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TGF-β–Exposed Plasmacytoid Dendritic Cells Participate in Th17 Commitment

Francis Bonnefoy,*‡ Mélanie Couturier,*‡ Amandine Clauzon,*‡ Jean-Paul Rémy-Martin,*‡ Beatrice Gaugler,*‡ Pierre Tiberghien,*‡ Wanjun Chen,§ Philippe Saas,*‡,† and Sylvain Perruche*‡,†,‡,1

TGF-β is required for both Foxp3+ regulatory T cell (Treg) and Th17 commitment. Plasmacytoid DCs (pDC) have been shown to participate to both Treg and Th17 commitment as well. However, few studies have evaluated the direct effect of TGF-β on pDC, and to our knowledge, no study has assessed the capacity of TGF-β–exposed pDC to polarize naïve CD4+ T cells. In this paper, we show that TGF-β–treated pDC favor Th17 but not Treg commitment. This process involves a TGF-β/Smad signal, because TGF-β treatment induced Smad2 phosphorylation in pDC and blockade of TGF-β signaling with the SD208 TGF-βRI kinase inhibitor abrogated Th17 commitment induced by TGF-β–treated pDC. Moreover, TGF-β mRNA synthesis and active TGF-β release were induced in TGF-β–treated pDC and anti–TGF-β Ab blocked Th17 commitment. Unexpectedly, TGF-β treatment also induced increased IL-6 production by pDC, which serves as the other arm for Th17 commitment driven by TGF-β–exposed pDC, because elimination of IL-6–mediated signal with either IL-6– or IL-6Rα–specific Abs prevented Th17 commitment. The in vivo pathogenic role of TGF-β–treated pDC was further confirmed in the Th17-dependent collagen-induced arthritis model in which TGF-β–treated pDC injection significantly increased arthritis severity and pathogenic Th17 cell accumulation in the draining lymph nodes. Thus, our data reveal a previously unrecognized effect of TGF-β–rich environment on pDC ability to trigger Th17 commitment. Such findings have implications in the pathogenesis of autoimmune diseases or immune responses against mucosal extracellular pathogens. The Journal of Immunology, 2011, 186: 6157–6164.

Plasmacytoid dendritic cells (pDC) are the main IFN-α–producing cells (1, 2); as such, they participate to antiviral immune responses (3). Through IFN-α secretion, pDC can play a role in the antimicrobial effects induced by TLR7/9 ligand agonist (4–6) as well as in some autoimmune diseases (e.g., psoriasis and lupus) (3). However, pDC functions are not limited to IFN-α secretion because human and mouse pDC may secrete inflammatory cytokines such as IL-6 (7, 8). Depending on the environmental signals, pDC have been described to initiate effective Th1 or Th2 responses (9). Moreover, data supported a role of pDC in tolerance induction through several mechanisms including IDO activity, IL-10 secretion, or regulatory T cell (Treg) induction (10–12). In contrast, data are emerging implicating mouse and human pDC in IL-17–secreting T cell (Th17) differentiation (13, 14). Recently, Th17 cells have been described as the major pathological T cells in several inflammatory diseases such as in experimental autoimmune encephalomyelitis or arthritis (15). Th17 cells are important in host defense against pathogens because Th17 and IL-17A are required for the control of mucosal pathogens (16). Th17 differentiation requires at least IL-6 and TGF-β (17, 18). TGF-β also participates to Treg polarization (19). As such, TGF-β plays a dual role, and for instance, it is implicated in both the resolution of inflammation and in the process of chronic inflammation (20). Indeed, TGF-β participates to fibrosis by stimulating extracellular matrix production and deposition as well as fibroblast proliferation and their differentiation into myofibroblasts (21). To date, limited data are available concerning TGF-β and pDC (22, 23). TGF-β participates to decrease IFN-α production by pDC in response to TLR ligands (22, 23). Studies mainly reported that TGF-β modifies pDC functions in cooperation with other immunosuppressive molecules, including IL-10 or PG-E2 (22–24). In this study, we asked how pDC respond to a TGF-β–enriched milieu and how such environment influences the capacity of pDC to polarize naïve CD4+ T cells.

