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Suppression of Th2 Cell Development by Notch Ligands Delta1 and Delta4

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Notch signaling plays important roles in Th cell activation. We show that in response to TLR ligation, dendritic cells up-regulate expression of Notch ligands Delta1 and Delta4 via a MyD88-dependent pathway. Expression of Delta1 or Delta4 by dendritic cells enhanced their ability to activate naive Th cells and promote Th1 cell development, and allowed them to strongly inhibit Th2 cell development. Promotion of Th1 cell development was dependent on IFN-γ and T-bet expression by responding Th cells. However, the inhibition of Th2 cell development occurred independently of IFN-γ or T-bet, and resulted from a block in IL-4-initiated commitment to the Th2 lineage. The promotion of Th1 cell development by Delta is not a reflection of the delivery of pro-Th1 instructional signal, but rather it is the result of a block in the downstream effects initiated by IL-4 signaling. The Journal of Immunology, 2008, 180: 1655–1661.

Following activation, CD4 T cells can differentiate into Th1, Th2, Th17, or regulatory T cells (1–3). The appropriate polarization of Th responses during infection is critical for host immunity. Multiple factors, including dendritic cell (DC) type, Ag type and dose, cytokine milieu, and costimulatory signals, have been implicated in this decision process (4).

Recent evidence has highlighted the role of Notch pathways in peripheral Th cell activation and differentiation (5–8). Notch signaling is an evolutionarily conserved pathway involved in cell fate choice during development. In mammalian cells, there are four Notch receptors (Notch1–4) and five Notch ligands (Jagged1, Jagged2, Delta1, Delta3, and Delta4). Upon Notch ligand–receptor interactions, the intracellular domain of Notch (NICD) is cleaved by γ-secretases, released from the membrane, and translocated to the nucleus, where it complexes with CSL/RBP-Jκ/MAML/p300 to act as a transcriptional activator (5). It has been proposed that differential expression of Notch ligands by DCs in response to exposure to different classes of pathogens underlies the ability of DCs to promote pathogen-appropriate Th responses, with Jagged and Delta promoting Th2 and Th1 differentiation, respectively (7). Consistent with the view that Delta promotes Th1 responses, Delta1-Fc and Delta4-Fc fusion proteins have been shown to promote Th1 cell development (9, 10). However, questions remain as to the exact roles of Notch signaling in Th response polarization. Using a γ-secretase inhibitor, Osborne and colleagues showed that Notch signaling is required specifically for Th1 cell differentiation (11). In contrast, using dominant negative MAML transgenic mice, Pear and colleagues showed that Notch signaling was required only for Th2 cell responses (12). The role for Notch signaling in Th2 cell development was emphasized by recent reports showing that Notch-mediated binding of RBPKκ to the GATA-3 promoter enhances GATA-3 expression, and therefore Th2 commitment, in the absence of IL-4 (13, 14).

We herein report that Delta1 and Delta4 are up-regulated on bone marrow-derived DCs in response to TLR-mediated activation, a process that has been suggested to be critical for Th1 response development (15), and which we have found to inhibit Th2 cell development (16, 17). We show that Delta4 protein, and Delta1 and Delta4 expressing DCs, profoundly inhibit Th2 cell development, and that this is likely to underlie the emergence of Th1 cells following immune priming in the presence of Delta.

