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Cutting Edge: Plasmacytoid Dendritic Cells Induce IL-10 Production in T Cells via the Delta-Like-4/Notch Axis

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Proinflammatory Th1 cells can produce large amounts of the immunosuppressive cytokine IL-10, thereby facilitating the self-limitation of inflammatory responses. Recently, we identified the Notch pathway as a main regulator of IL-10 production by Th1 cells. In this study, we show that plasmacytoid dendritic cells (pDCs), by means of their unique high-level expression of the Notch ligand Delta-like (Dll)-4, activate the Notch receptor on T cells to induce robust IL-10 production in vitro and in vivo. pDCs display a distinct pattern of Notch ligands compared with conventional dendritic cells, marked by the constitutive expression of Dll-4, the only Notch ligand to induce IL-10 expression in vivo, and Dll-1, while at the same time lacking the expression of Jagged. We provide a new mechanism for IL-10 induction by pDCs underlining the importance of the Dll-4/Notch axis in the regulation of inflammatory T cell responses. The Journal of Immunology, 2010, 184: 550–554.

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FN-γ-secretingTh1 cellsaresharceffectorsinthedefenseagainstintracellularpathogens. The immunosuppressive cytokine IL-10 is an effective negative regulator of Th1 responses, which helps to prevent excessive inflammation and tissue damage (1). Interestingly, proinflammatory Th1 cells produce large amounts of IL-10 (2, 3). This Th1-derived IL-10 was found to be critical for the prevention of immunopathology in a variety of infection models (4, 5) and represents a negative feedback mechanism that is independent of regulatory T cells. Recently, we identified the Notch pathway as a key regulator of IL-10 production in Th1 cells (6). The triggering of Notch on T cells acts synergistically with the proinflammatory cytokines IL-12 and -27, leading to greatly enhanced IL-10 production in Th1 cells (6). IL-12 and -27 are produced by APCs and have been implicated before in IL-10 induction (7–10).

Notch receptors, which are constitutively expressed on T cells, can be triggered by ligands belonging to the Delta-like (Dll)-1, -3, and -4 or the Jagged-1 and -2 family. Interestingly, only Dll induces IL-10 production in vitro, whereas Jagged is ineffective (6). Although dendritic cells (DCs), the main APC, were shown to express mRNA for various Notch ligands (11), the expression pattern of individual ligands on different subsets of DCs has not been determined.

Herein we report that plasmacytoid DCs (pDCs) exploit the Notch pathway to induce IL-10 production in T cells in vitro and in vivo. Consistently, pDCs exhibit a unique pattern of Notch ligand expression. pDCs are the only subset to constitutively express Dll-4, whereas Jagged, the most abundant ligand on conventional DCs, is absent. Interestingly, Dll-4 was the only Notch ligand to trigger IL-10 expression in vivo. Our findings identify pDCs and the Notch pathway as attractive targets for the therapeutic modulation of inflammatory T cell responses.

Materials and Methods

Mice

BALB/c, OVA-TCRαβ/β, DO11.10, C57BL/6, B6.PL-OT-2, B6.PL-OT-2/ICOS−/− (Federal Institute for Risk Assessment, Berlin, Germany). CD4-Cre (gift from Dr. W. Müller), and RBPs (gift from Dr. T. Honjo) mice were housed under specific pathogen-free conditions and used at 8–12 wk of age.

Abi

Anti–CD3 (145-2C11), anti–CD28 (37.51), anti–CD4 (RM4-5), anti–CD62L (MEL-14), anti–CD25 (PC61), anti–IL-10 (JES5-16E3), anti–IFN-γ (XMG1.2) (all from BD Pharmingen, San Diego, CA), anti–OVA-TCR (KJ1.26, in-house), and anti–Ly-6C (Miltenyi Biotec, Bergisch Gladbach, Germany) were used.

T cell purification

For in vitro studies, naive CD4+ T cells were isolated by FACS sorting for CD4+CD62L−CD25− (FACS Aria, BD Biosciences, San Jose, CA). For adoptive transfer studies, naive CD62L+ T cells were isolated by MACS (Miltenyi Biotec).

DC purification

DCs were isolated by FACS sorting of plasmacytoid dendritic cell Ag (PDCA)-1/B220+ (pDCs), CD11c+CD11b+ (myeloid DCs), or CD11c−CD11b− (lymphoid DCs) cells after tissue digestion with Dnase I (0.5 mg/ml, Sigma-Aldrich, St. Louis, MO) and collagenase IV (1 mg/ml, Sigma-Aldrich).

