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SHORT COMMUNICATION

Dendritic cell expression of the Notch ligand *jagged2* is not essential for Th2 response induction *in vivo*

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We have addressed the hypothesis that Notch ligands play a decisive role in determining the ability of antigen-presenting cells to influence T cell polarization. Dendritic cells displayed distinct expression profiles of Delta and Jagged ligands for Notch when exposed to biologically relevant pathogen preparations associated with Th1 or Th2 responses. Expression of *delta4* was increased, and *jagged2* decreased, after dendritic cell exposure to the Th1-promoting bacterium *Propionibacterium acnes*. In contrast, soluble egg antigen (SEA) from the parasitic helminth *Schistosoma mansoni*, a potent Th2 inducer, failed to significantly alter dendritic cell expression of any of the Notch ligands measured. Irrespective of this, *jagged2*-deficient dendritic cells were severely impaired in their ability to instruct Th2 polarization of naive T cells *in vitro*. However, the ability of SEA-pulsed *jagged2*-deficient dendritic cells to induce a Th2 response *in vivo* was unimpaired relative to *jagged2*-sufficient dendritic cells. Further, *jagged2*-deficient dendritic cells activated by *P. acnes* exhibited no evidence of enhanced (or impaired) Th1 induction *in vivo*. These data suggest that, although involved in Th2 direction *in vitro*, *jagged2* is not fundamentally required for Th2 induction by SEA-activated dendritic cells *in vivo*.

Key words: Cell differentiation · Dendritic cells · Infectious diseases · Notch signalling · T helper cells

Introduction

Conserved throughout the metazoan kingdom, the Notch signalling pathway is remarkable for its extensive versatility, being utilized for exchanging amplification signals, determining cell lineages, and even inducing apoptosis [1, 2]. It has recently been suggested that a possible role for Notch signalling may be to provide a mechanism by which APC can influence T cell polarization [3–7], a situation that is complicated by the existence of four Notch receptors (Notch receptors 1–4) and five Notch ligands (Delta-like1, Delta-like3 and Delta-like4, and Jagged1 and Jagged2) in mammals [1].

Several lines of evidence suggest that signalling through Notch receptors has an effect on both T cell proliferation and mature T cell commitment [3, 4, 6, 8, 9]. In the context of $CD4^+$ T cell polarization, a recent *in vivo* study showed that blocking the signalling ability of Notch receptors 1–4 resulted in impaired Th2, but not Th1, responses [10]. In addition, studies *in vitro* have suggested that both Delta and Jagged ligand families may be associated with T cell differentiation but that Delta ligands promote Th1 whereas Jagged ligands promote Th2 polarization [4, 6, 11]. However, this contention remains controversial, with other reports suggesting that no such association exists, that Delta

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can inhibit T cell cytokine production rather than promote Th1 differentiation [5, 12], or that Jagged may induce Treg, rather than Th2, differentiation [5, 8, 9, 13–15]. Thus, although evidence is emerging that Notch has a function in immunity beyond its developmental role, the exact nature of Notch signalling and the relative contribution of the two ligand families in the adaptive immune response is far from clear [6, 16–18].

Dendritic cells (DC) are the most proficient APC at activating naive T cells. Their ability to dictate the tone of the developing $CD4^+$ T cell response is thought to be largely determined by the nature of the stimulus they encounter. However, the molecular mechanisms that are employed by DC to influence and instruct T cell polarization are not completely understood, particularly in the case of Th2 development [19].

In this study we first assessed whether the profile of expression of Notch ligands by DC was altered in response to Th1- or Th2-polarizing pathogens. We then used both *in vitro* and *in vivo* approaches to examine the direct effect of a specific Notch ligand deficiency in DC on polarization of transgenic and non-transgenic, naturally occurring polyclonal populations of T cells. We found that DC expression of the Notch ligand *jagged2* was unchanged in response to the Th2-associated pathogen *Schistosoma mansoni* and down-regulated in response to the Th1-associated pathogen *Propionibacterium acnes*, and that *jagged2*$^{-/-}$ DC were severely impaired in their ability to direct Th2 polarization of OVA-specific TCR-transgenic T cells *in vitro*. However, the ability of *jagged2*$^{-/-}$ DC to induce either Th2 or Th1 responses *in vivo* was unaffected. Thus, our results support an association of defined patterns of Notch ligand expression by DC responding to different T cell-polarizing pathogens, but suggest a redundant role for *jagged2* in T cell polarization by DC *in vivo*.