Materials and Methods

Animals and reagents

C57BL/6 (B6) mice, BALB/c, and B6 IL-12 p40−/− mice were purchased from The Jackson Laboratory. OTII, IL4−/−, OTII, T-bet−/−, OTII, and 4get/DO.11 transgenic mice were bred and maintained under specific pathogen-free conditions at the University of Pennsylvania under approved Institutional Animal Care and Use Committee protocols. Listeriolysin O (LLO)190–201 (NEKYAQAYPNVS 18) was synthesized by Invitrogen. Heat-killed Propionibacterium acnes was purchased from The Van Kampen Group. CpG ODN 1826 was obtained from Coley Pharmaceutical Group. Delta4 protein and all mouse cytokines were recombinant and were provided by Dr. Antonia Freitas at the Pasteur Institute (19). Delta1 and Delta4 were then subcloned into MSCV-IRES-hCD8 vector. Empty (vector) or recombinant (Delta1 and Delta4) retrovirus was obtained after the transfection of 293T packaging cells using Lipofectamine (Invitrogen). Retrovirus-containing supernatants were collected 48 h after transfection and used for spin infection (2,500 rpm, 2 h) of day 1 IL-12−/− bone marrow dendritic cell cultures in 6-well plates (20). After an additional 7 days in culture with GM-CSF, DCs were harvested and transduction efficiency
was assessed using anti-human CD8 Ab and flow cytometry; we typically obtained transduction rates >80%.

**DC preparation and DC/CD4 T cell coculture**

On day 8, control DCs or transduced DCs were harvested and pulsed overnight with OVA (1 mg/ml) with or without *P. aeruginosa* (10 μg/ml), LPS (200 ng/ml), or CpG (1 μg/ml) in DC media (RPMI 1640 (Mediatech) supplemented with 10% heat-inactivated and filtered FCS (HyClone), 2 mM L-glutamine (Mediatech), 100 U/ml penicillin plus 100 μg/ml streptomycin (Mediatech), and 50 μM 2-mercaptoethanol (Mediatech)). DCs were then washed and mixed with OTII TCR transgenic CD4 T cells that had been negatively selected by MACS (Miltenyi Biotech). Where necessary, anti-IL-4 mAb 1B11 or anti-IFN-γ mAb XMG6 was added at 20 and 50 ng/ml, respectively, every 2 days to achieve the complete neutralization of cytokine activity. In experiments using recombinant Delta4 protein, plates were coated with 10 μg/ml recombinant proteins for 3 h at 37°C and then washed twice with PBS before using. We used a ratio of 2 × 10^5 DCs to 2 × 10^5 CD4 T cells per well of a round-bottom, 96-well plate, and used medium that contained 30 U/ml human IL-2, as described (16). In some experiments, OTII CD4 T cells were labeled with CFSE (Invitrogen: Molecular Probes) before mixing with DCs (21). After different times of culture, cells were stimulated with PMA and ionomycin in the presence of Golgi-Plug, fixed, and permeabilized, and intracellular cytokine staining was performed using cytokine-specific fluorochrome-labeled mAbs and flow cytometry, as described (16). Staining for intracellular T-bet and GATA-3 was based on a previously described protocol (17, 22).

**Real-time RT-PCR**

To measure Notch ligand expression, DCs were pulsed with different stimuli, as described above, for 6 h before RNA extraction. For cytokine expression, CD4 T cells were harvested from tissue culture plates at 36 h and stained with biotinylated anti-CD69 mAb. CD69+ cells, which comprised the activated CD4 T cells, were purified using anti-biotin microbeads (Miltenyi Biotech) for RNA extraction. RNA was extracted using RNeasy (QiaGen) and treated with DNase I (Invitrogen). Oligo(dT) (Promega) and Superscript II (Invitrogen) were used to synthesize first-strand cDNAs (Qiagen) and treated with DNase I (Invitrogen). Data were normalized to hypoxanthin phosphoribosyltransferase. Sequences of primers used in the studies are available on request.

**Th response priming in vivo**

Vector-, Delta1-, or Delta4-transduced DCs were activated in vitro with IFN-γ (6,000 U/ml, R&D Systems) and incubated overnight with or without LLO_190 peptide (10 μg/ml). All DCs were washed twice in HBSS to remove excess Ags and FCS, resuspended in HBSS, and injected i.p. via a 25-gauge needle into naive mice (5 × 10^6 DCs/mouse). After 7 days, splenocytes from the injected mice were suspended at 10^7/ml and cultured in media with or without LLO_190 peptide (1 μg/ml), and with human IL-2 (50 U/ml) plus Golgi-Plug (1 μl/ml, Pharmingen) for 6 h, after which cells were fixed and permeabilized for the detection of intracellular cytokines by flow cytometry.

