions. We can speculate that the low level of JAG1 expression on MHC class II^{+} cells, including tissue-resident CD103^{+} DCs and nonprofessional APCs in the lung such as type 2 alveolar epithelial cells, may favor the expansion of some subsets of CD4^{+} T cells over others. The encounter of a familiar Ag in an otherwise uninflamed organ would likely trigger rapid memory cell proliferation. However, encountering the same Ag in the context of an ongoing inflammatory process, complete with Ag presentation by recruited DCs, may favor effector cell proliferation with the goal of developing a new generation of memory cells that can prevent the same set of Ags from causing inflammation in the future. Although it may be undesirable to alter memory and effector cell populations during allergic inflammation, stimulating the proliferation

of memory cells during the sterile inflammation that accompanies cancer, which may also lack expression of DLL1/4, may be of benefit. Preliminary studies in this area indicate that increased JAG1 expression is associated with poor prognosis in head and neck cancers (30) and that a blockade of JAG1 is able to break T cell tolerance to cancer in mice (31).

DISCLOSURES

The authors have no financial conflicts of interest.

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