Results and discussion

DC express a defined pattern of expression of Notch ligands in response to diverse pathogens

Previous work has shown that stimulation with the Th1-associated bacterial product LPS resulted in substantial up-regulation of Delta by DC, while Th2-associated molecules such as prostaglandin E_2 and cholera toxin promoted Jagged expression [6]. We used two pathogen preparations that are well-characterized as being able to drive either Th1 (heat-killed *P. acnes*, Pa) or Th2 (soluble egg antigen from *S. mansoni*, SEA) induction *via* DC [20, 21] to determine whether we could see any clear pattern of Notch ligand expression associated with either type of stimulus. Over a 12-h time course, DC were activated by these stimuli in a manner consistent with previously published reports [20, 21]. Pa induced DC maturation, provoking up-regulation of MHC class II and co-stimulatory molecules (data not shown), as well as secretion of a range of cytokines including IL-12, IL-6, TNF and IL-10 (Fig. 1A). In contrast, and in keeping with previously published reports [22], SEA-activated DC showed little evidence of phenotypic maturation (data not shown) or cytokine secretion (Fig. 1A) when compared to unstimulated cells.

The influence of the same stimuli on Notch ligand expression by DC was determined using quantitative PCR to measure mRNA levels 6 and 12 h after stimulation (Fig. 1B–D). *delta4* expression was significantly increased over unstimulated control levels in DC activated with Pa ($p<0.03$; Fig. 1B), and this was evident from 6 h post-stimulation. In contrast to this, although showing a trend for elevated expression by 6 h that returned to control levels by 12 h post-stimulation, *delta1* was not significantly increased in response to Pa. Although expression of *jagged1* was unchanged in DC responding to Pa, *jagged2* expression showed a different profile, being strikingly decreased by 6 h post-stimulation ($p<0.001$; Fig. 1B), and returning to a similar level to unstimulated cells by 12 h.

Contrary to the dramatic up-regulation that was evident in response to Pa, expression of *delta4* was not significantly altered in DC exposed to SEA (Fig. 1B). Further, *delta1*, *jagged1*, and *jagged2* were maintained at equivalent levels to unstimulated controls in SEA-activated DC at both 6 and 12 h time points. *delta3* expression in response to either Pa or SEA failed to show a consistent pattern in any of the experiments carried out (data not shown). Although SEA failed to have a marked impact on Notch ligand expression, exposure to SEA resulted in DC that express higher levels of *jagged2* relative to *delta4*, whereas exposure to Pa resulted in DC with the converse phenotype, expressing higher levels of *delta4* relative to *jagged2* (Fig. 1B).

These data suggest that DC expression of a restricted cohort of Notch ligands can be associated with pathogens that induce distinct Th responses. However, they also reveal that the initial description of Jagged and Delta ligands as being Th2- or Th1-associated, respectively, is an oversimplification, and that expression of related members within the same ligand family does not appear to be regulated identically.

Th2 and Th1 responses are capably induced *in vivo* by *jagged2*$^{-/-}$ DC

jagged2 deficiency is embryonic lethal [23]. In order to address the role of expression of *jagged2* in BMDC development and Th induction, *jagged2*$^{-/-}$ chimeras were generated by reconstituting irradiated congenic $Ly5.1^+$ C57BL/6 mice with fetal liver from $Ly5.2^+$ *jagged2*$^{-/-}$ (or *jagged2*$^{+/+}$ control) embryos. DC were then grown from BM isolated from *jagged2*$^{-/-}$ or control chimeras, pulsed overnight with either Pa or SEA, and their activation status compared. The absence of *jagged2* did not significantly affect growth, development or activation of the DC *in vitro* (Fig. 2). Secretion of cytokines (Fig. 2A) and expression of co-stimulatory molecules (Fig. 2B) was similar for both control and *jagged2*$^{-/-}$ DC in response to SEA or Pa. Further, *jagged2*$^{-/-}$ DC did not compensate for *jagged2* deficiency by up-regulating expression of *jagged1*, *delta1*, or *delta4* in response to either stimulus (Fig. 2C). Thus, *jagged2*$^{-/-}$ DC appeared similar to their wild-type counterparts other than lacking *jagged2* expression.