**Results**

**Delta is expressed by DCs in response to TLR ligands and inhibits Th2 cell development**

It has been reported that the differential expression of Notch ligands Jagged and Delta promotes Th2 and Th1 cell differentiation, respectively (7). Consistent with reported findings that LPS in-

![Image](http://www.jimmunol.org/)

**FIGURE 1.** Delta4 is up-regulated on DCs by activation through TLRs and inhibits Th2 cell development. **A**. Wild-type DCs or MyD88^−/−^ DCs were left unstimulated in media (Media) or stimulated with *P. aeruginosa* or CpG. Six hours later, RNA was extracted and real-time RT-PCR was performed to measure Delta4 mRNA levels. The means and SEs of expression from two experiments are shown. **B**. OTII CD4 cells were stimulated with OVA-pulsed IL-12^−/−^ DCs (OVA/DC) in the absence or presence of recombinant Delta4 protein. Four days later, CD4 cells were restimulated with PMA/ionomycin in the presence of Golgi-Plug, and intracellular staining was performed to detect IL-4-producing cells. Numbers indicate the percentages of cytokine-positive cells within gates. Data are from one experiment (and are typical of three experiments).

**Delta expression by DCs promotes T cell IL-2 production and proliferation**

To begin investigating the role of Delta on Th cell priming in greater detail, we developed a Delta4 retroviral construct and a procedure for transducing DCs without activating them. We additionally constructed a Delta1 retrovirus, because we found that TLR stimulation of DCs strongly promotes Delta1 as well as Delta 4 expression (data not shown). After 4 days in culture, we restimulated the T cells with PMA/ionomycin in the presence of Golgi-Plug and stained for intracellular IL-4. As expected from our previous work (16), we observed strong Th2 cell differentiation in the absence of Delta. However, the addition of Delta4 profoundly inhibited the development of cells capable of making IL-4 (Fig. 1B).

Delta expression by DCs promotes T cell IL-2 production and proliferation

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**Delta4 can facilitate Th cell priming.**
Expression of Delta on DCs promotes T-bet-dependent Th1 cell development and inhibits Th2 cell development via a T-bet-independent mechanism

We next focused on the effects of Delta1 or Delta4 expression by DCs on Th cell polarization. We stimulated OTII CD4+ Th cells with OVA-pulsed vector-, Delta1 retrovirus-, or Delta4 retrovirus-transduced IL-12-/- DCs (we used IL-12-/- DCs for these and subsequent experiments specifically to avoid the effects of IL-12 in promoting Th1 and inhibiting Th2 cell development). After 4 days in culture, we restimulated the T cells with PMA/ionomycin in the presence of Golgi-Plug and stained them for intracellular IFN-γ and IL-4. We found that both Delta1 and Delta4 strongly promote IL-12-independent IFN-γ expression and Th1 cell differentiation while simultaneously inhibiting IL-4 production and Th2 cell differentiation (Fig. 3A).

Notch has been suggested to promote IFN-γ production and Th1 cell differentiation through an IFN-γ- (and cell proliferation-) independent manner (9, 11). To examine whether this was the case in our system, we stimulated OT II CD4+ Th cells with Delta1 or Delta4 retrovirus-transduced IL-12-/- DCs under conditions where IFN-γ was neutralized. We found that Delta1- and Delta4-induced Th1 cell development was highly dependent on IFN-γ (Fig. 3B). However, the ability of Delta1- and Delta4-expressing IL-12-/- DCs to inhibit Th2 cell development is clearly IFN-γ independent (Fig. 3B).