Abbreviations used in this paper: DC, dendritic cell; Dll, Delta-like; GSI, γ-secretase inhibitor; MFI, mean fluorescence intensity; pDC, plasmacytoid dendritic cell; PDCA, plasmacytoid dendritic cell Ag; RBPs, recombination binding protein.

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Immunization
Naïve CD62L<sup>+</sup> OVA-TCR transgenic T cells (1 × 10<sup>6</sup>) from DO11.10 mice were adoptively transferred into BALB/c recipients. Mice were immunized s.c. with 50 μg OVA<sub>232–339</sub> peptide and 30 μg CpG (ODN-1826, TIB Molbiol, Berlin, Germany); γ-Secretase inhibitor (GSI) dibenzapine (synthesized by Syncom, Groningen, The Netherlands) was used at 10 μM/kg in 0.1% Tween 80, 0.5% Methocel 60 HG (Sigma-Aldrich) and injected daily i.p.

DC vaccination
Purified DCs were activated with CpG (ODN-1826, TIB Molbiol) and pulsed with 10 μg OVA<sub>232–339</sub> peptide overnight. Thereafter, 1 × 10<sup>6</sup> pulsed DCs were injected i.v. into recipient mice.

pDC depletion
Mice were treated by two i.v. injections of 0.5 mg anti–PDCA-1 on days 1 and 2. Controls received 0.5 mg an isotype control (rat IgG).

Blockade of Notch ligands in vivo
Mice were treated by two i.v. injections of 0.25 mg αJagged-1 (HMIJ1-29), αJagged-2 (HMIJ2-1), αDil-1 (HMD1-5) (12, 13), or αDil-4 (14). Controls were untreated or received an isotype control (hamster IgG) (eBiosciences, San Diego, CA)/human IgG ([DiaNovia, Hamburg, Germany]).

Statistical analysis
Group differences were evaluated by the two-tailed Mann–Whitney t test. p values <0.05 were considered significant.

**Results and Discussion**

**The Dll-4/Notch axis determines IL-10 expression by Th1 cells in vivo**

Recently, we reported that IL-10 is strongly induced in Th1 cells by simultaneous activation of the Notch and STAT4 pathways (6). High frequencies of OVA-specific IFN-γ/IL-10<sup>+</sup> T cells were observed following OVA/CpG vaccination. Interestingly, the administration of a GSI, a highly specific inhibitor of the Notch pathway, drastically reduced IL-10 but not IFN-γ production, clearly demonstrating the involvement of Notch in the regulation of IL-10 expression in vivo (6).

However, GSI blocks the Notch pathway altogether and cannot be used to determine the role of individual Notch ligands in IL-10 induction. To address this question, we applied neutralizing Abs specific for the various Notch ligands. Naïve OVA-TCR transgenic CD4<sup>+</sup> T cells were adoptively transferred into recipient mice, followed by s.c. immunization with OVA/CpG. As previously demonstrated, the complete blockade of the Notch pathway by GSI administration reduced IL-10 expression by >80% (Fig. 1A), whereas IFN-γ production was enhanced compared with the controls. We could not detect any IL-4 or -13 (Fig. 1B), suggesting that the IL-10 single-positive T cells induced by OVA/CpG immunization are not Th2 cells.

When we analyzed IL-10 production by OVA-TCR transgenic T cells isolated from mice treated with blocking Abs against the various Notch ligands, we found only the neutralization of Dll-4 reduced IL-10 expression by ~70%, which is comparable to complete Notch blockade by GSI (Fig. 1C). In contrast, neutralizing Dll-1 was ineffective. We previously found that Dll-1 induced IL-10 production upon overexpression on APCs in vitro (6). However, Dll-1 does not seem to contribute in vivo. It is known that Dll-4 preferentially interacts with Notch-1, whereas Dll-1 binds mostly to Notch-2 (15). Therefore, it is conceivable that in vivo, where the duration of APC–T cell interaction and the expression level of Notch ligands per cell are limited, an effective Notch signal might only be induced by Dll-4–mediated triggering of Notch-1, which shows the highest expression on T cells. Neither blockade of Jagged-1 nor Jagged-2 reduced IL-10 production by Th1 cells (Fig. 1C).