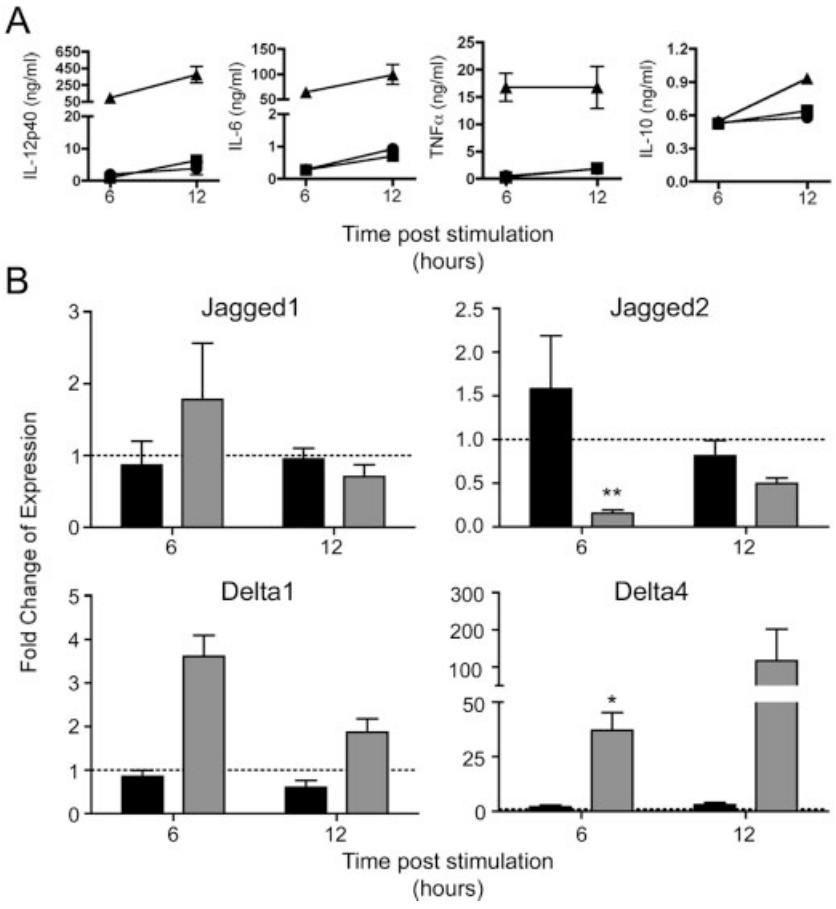

Figure 1. DC response to SEA or Pa. (A) DC were treated for up to 12 h with either medium alone (squares), 25 μg/mL SEA (circles), or 10 μg/mL Pa (triangles) in 24-well plates. Cytokines were measured in culture supernatants. Data shown indicate mean ± SEM of cytokine measured by ELISA of duplicate wells, and are representative of five separate experiments. (B) Notch ligand expression by DC in response to Pa or SEA as detailed in (A) was measured by quantitative PCR. Expression of *delta* and *jagged* mRNA was normalised to 18S RNA. Fold change of Notch ligand expression relative to unstimulated cells (dotted lines) by DC exposed to SEA (black bars) or Pa (grey bars) is shown. Data are mean + SEM of three to six combined experiments; $^{*}p<0.03$, $^{**}p<0.001$, comparing expression by SEA- or Pa-stimulated groups relative to medium controls.

We have previously shown that although DC activated with SEA display a muted activation phenotype, they remain potent inducers of a Th2 response both *in vivo* and *in vitro* [22]. While the exact mechanism by which this occurs remains unclear, Th2 induction by *in vivo* transfer of SEA-stimulated DC is an active process requiring DC expression of MHC class II, CD40, and NF-κB1 [22]. We first assessed the ability of *jagged2*-deficient DC to activate and polarize OVA-specific OTII TCR-transgenic T cells *in vitro*. Although equally proficient at stimulating T cell proliferation (Fig. 3A), *jagged2*$^{-/-}$ DC displayed a striking impairment in their ability to provoke Th2 polarization *in vitro*, in comparison to their *jagged2*$^{+/+}$ counterparts (Fig. 3B).

We then asked whether expression of *jagged2* was also important for polarization of Th cells by DC in a more complex *in vivo* setting. *jagged2*$^{+/+}$ or *jagged2*$^{-/-}$ DC were activated with Pa or SEA and then injected into the footpads of naive C57BL/6 mice. Four days later draining LN were removed and examined for cytokine secretion following restimulation with antigen *in vitro* (Fig. 3C).

Pa-activated DC induced a marked Th1 response with high Pa-specific IFN-γ levels detected in culture supernatants irrespective of whether transferred DC were *jagged2*$^{+/+}$ or *jagged2*$^{-/-}$ (Fig. 3C). Contrary to expectation, SEA-stimulated *jagged2*$^{-/-}$ DC induced an equivalent or higher IL-4 response after transfer into naive wild-type animals (Fig. 3C). This was irrespective of the route of immunization as *jagged2*$^{+/+}$ and *jagged2*$^{-/-}$ DC given i.p. also showed equivalent ability to induce a Th2 response whether measured by IL-4 (data not shown) or IL-5, IL-13, and IL-10 (Fig. 3D). Thus, despite the fact that DC maintain expression of *jagged2* after exposure to SEA *in vitro* (Fig. 1B), and *jagged2*$^{-/-}$ DC are severely impaired in their ability to instruct Th2 polarization *in vitro* (Fig. 3B), our data suggest that this Notch ligand is not essential for the establishment of a Th2 response *in vivo*.