T-bet, the Th1 cell lineage specific transcription factor, plays an important role in IFN-γ expression and Th1 cell differentiation (23). T-bet-deficient mice exhibit multiple symptoms characteristic of asthma, suggesting that T-bet is a potent negative regulator of Th2 cell development (24). Because Delta1 has been shown to promote T-bet expression (9), we next asked whether the observed effects of Delta1 and Delta4 on Th cell differentiation are T-bet dependent. Consistent with the ability of Delta-expressing IL-12-/- DCs to promote Th1 cell development, we found that OTII CD4+ Th cells stimulated by OVA-pulsed Delta1 or Delta4 retrovirus-transduced DCs for 2 days expressed more T-bet than did the Th cells stimulated by OVA-pulsed control IL-12-/- DCs (Fig. 3C). However, neither Delta1 nor Delta4 directly promoted sustained T-bet expression. Rather, persistent T-bet expression was a consequence of increased IFN-γ production, because neutralizing IFN-γ blocked early increases in expression of this transcription factor (Fig. 3C). These results do not rule out the possibility that Delta directly stimulates early T-bet expression (11), which is subsequently maintained by IFN-γ-initiated signaling. To further explore whether T-bet is needed for the promotion of Th1
and the inhibition of Th2 cell development by Delta1 or Delta4, we used Delta1 or Delta4 retrovirus-transduced IL-12−/− DCs to stimulate T-bet−/− OTII CD4+ Th cells. Consistent with the role of T-bet in Th1 cell differentiation, the promotion of Th1 cell differentiation by Delta1- or Delta4-expressing IL-12−/− DCs was T-bet dependent (Fig. 3D). However, Delta1- and Delta4-expressing IL-12−/− DCs retained the ability to inhibit IL-4 production and Th2 cell development even in the absence of T-bet (Fig. 3D). Importantly, in these experiments CD4 cells stimulated with Delta1- or Delta4-expressing IL-12−/− DCs proliferated at least as much as did CD4 cells stimulated with control IL-12−/− DCs (Fig. 3D). These data indicate that Delta expression by DCs has two distinct but complementary effects on Th cell polarization: the promotion of IFN-γ production, and the inhibition of Th2 cell development, both of which serve to favor the establishment of polarized Th1 responses.

**Delta inhibits Th2 cell development in vivo**

We next asked whether Delta1 or Delta4 could modulate Th2 responses in vivo. To address this issue, we loaded IFN-α-activated vector-, Delta1 retrovirus-, or Delta4 retrovirus-transduced IL-12−/− DCs with LLO190 peptide and injected them into B6 mice. This protocol has been shown to induce LLO190-specific Th2 cell differentiation in vivo (unpublished data). One week postinjection we recovered splenocytes and restimulated cells through the addition of LLO190, and 6 h later we measured cytokine production by intracellular cytokine staining. As expected, we found that the peptide-loaded control IL-12−/− DCs induced the development of LLO190-specific Th2 cells (Fig. 4A). However, the forced expression of either Delta1 or Delta4 on IL-12−/− DCs significantly inhibited their ability to promote LLO190-specific Th2 response development in vivo (Fig. 4, A and B).

**FIGURE 4.** Delta expression on DCs suppresses Th2 cell development in vivo. IL-12−/− DCs transduced with either vector, Delta1 retrovirus, or Delta4 retrovirus were activated with IFN-α and pulsed with LLO190 peptide and then were injected into B6 mice. Seven days later, splenocytes from the immunized mice were recovered and left unstimulated in media (Media) or restimulated in vitro with LLO190 peptide (LLO190) in the presence of Golgi-Plug, and intracellular staining was performed to measure the production of IL-4 by flow cytometry. A, FACS plots of IL-4+ cells in splenocytes. Numbers indicate the percentages of cytokine-positive cells in the gates within the whole CD4+ population. B, The mean percentages of IL-4+ cells in CD4+ population ± SD of duplicate evaluations with six individual mice per group in a total of two experiments. *p < 0.05.