Materials and Methods

Mice

C57BL/6 and DBA/1 mice were purchased from Charles River Laboratories, and C57BL/6 OTII αBcr-transgenic RAG-1–deficient (OTII/RAG-1−/−) and C57BL/6 RAG-2– and common cytokine receptor γ-chain–deficient (RAGγc−/−) (alymphoid [B−, T−, and NK−] mouse strain) mice (25) were issued from our own colonies (some OTII/RAG-1−/− mice were also obtained from Taconic, Lille Skensved, Denmark). Mice were housed

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in a specific pathogen-free rodent facility at the Unité Mixte de Recherche 645 (agreement number C25-056-7) with food and water available ad libitum. Approval for animal experimentation and care was received from the Services Vétérinaires de la Santé et de la Protection Animale delivered by the Ministère de l’Agriculture, Paris, France.

**Abs and reagents**

The Abs specific for the following molecules were obtained from eBioscience (San Diego, CA) or BioLegend (San Diego, CA): CD4 (PerCP or Pacific Blue; clone RM4-5; rat IgG2a), CD25 (allophycocyanin; clone PC61; rat IgG1), CD11c (PE-Cy7; clone HL3; American Hamster IgG1), CD19 (allophycocyanin; clone 1D3; rat IgG2a), CD45R/B220 (PerCP; clone RA3-6B2; rat IgG2a), I-A/I-E (FITC; clone 2G9; rat IgG2a), CD86 (PE; clone GL1; rat IgG2a), Foxp3, PE; clone FJK-16s; rat IgG1, IFN-γ (FITC; clone XMG1.2; rat IgG1), IL-17A (allophycocyanin; clone TC 11-18H10.1; rat IgG1), NK1.1 (allophycocyanin-Cy7; clone PK136; mouse IgG2a x), Siglec-H (PE; clone eBio440c; rat IgG2b). Anti–IFN-α mAb (FITC; clone RMMA-1; rat IgG1) was obtained from MBL (Piscataway, NJ), and anti-mPDCA-1/CD317 mAb (allophycocyanin; clone JF05-1C2.4.1; rat IgG2b) was purchased from Miltenyi Biotec (Paris, France). To block FcγRs, purified anti-CD16/32 mAb (hybridoma supernatant from clone 2.4G2) was used. Human rTGF-β1 from R&D Systems (Minneapolis, MN; 99% aa identity with mouse TGF-β1) was used in culture as mentioned. The synthetic oligodeoxynucleotide containing CpG motifs (CpG ODN) 2216 from InvivoGen (Cayla, Toulouse, France) was used in culture at 12 μg/ml. For neutralizing culture assays, either purified IL-6-specific mAb (1 μg/ml; PeproTech, Rocky Hill, NJ) or IL-6Ra-chain (CD126)-specific mAb (1 μg/ml; BD Biosciences, Le Pont de Claix, France) were used. To inhibit TGF-β signaling, the SD208 inhibitor (1 μM; Sigma-Aldrich, St. Quentin Fallavier, France) was used.