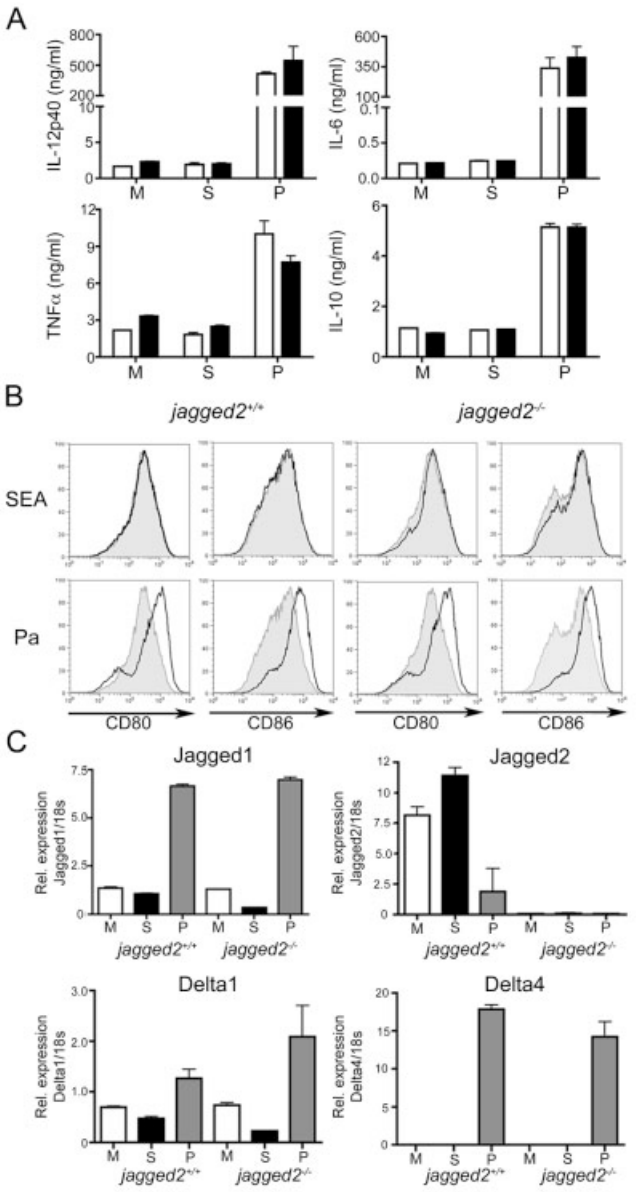

Figure 2. Deficiency in *jagged2* does not interfere with BMDC generation or activation. BMDC were grown from BM derived from *jagged2*$^{+/+}$ or *jagged2*$^{-/-}$ chimeras and exposed to either medium alone (M), SEA (S, 25 μg/mL) or Pa (P, 10 μg/mL) for 24 h. (A) Cytokine production by *jagged2*$^{+/+}$ (open bars) or *jagged2*$^{-/-}$ (black bars) DC. Data shown are mean + SEM of cytokine measured by ELISA of duplicate wells and are representative of two separate experiments. (B) Surface expression of CD80 and CD86 on *jagged2*$^{+/+}$ or *jagged2*$^{-/-}$ DC. Light grey filled histograms indicate unstimulated DC, black unfilled histograms indicate DC stimulated with SEA or Pa. Graphs are representative of two separate experiments. (C) Expression of *jagged1*, *jagged2*, *delta1*, and *delta4* by DC exposed to medium (open bars), Pa (grey bars), or SEA (black bars) was assessed by quantitative PCR. Data shown are mean + SEM of expression levels measured in duplicate relative to 18S, and are representative of five separate experiments.

Concluding remarks

Studies examining the effect of wholesale inhibition of Notch signalling to T cells [6, 10, 15] have demonstrated that signalling through Notch receptors is required for the establishment of an effective T cell response. However, the mechanism by which Notch signalling translates into polarized CD4$^+$ T cell differentiation remains unclear. Tu *et al.* [10] described how inhibition of Notch signalling using dominant-negative MAML-transgenic mice during live infections of either *Leishmania major* or *Trichuris muris* resulted in a fully capable *L. major* Th1 response but an impaired Th2 response and ineffective clearance of *T. muris*. This suggests that in complete biological systems Notch signalling is required for Th2 establishment, but does not reveal which ligands are required to initiate this immunological response *via* Notch, or indeed whether APC expression of Notch ligands is required.

As shown by our expression data, Delta and Jagged ligands are expressed concurrently by DC in either Th1- or Th2-priming conditions, but their relative expression changes dramatically depending upon the stimulus encountered. Conceivably the ratio of different Notch ligands expressed by APC may ultimately determine the manner in which Notch signalling affects the differentiation of a naive T cell. Our results clearly show that DC expression of *jagged2* alone can be critical for Th2 polarization of naive CD4$^+$ T cells *in vitro* (Fig. 3B). The presence of the other Notch ligands (Fig. 2C) could not compensate for the absence of *jagged2* to enable Th2 polarization *in vitro* (Fig. 3B), indicating that *jagged1*, *delta1*, and *delta4* cannot fulfil the signalling requirement by Notch receptors on CD4$^+$ T cells to establish a Th2 response *in vitro*.