**FIGURE 5.** Delta expression on DCs suppresses IL-4 driven GATA-3 expression and Th2 cell development, but not early IL-4 production by CD4 cells. A, OTII CD4 cells were stimulated with OVA-pulsed control untransduced IL-12−/− DCs, or vector, Delta1 retrovirus-, or Delta4 retrovirus-transduced IL-12−/− DCs. After 36 h of stimulation, CD69+ cells were sorted and RNA was extracted. IL-4 mRNA levels were measured by real-time RT-PCR. The means and SEs of expression levels, relative to IL-4 mRNAs levels in CD4 cells cultured with DCs alone, from two experiments are shown. B, OTII CD4 cells were stimulated with OVA-pulsed vector, Delta1 retrovirus-, or Delta4 retrovirus-transduced IL-12−/− DCs in the absence or presence of anti-IL-4 mAbs in conditions where IFN-γ was neutralized. After 2 or 4 days of stimulation, GATA-3 expression levels were measured by intracellular staining. Gray line, isotype control; dark line, GATA-3. Numbers indicate the mean fluorescent intensities of GATA-3 levels. Data are from one experiment (and are typical of three experiments).

**The suppression of Th2 cell development by Delta expression on DCs is mediated by a block in IL-4-induced events**

Th2 cell development is dependent on IL-4/Stat6-mediated signaling, and in situations where CD4+ T cells are the only source of IL-4, as is the case in our DC/CD4+ Th cell cocultures, IL-4 made by CD4+ Th cells is the only cytokine capable of promoting Th2 cell development (17). One possibility to explain how Delta inhibits Th2 cell development, then, is that exposure to these Notch ligands inhibits early IL-4 production by responding CD4+ Th cells. To explore this possibility we used real-time RT-PCR to measure IL-4 transcripts in OTII CD4+ Th cells that had been stimulated by OVA-pulsed control untransduced IL-12−/− DCs or vector-, Delta1 retrovirus-, or Delta4 retrovirus-expressing IL-12−/− DCs for 36 h, and we found no differences in IL-4 mRNA levels related to exposure to Delta (Fig. 5A). These results suggest that Delta1 and Delta4 inhibit Th2 cell development through mechanisms that are independent of the inhibition of early IL-4 production.

One of the major consequences of IL-4 signaling via the STAT6 pathway in CD4+ Th cells is the up-regulation of expression of GATA-3 (25). We found that OTII CD4+ Th cells stimulated with OVA-pulsed Delta1 or Delta4 retrovirus-transduced IL-12−/− DCs expressed the same levels of GATA-3 as did CD4+ Th cells...
stirulated with OVA-pulsed control IL-12−/− DCs at day 2 (Fig. 5B), which was consistent with the fact that Delta expression on DCs did not suppress early IL-4 production (Fig. 5A). However, after 4 days of stimulation, CD4+ Th cells stimulated with Delta1 or Delta4 retrovirus-transduced IL-12−/− DCs expressed significantly lower amounts of GATA-3 than did Th cells stimulated with OVA-pulsed control IL-12−/− DCs (Fig. 5B). At both early and late time points, GATA-3 up-regulation was inhibited by inclusion of mAb anti-IL-4 in the cultures, illustrating the essential role of IL-4 in the commitment of cells to the Th2 lineage under these conditions. These data suggest that in Th cells activated by Delta-expressing IL-12−/− DCs, responsiveness to IL-4 is progressively lost, and, as a consequence, GATA-3 expression is never up-regulated and Th2 commitment aborts.

To examine the ability of Th cells primed in the presence of Delta to respond to IL-4, we stimulated OTII CD4+ Th cells with OVA-pulsed IL-12−/− DCs under conditions where IL-4 was added or neutralized, and in the absence or presence of recombinant Delta4. Four days later we restimulated the Th cells with PMA/ionomycin and then assessed Th cell differentiation by measuring intracellular IL-4 and IFN-γ. As expected, Delta4 promoted Th1 cell development and inhibited Th2 cell development, as indicated by enhanced production of IFN-γ and decreased IL-4 production (Fig. 6A). In the presence of increasing doses of rIL-4, IL-4 production by CD4+ Th cells stimulated with Delta4 gradually increased, but to a lesser extent than was the case for CD4+ Th cells stimulated with OVA/DCs alone (Fig. 6, B and C), which is consistent with Delta inhibiting responsiveness to IL-4. Importantly, rIL-4 treatment dramatically dampened the role of Delta in promoting Th1 cell differentiation, as indicated by the very low number of IFN-γ+ cells (Fig. 6, B and C). In conditions where IL-4 was neutralized, CD4+ Th cells stimulated with OVA-pulsed IL-12−/− DCs or OVA-pulsed IL-12−/− DCs plus Delta4 exhibited similar levels of Th1 cell development (Fig. 6D), suggesting that the effect of the Delta Notch ligands in promoting Th1 cell development is dependent on their ability to counteract the function of IL-4. Furthermore, IFN-γ neutralization blocked the effect of IL-4 neutralization in promoting Th1 cell development (Fig. 6E). Together, these results suggest that Delta promotes Th1 cell development by inhibiting the positive effects of IL-4 on Th2 cell differentiation.