**pDC enrichment and culture**

pDC were immunomagnetically enriched either from C57BL/6 wild-type (WT) or RAG2−/− bone marrow (BM) using anti-mPDCA-1 microbead kit according to Miltenyi Biotec procedures. Briefly, BM were digested with collagenase D (2 mg/ml; Roche, Penzberg, Germany), and cells were labeled with anti-mouse PDCA-1 microbeads and then washed and positively selected using AutoMACS system (Miltenyi Biotec) or column (Miltenyi Biotec). Purity was measured by FACS staining using anti-Siglec-H and mPDCA-1 mAb combination in NK1.1+ CD19− cells. After isolation, purity was >85% (contaminant cells were mostly CD19+ B and activated NK1.1+ cells when BM from WT mice was used). To obtain enough enriched pDC for individual experiments, pDC were enriched from BM pooled from 8 to 12 mice. Enriched pDC were rested overnight in complete medium (RPMI 1640 medium supplemented with 10% FBS, 1% penicillin/streptomycin, 10 mM HEPES, 10 mM nonessential amino acids, 1 mM sodium pyruvate, 0.05 mM 2-ME, 37°C, 5% CO₂, in the presence of mouse rIL-3 (10 ng/ml; PeproTech). Then, pDC were cultured 24 h with either complete medium supplemented with rTGF-β1 and/or CpG ODN2216 or medium alone, washed, and cocultured for 4 d with naive CD4+CD25− T cells from OTII/Rag2−/− mice and OVA223–239 peptide (2 μg/ml; NeoMPS, Strasbourg, France) at a one pDC for two CD4+ T cell ratio. CD4+CD25− T cells were isolated following Miltenyi Biotec’s procedure (>95% purity). Polarization of CD4+ T cell was then addressed using FACS analysis using CD4+, CD25−, IFN-γ−, IL-17A−, and Foxp3-specific mAb following manufacturer’s recommendations.

**Cytokine measurement**

Cytokines were quantified in culture supernatants by ELISA using commercial kits following the manufacturer’s instructions: IL-17A (eBioscience), IL-6 (R&D Systems), TGF-β1 (Promega, Madison, WI), and IFN-γ (BioLegend).

**Quantitative real-time PCR**

RNA were extracted from cells using the RNeasy mini kit (Qiagen, Cergy Pontoise, France), subjected to reverse transcription (Invitrogen Life Technologies, Gaithersburg, MD), and quantified by real-time quantitative PCR using the iCycler CFX real-time PCR system (Bio-Rad) using TGF-β1 (Mm00441139 m1), retinoic acid receptor-related orphan receptor-γ (ROR-γt) (Mm00441139 m1), and G6PDH primers (all from Applied Biosystems, Courtaboeuf, France). Relative expression for the mRNA transcripts was calculated using the ΔΔCt method using G6PDH mRNA transcript as reference.

**Immunoblot**

Enriched pDC were cultured for 4 h and then pelleted and lysed with sample buffer (62.5 mM Tris-HCl [pH 6.8], 2% SDS, and 10% glycerol). Proteins

![FIGURE 1. TGF-β treatment does not influence pDC maturation. A–C. Percentage of CD86+IA-IE+ (A), IA-IE+ (B), or IFN-α+ cells (C) within Siglec-H+ mPDCA+ pDC assessed by flow cytometry at 0, 12, 24, and 48 h after stimulation (mean of triplicates, error bars represent SEM) with either rTGF-β1 (5 ng/ml, ○) or CpG ODN2216 (12 μg/ml, △) or cultured in medium only (■). Data in A–C are from one representative experiment out of five showing similar results. *p < 0.05 unstimulated versus TGF-β/pDC; #p < 0.05 unstimulated versus CpG/pDC. D. Eight-color FACS analysis showing pDC analysis before (upper panels, whole BM) and after enrichment (lower panels, mPDCA+ fraction). Siglec-H and mPDCA expressions were assessed on CD19− NK1.1+ cells (left panels). pDC percentage was obtained using such gating strategy (right panels, red cells). Such gated pDC were then confirmed as CD11cf-“B220” cells (middle panels, red cells).
were extracted from pDC, quantified and separated by electrophoresis on 8.5% SDS polyacrylamid gel, and transferred to polyvinylidene difluoride membrane (GE Healthcare, Orsay, France). The blots were then blocked for 90 min using TBS 0.1% Tween 20, 5% milk before incubation with rabbit anti-Smad2/3 polyclonal Ab (Cell Signaling Technology, Beverly, MA), rabbit anti–phospho-Smad2 mAb (Cell Signaling Technology), or mouse anti–β-actin mAb (clone AC15; Sigma-Aldrich). Blotted proteins were detected and quantified using a bioluminescence imager and BIO-1D advanced software (Wilber-Lourmat, Marne-la-Vallée, France) after incubation with appropriated anti-mouse or anti-rabbit HRP-conjugated secondary Ab (Jackson ImmunoResearch Laboratories, West Grove, PA).