In stark contrast to this, in the *in vivo* setting, DC expression of *jagged2* was dispensable for Th2 induction (Fig. 3C, D). Taken together, our *in vitro* and *in vivo* results suggest that *jagged2* signalling is important for Th2 polarization, yet Jagged2 expression need not be limited to the APC driving the response. For example, the important interaction *in vivo* may not actually be between a Notch ligand-bearing APC and a naive CD4$^+$ T cell, but rather between APC and NKT cells, or a subset of memory CD4$^+$ T cells capable of producing IL-4 rapidly and independently of STAT6 [24, 25]. It is therefore possible that *in vivo* the multivariant expression of Notch ligands by APC involves interactions with multiple cell types since both Notch receptors and ligands can be found in diverse cell types in addition to T cells and DC [6, 26].

Our *in vivo* experiments, in which all such cells are present, reveal that DC expression of the Notch ligand *jagged2* plays no major role during SEA-specific Th2 response induction. *jagged2* deficiency impaired neither DC generation nor activation, with *jagged2*$^{-/-}$ DC producing equivalent levels of cytokine and expressing the same levels of surface markers as similarly stimulated *jagged2*$^{+/+}$ controls. Furthermore, transfer of either *jagged2*$^{+/+}$ or *jagged2*$^{-/-}$ SEA-activated DC resulted in production of similar quantities of SEA-specific IL-4, IL-5, IL-10, and IL-13 in recipient mice. Whether this finding is indicative of the provision of *jagged2* by additional cell types, a redundant pathway, or compensation *via* other Notch ligands remains to be determined.