Discussion

The role of the Notch pathway in Th cell activation and differentiation has received much recent attention. In particular, the Delta Notch ligands have been implicated in Th1 response development (7, 10). We herein show that ability of Delta4 and Delta1 to promote Th1 cell development is a result of their profound ability to suppress Th2 cell development. It is the inhibition of IL-4 production that allows Th cells activated by Delta to develop into Th1 cells through a pathway that is IL-12 independent.

We show that Delta1 or Delta4 expression on DCs strongly promotes Th1 cell differentiation, which is consistent with the findings that Delta1-Fc protein (9) and Delta4-Fc protein (10) promote Th1 cell responses, and that expression of these Notch ligands in fibroblast APCs favors Th1 cell development (7, 10). We herein show that ability of Delta4 and Delta1 to promote Th1 cell development is a result of their profound ability to suppress Th2 cell development. It is the inhibition of IL-4 production that allows Th cells activated by Delta to develop into Th1 cells through a pathway that is IL-12 independent.

All Notch receptors appear to share the one NICD/MAML/RBP-Jκ pathway to transcribe downstream genes (5). Therefore, our finding that Notch ligands Delta1 and Delta4 inhibit Th2 cell development appears to contradict earlier reports that RBP-Jκ-deficient Th cells and dominant negative MAML transgenic Th cells have defects in Th2 cell development (7, 12), and that Notch signaling directly promotes GATA-3 expression (13, 14). However, our data are strongly consistent with recent findings from Skokos and Nussenzweig showing that CD8+ splenic DCs express Delta4 in response to LPS and promote Th1 responses both in vitro and in vivo through an IL-12-independent, Delta4/Notch-dependent pathway (10). The fact that different Notch ligands and receptors can, in different settings, elicit apparently contradictory responses, suggests that the Notch pathway is more complex than currently envisaged (28). Consistent with this view, it has been suggested that
Notch can elicit additional pathways that are independent of the transcriptional activity of MAML/RBP-Jκ (29). It is possible that Delta uses such alternative pathways to suppress Th2 cell development. Another possibility, given the reported role of Jagged in promoting Th2 cell development (7), is that Delta competes with Jagged for the NICD/MAML/RBP-Jκ pathway to suppress Th2 cell development. The effect of the Notch receptor pathway in Th cell activation per se is somewhat controversial, with reports of Notch signaling having generally positive (30–32) or negative (9, 33) effects. Our data support a positive role for Notch signaling in Th cell activation, because we found that expression of either Delta1 or Delta4 on DCs had costimulatory effects on Th cell priming. This is consistent with the reports that γ-secretase inhibition blocks Th cell activation (30) and that RBP-Jκ-deficient Th cells exhibit a proliferation defect (32).