**Induction and assessment of collagen-induced arthritis**

DBA/1-susceptible mice were immunized by s.c. injection at the tail base with 100 μl bovine type II collagen (200 μg/ml; MD Bioproducts, Zurich, Switzerland) emulsified in CFA (4 mg *Mycobacterium* toxin resuspended in 1 ml IFA; Sigma-Aldrich). Arthritis developed at days 25–28 after collagen immunization in all mice. At day 26 after immunization, the mice received PBS, pDC, or TGF-β–treated pDC either enriched or FACS sorted from BM (10^7 pDC per mouse, i.v.). Arthritis severity was determined by visual examination of the paws as follows: each limb was scored on a scale of 0–4, where 0 = normal paw, 1 = swelling of one digit, 2 = swelling of one or more digits or mild swelling of the entire paw, 3 = moderate erythema and swelling of the entire paw, and 4 = erythema and severe swelling involving the entire paw. The clinical score for each mouse was the result of the sum of the four limbs (maximum score 16). Lymphoid organs were harvested at time of sacrifice for effector CD4+ T cells analysis by FACS. T cells from lymphoid organs were also stimulated for 24 and 48 h in culture in the presence of collagen (2 μg/ml), T cell polarization was assessed by FACS, and supernatants were collected. Joint destruction was determined by immunohistochemistry of ankle joints; back limbs were harvested, decalcified using rapid decalifier solution (Eurobio, Les Ulis, France) and fixed with 10% neutral formalin, embedded in paraffin, and cut in 5-μm sections for hematoxylin/eosin/saffron staining.

**FIGURE 2.** TGF-β–treated pDC induce IL-17+ CD4+ T cells. A, FACs analysis of naive CD4+CD25− OVA-specific OTII T cells cultured for 4 d with TGF-β (5 ng/ml)-treated or untreated pDC pulsed with OVA323–339 peptide. Dot plots from a representative experiment show IL-17A, IFN-γ, and Foxp3 expression in CD4+ T cells. The corresponding percentages of cells are depicted. B, IFN-γ, IL-17A, and Foxp3 expression by FACs in cultured CD4+ T cells as in A from triplicates (*p = 0.027, **p = 0.001; error bars represent SEM). Data in A and B are from one representative experiment out of nine showing similar results. Similar results were obtained using an allogeneic pDC/CD4+ T cell coculture system (data not shown). C, Intracellular expression of IFN-γ by CD4+ T cells (Th1) after coculture of naive CD4+ T cells with untreated pDC or pDC treated for 24 h before coculture with CpG ODN2216 (12 μg/ml). Data obtained by flow cytometry show mean of triplicates ± SEM and are representative out of six experiments showing similar results. *p = 0.019. D, FACs analysis of naive CD4+ T cells cultured (as in A, B) with untreated pDC or pDC treated with increasing concentrations of TGF-β (0–100 ng/ml). Dot plots from a representative experiment show IL-17A and IFN-γ expression in CD4+ T cells. The corresponding percentages of cells are depicted. E, IFN-γ, IL-17A, and Foxp3 expression by FACs in cultured CD4+ T cells as in D from triplicates (error bars represent SEM). Data in D and E are from one representative experiment out of three showing similar results. F, FACs analysis of naive CD4+ T cells cultured (as in A–E) with decreasing numbers of untreated or TGF-β–treated pDC (TGF-β = 50 ng/ml). Dot plots are from one representative experiment out of three and show IL-17A and IFN-γ expression in CD4+ T cells. The corresponding percentages of cells are depicted.
Results
TGF-β-stimulated pDC induce Th17 commitment