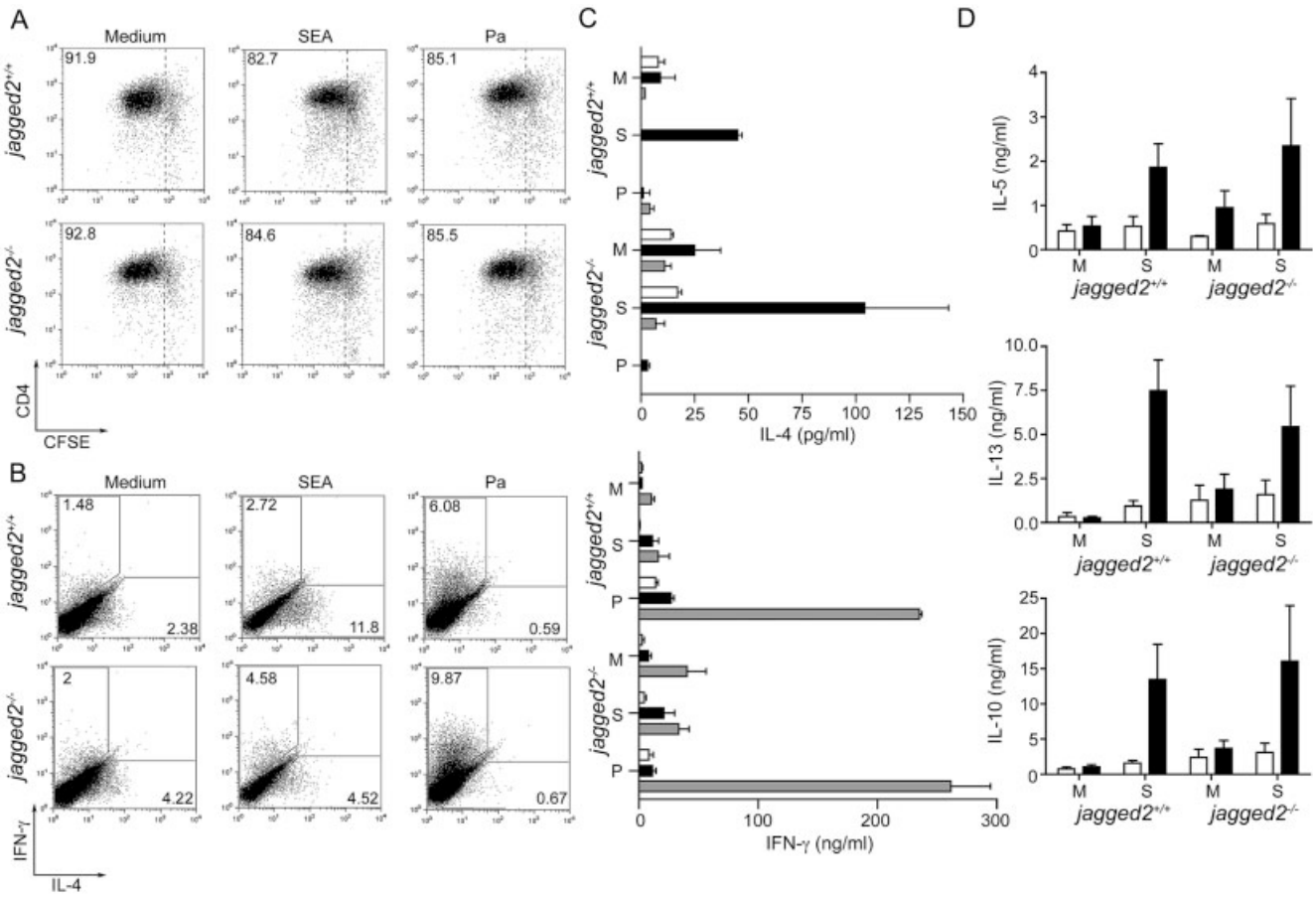

Figure 3. DC expression of *jagged2* is dispensable for Th2 or Th1 induction *in vivo*. (A, B) CD4-purified OTII cells were co-cultured with *jagged2*$^{+/+}$ or *jagged2*$^{-/-}$ DC in the presence of $OVA_{323–339}$ peptide (50 ng/mL) alone (medium) or in conjunction with SEA (50 μg/mL) or Pa (10 μg/mL). OTII cells were labelled with CD4 and CFSE to assess proliferation (A) or were examined for intracellular staining (B). Dotted lines in (A) represent CFSE levels on cells cultured in the absence of peptide. Plots depicting intracellular staining in (B) are gated on live CD4 cells. Figures refer to percentage of dividing cells (A) or cytokine-producing cells (B) and results are representative of three independent experiments. (C, D) *jagged2*$^{+/+}$ or *jagged2*$^{-/-}$ DC were exposed to medium alone (M), Pa (P, 10 μg/mL), or SEA (S, 25 μg/mL), then injected into the footpad (C) or i.p. (D) of naive C57BL/6 recipient mice. Popliteal LN cells (C) or splenocytes (D) were removed 4 days (C) or 7 days (D) later and then stimulated *in vitro* with medium (open bars), SEA (black bars), or Pa (grey bars). Data shown are mean + SD of cytokine measured by ELISA of triplicate wells of combined LN cells (C) or mean + SEM of cytokine measured by ELISA of three to five mice per group (D), and are representative of three (C) or two (D) separate experiments.

Even so, these results challenge the model that selective expression of Jagged ligands by DC is responsible for determining Th2 differentiation.

Materials and methods

Animals and reagents

C57BL/6 mice were bred and maintained in the animal facilities at the University of Edinburgh or at Cancer Research UK. A heat-killed preparation of the Gram$^+$ bacterium *P. acnes* (ATCC No. 6919) was used as a Th1 stimulus, while SEA (prepared in-house [20]) was used for its Th2-driving capacity. Animal work was carried out under UK Home Office Project license, and was approved locally by Ethical Review Committee.

Dendritic cell culture

Murine BMDC were generated in the presence of rGM-CSF (Peprotech, London, UK) as previously described [20]. DC were stimulated with 25 μg/mL SEA or 10 μg/mL Pa (measured by Bradford assay) over a time course, supernatants assessed for cytokine levels by ELISA, and cells harvested for RNA extraction.

Generation of chimeric bone marrow

Fetal livers from Ly5.2$^+$ *jagged2*$^{+/-}$ × *jagged2*$^{+/-}$ matings were removed from day-14.5 embryos, and *jagged2*$^{-/-}$ fetuses were identified by PCR [23]. Irradiated Ly5.1$^+$ recipients were reconstituted with cells from *jagged2*$^{-/-}$ or *jagged2*$^{+/+}$ womb mates. DC were grown from BM 8–20 wk later. Donor origin of DC was verified by flow cytometry (data not shown).

Determination of DC activation status

Cytokine levels were measured in DC supernatants by ELISA using commercial mAb (BD Pharmingen or R&D Systems). Phenotype was assessed by flow cytometry using mAb for CD11c, CD80 and CD86 (Pharmingen). Samples were acquired by FACSCalibur using CellQuest software and analysed using FlowJo software (TreeStar, Ashland, OR).

Assessment of DC priming ability *in vitro*

$CD4^+$ T cells were purified from the spleen and LN of OTII mice by positive selection using magnetic sorting (Miltenyi). For studies measuring proliferation, OTII $CD4^+$ cells were stained with carboxyfluoroscein succinimidyl ester (CFSE, 5 μM). $CD4^+$ cells (2×10^5) were co-cultured for 3 days with 2×10^4 *jagged2*$^{+/+}$ or *jagged2*$^{-/-}$ DC in the presence of $OVA_{323–339}$ peptide (50 ng/mL) alone or in conjunction with SEA (50 μg/mL) or Pa (10 μg/mL). On day 3, CFSE-labelled $CD4^+$ cells were washed, stained with anti-CD4-allophycocyanin (Pharmingen), then assessed by flow cytometry as described above. For measurement of intracellular cytokine, non-CFSE-labelled $CD4^+$ cells were stimulated for 4 h with PMA (10 ng/mL), ionomycin (1 μg/mL), and Brefeldin A (10 μg/mL). Cells were then washed and stained with anti-CD4-allophycocyanin before being fixed using Cytofix/Cytoperm (BD Pharmingen), according to manufacturer's protocol. Intracellular cytokines were labelled with anti-IFN-γ-FITC and anti-IL-4-PE (both from Pharmingen).