**Dll-4 is selectively expressed at high levels by pDCs**

In light of the nonredundant role of Dll-4 in vivo, we wanted to characterize its expression by various DC subsets. Notch ligand expression by DCs has primarily been analyzed on the level of mRNA isolated from total CD11c<sup>+</sup> cells (6, 11). We compared, by flow cytometry, the expression of Dll-4 and the other Notch ligands against the various Notch ligands, we found only the neutralization of Dll-4 reduced IL-10 expression by ~70%, which is comparable to complete Notch blockade by GSI (Fig. 1C). In contrast, neutralizing Dll-1 was ineffective. We previously found that Dll-1 induced IL-10 production upon overexpression on APCs in vitro (6). However, Dll-1 does not seem to contribute in vivo. It is known that Dll-4 preferentially interacts with Notch-1, whereas Dll-1 binds mostly to Notch-2 (15). Therefore, it is conceivable that in vivo, where the duration of APC–T cell interaction and the expression level of Notch ligands per cell are limited, an effective Notch signal might only be induced by Dll-4–mediated triggering of Notch-1, which shows the highest expression on T cells. Neither blockade of Jagged-1 nor Jagged-2 reduced IL-10 production by Th1 cells (Fig. 1C).

**FIGURE 1.** Dll-4/Notch axis determines IL-10 expression in Th1 cells in vivo. Analysis of IL-10 and IFN-γ production by OVA-TCR transgenic T cells isolated from draining lymph nodes on day 6 after adoptive transfer and immunization with OVA/CpG. A, Mice received GSI daily or solvents as control. Intracellular staining for IFN-γ/IL-10<sup>+</sup> double-positive cells (data summarize four independent experiments; n = 12, mean ± SD). B, Intracellular staining for IFN-γ and -13 after PMA/ionomycin restimulation. C, Mice received neutralizing Abs against Dll-1 or -4, Jagged-1 or -2, or an isotype control. Intracellular stainings (gated on CD4<sup>+</sup>OVA-TCR<sup>tg/tg</sup> cells) and frequency of IFN-γ/IL-10<sup>+</sup>–producing cells among CD4<sup>+</sup>OVA-TCR<sup>tg/tg</sup> cells.
ligands on splenic “myeloid” CD11c−CD11b+ DCs, “lymphoid” CD11c+CD11b− DCs, and CD11c+ICOS−PDCA-1−B220− pDCs. Surprisingly, we found Dll-4 expression to be restricted to pDCs, which, at the same time, completely lack expression of the Jagged ligands (Fig. 2A). In contrast, Dll-1 was ubiquitously expressed on all populations tested. The highest expression was again found on pDCs. Jagged-1 is expressed on myeloid DCs, whereas Jagged-2 is expressed by myeloid and lymphoid DCs (Fig. 2A).

These data reveal a clearly distinct Notch ligand expression pattern for pDCs and suggest their involvement in Dll-4–dependent IL-10 induction observed after CpG vaccination. However, there is the possibility that Dll-4 is induced on other DC subsets following CpG stimulation. In fact, mRNA data show a strong induction of Dll-4 in total CD11c+ DCs, containing all three subsets, upon CpG treatment (6). Surprisingly, when we re-examined Dll-4 expression 48 h after immunization with OVA/CpG, we found that Dll-4 surface expression was only moderately elevated on myeloid DCs, whereas lymphoid DCs remained negative for surface Dll-4. Expression on pDCs was further increased following immunization (Fig. 2B), suggesting that pDCs, at least upon OVA/CpG immunization, are the main source for Dll-4 among DCs in vivo (see fluorescence intensity, Fig. 2B).

pDCs exploit the Notch pathway to induce IL-10 in Th1 cells

The high level of Dll-4 surface expression suggested that pDCs trigger Notch on T cells, leading to IL-10 production. To test this hypothesis, we cocultured naive OVA-specific CD4+ T cells for 5 d with highly purified pDCs, myeloid DCs, or lymphoid DCs in the presence of CpG, IL-12, and OVA peptide. In some samples, the Notch pathway was blocked by the addition of GSI. After 5 d, the T cells were restimulated with PMA/ionomycin, and IFN-γ/IL-10 coproduction was analyzed by intracellular staining. High frequencies of IFN-γ–producing T cells were induced under these conditions, independent of the type of APC used. However, after coculture with pDCs, almost 60% of IFN-γ–producing T cells coexpressed IL-10. About 70% of IL-10 production was abrogated in cultures in which Notch had been blocked by GSI (Fig. 3A). After coculture with myeloid DCs, ~20% of the IFN-γ–producing T cells coexpressed IL-10 (Fig. 3A). Again, IL-10 could be blocked, in part, by the addition of GSI, which is in accordance with the low-level expression of Dll-4 on CpG-treated myeloid DCs. Only 10% of IFN-γ producers coexpressed IL-10 in T cells cultured with lymphoid DCs (Fig. 3A). These data clearly show that pDCs very potently induce IL-10 production in T cells via the Notch pathway.