Because TGF-β contributes to Th17 commitment (17–19), we analyzed how a TGF-β–rich environment influences the capacity of pDC to polarize naïve CD4+ T cells. Enriched pDC were cultured overnight in the presence or absence of TGF-β. pDC were then washed, loaded with OVA253–319 peptide, and cocultured with purified naïve CD4+/CD25− OVA-specific T cells isolated from OTII TCR-transgenic RAG-deficient (OTII/RAG−/−) mice. First, the analysis of pDC revealed no major difference in the expression of MHC class II (Ia-IE) costimulatory (CD86 and CD80) molecules and intracellular IFN-α between untreated and TGF-β–treated pDC at 12, 24, and 48 h after culture (Fig. 1A–C). TGF-β treatment seemed to limit Ia-IE expression along culture in pDC (Fig. 1B). In marked contrast, we observed that pDC (enriched from BM; Fig. 1D) pretreated with TGF-β induced an increase of IL-17+CD4+ T cells after 4 d of culture, whereas the percentage of IFN-γ+CD4+ T cells decreased (Fig. 2A, 2B). The IL-17+CD4+ T cell induction was not due to changes in CD4+ T cell proliferation because similar proliferative responses were observed in the presence of TGF-β–treated pDC or untreated pDC (data not shown). As control, CpG ODN2216-treated pDC induced IFN-γ+ CD4+ T cells (Fig. 2C), as reported previously (9). IL-17+ cells represented between 6 and 30% in CD4+ T cells after culture with TGF-β–treated pDC, whereas they represented rare events (0.1–2% of CD4+ T cells) after culture with untreated pDC (Fig. 2A, 2B, Supplemental Fig. 1B). Surprisingly, pDC treated with TGF-β weakly increased Foxp3+ Tregs (Fig. 2A, 2B, Supplemental Fig. 1B). We observed that IL-17–secreting CD4+ T cell induction by pDC was TGF-β concentration dependent (Fig. 2D, 2E). We then determined whether the pDC to CD4+ T cell ratio influenced IL-17+CD4+ T cell differentiation. We showed that IL-17–secreting CD4+ T cells were positively correlated with the numbers of TGF-β–treated pDC in the coculture, whereas the frequency of IFN-γ+ CD4+ T cells was inversely correlated (Fig. 2F). Because pDC enrichment may contain contaminating cells such as activated NK or B cells (26, 27), pDC were enriched from aliphylmoid Ragγ−/− BM (Supplemental Fig. 1A) and treated with TGF-β or not and cultured with naïve CD4+CD25− OVA-specific T cells. TGF-β–treated pDC issued from Ragγ−/− mice demonstrated similar properties to favor IL-17 expression in CD4+ T cells after coculture as TGF-β–treated pDC from WT mice (Supplemental Fig. 1B). Using ELISA, we confirmed that supernatants from CD4+ T cells cocultured with TGF-β–treated pDC demonstrated an increase of IL-17A production associated with a decrease of IFN-γ production (Fig. 3A, 3B). IL-17A was almost undetectable in the supernatant of CD4+ T cells cocultured with untreated pDC (Fig. 3A). Th17 commitment was further confirmed at mRNA levels using quantitative real-time PCR showing that ROR-γt mRNA expression was detectable only in OTII/RAG−/− CD4+ T cells stimulated with TGF-β–treated pDC (Fig. 3C). Expression of ROR-γt mRNA was not detectable in CD4+ T cells cultured with either untreated or CpG-stimulated pDC (Fig. 3C). Altogether, the data demonstrated that pDC exposed to a TGF-β–rich environment promote Th17 commitment.