Assessment of DC priming ability *in vivo*

DC activated overnight with either SEA or Pa were injected into mice (2×10^5 per footpad or 3×10^5 i.p.). In some experiments, DC were also pulsed with OVA peptide (data not shown). Cell suspensions were prepared from spleens removed 7 days after i.p. DC transfer, or popliteal LN cells 4 days after footpad injection. In some experiments, mice received 4×10^6 OTII cells 1 day previously. Spleen and LN cells were cultured in X-Vivo 15™ serum free medium (Cambrex Bio Science, Wokingham, UK) with 2 mM L-glutamine (Gibco, Paisley, UK) and 50 μM 2-ME (Sigma, Poole, UK) without or with SEA at a final concentration of 15 μg/mL, Pa at 1 μg/mL, or OVA peptide at 1 μM. Supernatants were harvested after 72 h (splenocytes) or 48 h (LN cells) for cytokine analysis by ELISA. Results did not differ significantly when transferred DC had been activated with SEA or Pa for 6 h rather than overnight (data not shown). In OTII co-transfer experiments, SEA was not found to consistently act as an adjuvant for OVA peptide-specific IL-4 production (data not shown).

Determination of Notch ligand expression

Total RNA was extracted from 1×10^6 DC using Trizol (Invitrogen) and cDNA generated using Reverse Transcriptase System with random hexamers (Promega, UK). Notch ligand expression was assessed by quantitative PCR using SYBR green (Invitrogen), a Chromo4 detector and Opticon Monitor software (MJ Research). Relative expression values were calculated by dividing the acquired expression quantity for the gene of interest using SYBR by the expression quantity of 18S rRNA, and using a serially diluted standard of pooled cDNA or using the $2^{-\Delta\Delta C(t)}$ method. Primers used were (5′–3′): murine *jagged2* forward GTCGTCATTCCCTTTCAGTTCG, reverse AGTTCTCATCACAGCGTACTCG; murine *jagged1* forward GCAACGACCGTAATCGCATC, reverse TGCCTGAGTGAGAAGCCTTTTC; murine *delta4* forward AGGTGCCACTTCGGTTACACAG, reverse CAATCACACACTCGTTCCTCTCTTC; *delta1* forward GCACTACTACGGAGAAGGTTGCTC, reverse TCACACCCTGGAGACAGATTG; 18S forward GTAACCCGTTGAACCCCATT, reverse CCATCCAATCGGTAGTAGCG.

Statistical analysis

The one-sample *t*-test was used to determine whether means significantly differed in comparison to a standardized control value.

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Conflict of interest: The authors declare no financial or commercial conflict of interest.

References

1 **Artavanis-Tsakonas, S., Rand, M. D. and Lake, R. J.,** Notch signaling: Cell fate control and signal integration in development. *Science* 1999. **284:** 770–776.

2 **Osborne, B. and Miele, L.,** Notch and the immune system. *Immunity* 1999. **11:** 653–663.

3 **Hoyne, G. F., Dallman, M. J., Champion, B. R. and Lamb, J. R.,** Notch signalling in the regulation of peripheral immunity. *Immunol. Rev.* 2001. **182:** 215–227.

4 **Maekawa, Y., Tsukumo, S., Chiba, S., Hirai, H., Hayashi, Y., Okada, H., Kishihara, K. and Yasutomo, K.,** Delta1-Notch3 interactions bias the functional differentiation of activated $CD4^+$ T cells. *Immunity* 2003. **19:** 549–559.

5 **Wong, K. K., Carpenter, M. J., Young, L. L., Walker, S. J., McKenzie, G., Rust, A. J., Ward, G. *et al.*,** Notch ligation by Delta1 inhibits peripheral immune responses to transplantation antigens by a $CD8^+$ cell-dependent mechanism. *J. Clin. Invest.* 2003. **112:** 1741–1750.

6 **Amsen, D., Blander, J. M., Lee, G. R., Tanigaki, K., Honjo, T. and Flavell, R. A.,** Instruction of distinct CD4 T helper cell fates by different notch ligands on antigen-presenting cells. *Cell* 2004. **117:** 515–526.

7 **Osborne, B. A. and Minter, L. M.,** Notch signalling during peripheral T-cell activation and differentiation. *Nat. Rev. Immunol.* 2007. **7:** 64–75.

8 **Hoyne, G. F., Le Roux, I., Corsin-Jimenez, M., Tan, K., Dunne, J., Forsyth, L. M., Dallman, M. J. *et al.*,** Serrate1-induced notch signalling regulates the decision between immunity and tolerance made by peripheral CD4(+) T cells. *Int. Immunol.* 2000. **12:** 177–185.