The mechanism by which Delta-initiated signaling blocks Th2 cell development remains to be established. In a recent report we showed that DCs activated by TLR ligands block Th2 cell development at two distinct levels: 1) by inhibiting early IL-4 production, and 2) by suppressing the ability of IL-4 to up-regulate GATA-3 expression and promote commitment to the Th2 lineage (17). We herein show that Delta expression by DCs has the latter, but not former, effect. The data indicate that Delta selectively suppresses the positive effects of the IL-4 signaling pathway on Th2 cell development. Several molecules, such as ICOS-L and IFN-γ, have been shown to affect IL-4 responsiveness during Th polarization by regulating STAT6 phosphorylation (34, 35). However, we have not been able to show that Delta inhibits STAT6 phosphorylation in Th cells in response to IL-4 (data not shown). It is conceivable that Notch signaling initiated by Delta, rather than by Jagged, for example, leads to the development of a GATA-3 promoter binding complex that blocks rather than promotes GATA-3 expression.

We think that the ability of Delta to promote Th1 cell development is intimately linked to its ability to inhibit Th2 cell development. Th cells stimulated by IL-12−/− DCs without TLR engagement preferentially differentiate along the Th2 pathway (16). The effect of IL-4 in this process is not only to promote GATA-3 expression but also to inhibit IFN-γ production. We think that by inhibiting IL-4 function in responding Th cells, Delta favors IFN-γ production and therefore allows T-bet expression and Th1 commitment. Our data are consistent with a recent report by Strober and colleagues that proposed that the positive effect of T-bet in Th1 differentiation is primarily a result of the ability of this transcription factor to counteract IL-4-driven GATA-3 induction (36). Collectively, these data, along with the finding that GATA-3-deficient Th cells default to Th1 polarization (37), indicate that in neutral conditions Th cells favor differentiating along the Th2 pathway, and that Delta expressed by TLR-activated DCs interferes with this pathway to allow Th1 cell development.

Kapsenberg formally suggested that “Signal 3” should be used to describe the effects of signals elaborated by DCs in response to PAMPs on Th cell differentiation (4). IL-12 is the prototypic example of Signal 3 because it is made by TLR-stimulated DCs and promotes Th1 cell responses. In this context, the delivery of a negative signal for Th2 cell polarization by Notch ligands that are differentially expressed on DCs in response to PAMPs suggests a distinct fourth level of regulation of Th cell activation that we propose might be referred to as “Signal 4”.

Certain conditions, such as allergy and asthma, as well as infections with helminth parasites, are strongly associated with Th2 response development. There is ongoing debate about the nature of the signals delivered during these settings that favor Th2 cell differentiation, and the possibility that the Th2 response is a default that occurs in the absence of Th1-promoting Signal 3. Our data do not argue against such a default but indicate that the key regulatory event that dictates whether a Th2 response can develop is Signal 4. Clearly, Th1 responses are defective in the absence of cytokines such as IL-12, but even in such a setting Th2 responses cannot emerge if Signal 4 is present (an example of such a situation could be infection with Listeria monocytogenes, where the absence of IL-12 does not allow polarization to the Th2 pathway 38). We have found that schistosome egg Ag, an extract of the egg stage of the helminth parasite Schistosoma mansoni that is known to strongly induce Th2 responses in vivo, markedly inhibits the expression of Delta1 and Delta4 by TLR-activated DCs and simultaneously blocks the delivery of Signal 4 (data not shown), suggesting a mechanism through which Th2 responses might be favored following helminth infection.

In summary, we think that compared with the nonspecific targeting of Notch receptor activity in Th cells, our approach of using retroviral transduction to preferentially express Notch ligands in DCs represents a more specific technique to study the role of the Notch pathway in Th cell polarization. We have found that the Notch ligands Delta1 and Delta4 are expressed in DCs in response to exposure to TLR ligands. The expression of Delta on DCs has two linked effects on Th cell polarization: the inhibition of Th2 cell development and the promotion of Th1 cell development. Understanding the mechanisms underlying the suppression of Th2 cell differentiation by Delta may result in new therapeutic targets for treating asthma, allergies, and other Th2-mediated pathologies.

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Disclosures
The authors have no financial conflicts of interest.

References
10. Skokos, D., and M. C. Nussenzweig. 2007. CD8ε DCs induce IL-12-independent Th1 differentiation through Delta 4 Notch-like ligand in response to bacterial LPS. J. Exp. Med. 204: 1525–1531.