TGF-β signaling is required in Th17 commitment induced by TGF-β–treated pDC

We then determined whether and how TGF-β signaling pathway was implicated. We showed that TGF-β treatment substantially upregulated Smad2 phosphorylation in TGF-β–treated pDC compared with untreated pDC (Fig. 4A), suggesting the involvement of the TGF-β receptor signaling pathway. Triggering of TLR9 using CpG ODN2216 did not interfere with TGF-β–induced upregulation of Smad2 phosphorylation (Fig. 4A). In contrast, inhibition of the TGF-β receptor I (TβRI) kinase using the SD208 inhibitor efficiently blocked TGF-β–induced Smad2 phosphorylation in pDC (Fig. 4A). This suggests that pDC expressed TβRI and responded to TGF-β. We next determined whether TGF-β treatment induced TGF-β production by pDC. We showed that TGF-β treatment increases TGF-β mRNA expression in pDC, which depends on the TGF-β concentration used for pDC treatment (Fig. 4B). Importantly, TGF-β–treated pDC secreted active TGF-β protein in the supernatants (Fig. 4C). These data demonstrated that pDC produced ready-to-use active TGF-β, which can directly affect cultured CD4+ T cells. TGF-β was almost undetectable in untreated pDC or in control CpG-treated pDC (Fig. 4C). Similar results were obtained using serum-free X-vivo medium (data not shown). Intriguingly, CpG/TGF-β–treated pDC did not produce TGF-β (Fig. 4C), eliminating a possible contamination of exogenous rTGF-β. This also demonstrates that although TLR9 signaling does not block TGF-β–induced Smad2 signaling, it inhibits TGF-β production at both mRNA and protein levels. To confirm the mandatory role of TGF-β, we blocked TGF-β signaling during TGF-β treatment of pDC.

FIGURE 3. TGF-β–treated pDC induce IL-17+CD4+ T cells that demonstrate Th17 features. A and B, ELISA for IL-17A (A) and IFN-γ (B) quantification in the supernatants issued from CD4+ T cells cocultures with pDC treated with either TGF-β (1, 5, or 50 ng/ml) or untreated (medium) (error bars represent SEM). C, ROR-γt mRNA quantification using real-time PCR in CD4+ T cells 4 h after coculture with untreated pDC or pDC treated for 24 h before coculture with either TGF-β (5 ng/ml) or CpG ODN2216 (12 μg/ml). Data in A–C are representative of three experiments showing similar results. nd, not detectable.
or during pDC/CD4+ T cell coculture with either TβRI inhibitor SD208 or anti–TGF-β–neutralizing mAb and demonstrated that Th17 differentiation was abrogated (Fig. 4D, 4E, Supplemental Fig. 1C, 1D). Thus, we have provided evidence for the first time, to our knowledge, that pDC may be another cellular source of TGF-β for generating IL-17–secreting cells.

IL-6 is required in TGF-β–treated pDC induction of Th17

Th17 cells also require IL-6 for their differentiation (17, 18). Mouse pDC have been described as IL-6–producing cells (8). However, the factors triggering pDC to produce IL-6 were elusive. We therefore tested whether TGF-β treatment induced pDC to produce IL-6. Indeed, TGF-β treatment significantly increased IL-6 production by pDC (Fig. 5A). As control (8), CpG-treated pDC increased IL-6 production (Fig. 5A). To confirm a role of IL-6 secreted by TGF-β–treated pDC in Th17 commitment, we included either an anti–IL-6- or an anti–IL-6Rα-chain (CD126) neutralizing mAb in TGF-β–treated pDC/CD4+ T cell cocultures. In these settings, Th17 commitment through TGF-β–treated pDC was strongly abolished (Fig. 5B, 5C, Supplemental Fig. 1D). Overall, the data demonstrated that TGF-β treatment induces pDC to produce TGF-β and to increase IL-6 production that in turn promotes Th17 differentiation.