9 **Vigouroux, S., Yvon, E., Wagner, H. J., Biagi, E., Dotti, G., Sili, U., Lira, C. *et al.*,** Induction of antigen-specific regulatory T cells following overexpression of a Notch ligand by human B lymphocytes. *J. Virol.* 2003. **77:** 10872–10880.

10 **Tu, L., Fang, T. C., Artis, D., Shestova, O., Pross, S. E., Maillard, I. and Pear, W. S.,** Notch signaling is an important regulator of type 2 immunity. *J. Exp. Med.* 2005. **202:** 1037–1042.

11 **Napolitani, G., Rinaldi, A., Bertoni, F., Sallusto, F. and Lanzavecchia, A.,** Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. *Nat. Immunol.* 2005. **6:** 769–776.

12 **Stallwood, Y., Briend, E., Ray, K. M., Ward, G. A., Smith, B. J., Nye, E., Champion, B. R. and McKenzie, G. J.,** Small interfering RNA-mediated knockdown of notch ligands in primary $CD4^+$ T cells and dendritic cells enhances cytokine production. *J. Immunol.* 2006. **177:** 885–895.

13 **Yvon, E. S., Vigouroux, S., Rousseau, R. F., Biagi, E., Amrolia, P., Dotti, G., Wagner, H. J. and Brenner, M. K.,** Overexpression of the Notch ligand, Jagged-1, induces alloantigen-specific human regulatory T cells. *Blood* 2003. **102:** 3815–3821.

14 **Dallman, M. J., Champion, B. and Lamb, J. R.,** Notch signalling in the peripheral immune system. *Novartis Found Symp.* 2003. **252:** 268–276.

15 **Minter, L. M., Turley, D. M., Das, P., Shin, H. M., Joshi, I., Lawlor, R. G., Cho, O. H. *et al.*,** Inhibitors of gamma-secretase block *in vivo* and *in vitro* T helper type 1 polarization by preventing Notch upregulation of Tbx21. *Nat. Immunol.* 2005. **6:** 680–688.

16 **Eagar, T. N., Tang, Q., Wolfe, M., He, Y., Pear, W. S. and Bluestone, J. A.,** Notch 1 signaling regulates peripheral T cell activation. *Immunity* 2004. **20:** 407–415.

17 **Hoyne, G. F., Dallman, M. J. and Lamb, J. R.,** T-cell regulation of peripheral tolerance and immunity: The potential role for Notch signalling. *Immunology* 2000. **100:** 281–288.

18 **Tsukumo, S. and Yasutomo, K.,** Notch governing mature T cell differentiation. *J. Immunol.* 2004. **173:** 7109–7113.

19 **Reis e Sousa, C.,** Dendritic cells in a mature age. *Nat. Rev. Immunol.* 2006. **6:** 476–483.

20 **MacDonald, A. S., Straw, A. D., Bauman, B. and Pearce, E. J.,** $CD8^-$ dendritic cell activation status plays an integral role in influencing Th2 response development. *J. Immunol.* 2001. **167:** 1982–1988.

21 **Cervi, L., MacDonald, A. S., Kane, C., Dzierszinski, F. and Pearce, E. J.,** Cutting edge: Dendritic cells copulsed with microbial and helminth antigens undergo modified maturation, segregate the antigens to distinct intracellular compartments, and concurrently induce microbe-specific Th1 and helminth-specific Th2 responses. *J. Immunol.* 2004. **172:** 2016–2020.

22 **Perona-Wright, G., Jenkins, S. J. and MacDonald, A. S.,** Dendritic cell activation and function in response to *Schistosoma mansoni*. *Int. J. Parasitol.* 2006. **36:** 711–721.

23 **Jiang, R., Lan, Y., Chapman, H. D., Shawber, C., Norton, C. R., Serreze, D. V., Weinmaster, G. and Gridley, T.,** Defects in limb, craniofacial, and thymic development in Jagged2 mutant mice. *Genes Dev.* 1998. **12:** 1046–1057.

24 **Tanaka, S., Tsukada, J., Suzuki, W., Hayashi, K., Tanigaki, K., Tsuji, M., Inoue, H. *et al.*,** The interleukin-4 enhancer CNS-2 is regulated by Notch signals and controls initial expression in NKT cells and memory-type CD4 T cells. *Immunity* 2006. **24:** 689–701.

25 **van der Vliet, H. J., Nishi, N., Koezuka, Y., von Blomberg, B. M., van den Eertwegh, A. J., Porcelli, S. A., Pinedo, H. M. *et al.*,** Potent expansion of human natural killer T cells using alpha-galactosylceramide (KRN7000)-loaded monocyte-derived dendritic cells, cultured in the presence of IL-7 and IL-15. *J. Immunol. Methods* 2001. **247:** 61–72.

26 **Baron, M.,** An overview of the Notch signalling pathway. *Semin. Cell Dev. Biol.* 2003. **14:** 113–119.

Abbreviations: **Pa:** heat-killed *Propionibacterium acnes* · **SEA:** soluble egg antigen from *Schistosoma mansoni*

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