To demonstrate a T cell-intrinsic role of Notch, pDCs were cocultured with RBP-Jκ–deficient T cells, which are deficient in Notch signaling. T cells were stimulated polyclonally with anti-CD3/anti-CD28 in the presence of CpG and IL-12. Although the frequencies of IL-10–expressing cells are lower, which is probably due to the mixed genetic background of the mice, the T cell-restricted deficiency in Notch signaling again resulted in ~60% reduction in IL-10 expression compared with wild-type T cells (Fig. 3B), demonstrating that pDCs induce IL-10 in an RBP-Jκ– and Notch-dependent fashion.

Previous reports claimed an essential role for ICOS stimulation in pDC-mediated IL-10 expression in T cells (16, 17). To estimate the relative contributions of Notch and ICOS to IL-10 production in our coculture system, we compared wild-type and ICOS-deficient OVA-TCR transgenic T cells after coculture with pDCs with and without the addition of GSI. As shown in Fig. 3C, IL-10 production was slightly reduced in ICOS-deficient T cells compared with wild-type T cells, suggesting that ICOS accounts for ~10–20% of total IL-10 expression in this system. In contrast, blocking the Notch pathway downregulated IL-10 expression by ~60% in wild-type T cells. In ICOS-deficient T cells, Notch blockade led to an even greater reduction (~80%). It is conceivable that the Th1-polarizing conditions applied to the cultures minimize the contribution of ICOS to IL-10 induction. We conclude that the Dll-4/Notch axis is the main mechanism by which pDCs trigger IL-10 production in Th1 cells.

pDCs largely contribute to IL-10 induction following CpG/OVA immunization

To estimate their contribution to IL-10 expression observed after OVA/CpG immunization in vivo, pDCs were depleted by application of an anti–PDCA-1 Ab. Control stainings showed >90% reduction in pDCs in draining lymph nodes by this treatment (Fig. 4A). Analysis of cytokine production 6 d after transfer of OVA-TCR transgenic T cells in pDC-depleted mice revealed a 50% reduction in the frequency of IFN-γ/IL-10 double-producing T cells. In comparison, the neutralization of Dll-4 was slightly more effective in reducing IL-10, which might point to a minor contribution of other APCs or simply reflects the incomplete depletion of pDCs. However, this approach clearly demonstrates a major role for pDCs in the Notch-mediated IL-10 induction following OVA/CpG immunization.
corroborate this finding, we took a complementary approach and immunized mice with OVA peptide/CpG-pulsed pDCs or CD11c+ conventional DCs. The endogenous Notch pathway was blocked by GSI in some mice that had received pDCs. Strikingly, following pDC vaccination, almost 60% of the IFN-γ–producing OVA-specific T cells coexpressed IL-10, whereas conventional DCs induced a clear Th1 polarization, as evidenced by 50% of the cells producing IFN-γ but hardly any IL-10 (Fig. 4C). Again, pDC-induced IL-10 was almost completely abrogated in animals that had received GSI to block the Notch pathway (Fig. 4C).

In this study, we identified a new mechanism by which pDCs trigger IL-10 production in T cells. pDCs make use of their constitutive and high-level expression of Dll-4, the one Notch ligand crucial for IL-10 production in vivo, to induce IL-10 via activation of the Notch pathway. The expression profile of Notch ligands on pDCs is clearly distinct from conventional DCs. pDCs lack the expression of Jagged, which is otherwise abundantly expressed, a fact that might add to pDCs’ unique capacity as IL-10 inducers. A recent report showed that Jagged could antagonize Dll-4 by competing for receptor binding without triggering receptor signaling (18).

Although speculative at this point, it is conceivable that the ratio between Dll-4 and Jagged expression determines the capacity of the APCs to induce IL-10 production. The constitutive expression of Jagged on conventional DCs might counterbalance their short-term upregulation of Dll-4, as seen on the mRNA level, and prevent them from inducing IL-10 expression during priming of an immune response. It remains to be determined whether manipulation of the Dll-4/Jagged ratio in conventional DCs can be exploited to alter their proinflammatory versus tolerogenic potential. However, our findings suggest important roles for pDCs and Dll-4 in the regulation of the self-limiting program of inflammatory T cell reactions. Consequently, they represent interesting targets for therapeutic interventions aiming at the modulation of inflammatory processes on a cellular or molecular level, respectively.

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Disclosures
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