TGF-β–treated pDC injection aggravates arthritis severity and increases Th17-draining lymph node infiltration

To evaluate the in vivo relevance of TGF-β–treated pDC in Th17 commitment, we used an inflammatory experimental model implicating pathological Th17 cells, collagen-induced arthritis (28–30). To observe whether TGF-β–treated pDC injection influences Th17 cells, TGF-β–treated pDC were injected in mice at day 26 after immunization with collagen and compared with untreated pDC or PBS injection. As shown in Fig. 6A, collagen-immunized mice injected with TGF-β–treated pDC at the time of arthritis occurrence demonstrated a more severe arthritis, as attested by a 2-fold score compared with control collagen-immunized mice receiving PBS (p = 0.029; one way ANOVA analysis, Tukey test) (Fig. 6A). Joint destruction was attested by histological analysis (data not shown). Collagen-immunized mice receiving untreated pDC had less severe arthritis, similar to control PBS-receiving mice (Fig. 6A). Then, at time of sacrifice, secondary lymphoid organs were analyzed for CD4+ T cell differentiation. TGF-β–treated pDC injection, but not untreated pDC or PBS injection, resulted in more cells in the draining lymph nodes including leukocytes (Fig. 6B) and CD4+ T cells in particular (Fig. 6C). In contrast, no leukocyte or CD4+ T cell increase was observed in the peripheral lymph nodes or spleen (Fig. 6B, 6C). When the phenotype of the cells present in the draining lymph nodes was analyzed, we observed that TGF-β–treated pDC injection strongly increased the percentage of IFN-γ+CD4+ and IL-17+CD4+ T cells compared with untreated pDC or PBS injection (Fig. 6D). In contrast, the percentage of Foxp3+CD25+CD4+ T cells was similar between the three groups (Fig. 6D). When T cells from the draining lymph nodes were stimulated for 24 h with collagen, pathogenic IFN-γ+CD4+ and IL-17+CD4+ were again observed increased in mice receiving TGF-β–treated pDC injection (data not shown). This sustains the ability of TGF-β–treated pDC to prime pathogenic collagen-specific CD4+ T cells. We observed similar results using highly purified FACS-sorted pDC (data not shown). Altogether, the data confirmed in vivo the pathogenic role of TGF-β–treated pDC and that these cells participate to pathogenic Th17 cell increase.

Discussion

An APC microenvironment is known to influence APC functions such as CD4+ T cell differentiation. To our knowledge, no study has assessed the capacity of TGF-β–exposed pDC to polarize naïve CD4+ T cells. In this paper, we provided strong evidence that pDC, once stimulated by TGF-β, can induce commitment of naïve CD25+CD4+ T cells into fully differentiated Th17 cells. Th17 commitment was attested by acquisition of ROR-γt mRNA,
intracellular IL-17A* staining, and IL-17A secretion and support in vivo by aggravation of Th17-dependent collagen-induced arthritis. This commitment by TGF-β–treated pDC requires TGF-β/Smad signaling pathway in pDC, because TGF-β treatment induced Smad2/3 phosphorylation in pDC and TGF-β signaling blockade with the TβRI receptor I kinase inhibitor SD208 in pDC abrogated Th17 commitment. Moreover, TGF-β mRNA synthesis and bioactive TGF-β release were induced in TGF-β–treated pDC. Thus, pDC exposure to TGF-β induces active TGF-β release by pDC, suggesting the induction of a TGF-β autocrine loop. A study supported such data because, in an experimental model of tolerance induction, pDC issued from tolerized animals demonstrated an increase in TGF-β mRNA transcripts (31). TGF-β is one of mandatory cytokines for Th17 commitment. However, the cellular source of TGF-β leading Th17 commitment has not been fully identified yet. Previous studies reporting the capacity of human (14) and mouse (13) pDC to favor Th17 commitment did not directly assess either TGF-β secretion by pDC or TGF-β dependency. In this study, we clearly demonstrated that in addition to Tregs or conventional DCs (cDC) (32–34), pDC exposed to a TGF-β–rich environment, may represent another cellular source of TGF-β for generating IL-17–secreting cells.

Our data demonstrated also that in addition to TGF-β production, pDC exposed to TGF-β treatment also produce IL-6 leading to Th17 commitment. Taken together with TGF-β, IL-6 serves as the other arm for Th17 commitment (17, 18). Thus, in our setting, elimination of IL-6–mediated signal with either IL-6– or IL-6Rα–specific Abs prevented Th17 commitment. In addition, we observed that neutralization of IL-6 dramatically impaired OVA-specific CD4 T cell proliferation (data not shown), confirming previous study showing the capacity of IL-6 to stimulate IL-2 secretion by activated CD4 T cells (35). Therefore, in our conditions, the release of IL-6 by TGF-β–exposed pDC is essential to both favor Th17 proliferation and inhibits TGF-β–induced proliferation inhibition as described previously (36).

In infectious conditions or in response to “danger” signals, pDC secrete IL-6 (7, 8). In this paper, we reported increased IL-6 production by pDC in response to TGF-β, emphasizing the less recognized proinflammatory role of TGF-β (37). Indeed, disrupted
TGF-β regulation leads to fibrosis, an aberrant response to organ injury. For instance, TGF-β contributes to the pathogenesis of systemic sclerosis and scleroderma as well as pulmonary fibrosis (21). Thus, TGF-β–exposed pDC-induced Th17 may contribute to fibrosis-associated diseases (21). In contrast, TGF-β–exposed pDC-induced Th17 may also participate to tissue repair through IL-22 secretion (38) to antagonize fibrosis.

pDC are influenced by microenvironment signals like other APC. Whereas pDC have been largely implicated in tolerance induction through Threg generation, our data and others demonstrated their role in the development of pathogenic Th17 cells (13, 14). Indeed, human pDC stimulated through TLR7 have been demonstrated to both promote Th17 cell commitment and amplify Th17 effector functions (14). This further supported the role of pDC such as a cellular source of TGF-β, like TGF-β–producing Tregs (32, 34), to polarize Th17 cells. In contrast, human thymic pDC have been involved in Treg induction when stimulated by the thymic stromal lymphopoietin produced by the thymic epithelial cells (39). Therefore, depending on the microenvironment, pDC might be a key player in TGF-β–dependent T cell commitment such as Tregs and Th17 cells. In this study, we studied the impact of a TGF-β–rich milieu on pDC. We revealed that TGF-β–exposed pDC favor Th17. In contrast, simultaneous stimulation of pDC by TGF-β and TLR9 ligand rather favor Th1 differentiation by direct inhibition of TGF-β secretion by pDC. This might happen during an infectious challenge. Thus, our data and others (3, 9, 12) confirm pDC as an important player in adaptive immunity in addition to its recognized role in innate immunity.

pDC is an APC quite distinct from cDC, as shown for the Ag uptake, Ag presentation machinery (40) and CD4+ T cell differentiation (9). In this paper, we provided strong evidence that in contrast to TGF-β–treated cDC that do not induce Th17 polarization (data not shown), pDC, once stimulated by TGF-β, can induce Th17 commitment. Indeed, when exposed to TGF-β, cDC have been demonstrated to harbor tolerogenic functions (41, 42). cDC may also favor Th17 differentiation during the elimination of infected apoptotic cells (33). This requires both TLR triggering and TGF-β synthesis because of apoptotic cell uptake (33). Again, pDC and cDC demonstrated distinct properties because we demonstrated that TLR triggering associated with TGF-β limit Th17 commitment.

Altogether, our data described a previously unrecognized feature of pDC in CD4+ T cell differentiation in response to TGF-β (i.e., Th17 induction), which may have implications in understanding the pathogenesis of TGF-β–associated diseases such as fibrosis.

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Disclosures

The authors have no financial conflicts of interest.

References


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