Ag-Presenting CpG-Activated pDCs Prime Th17 Cells That Induce Tumor Regression

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Abstract
Plasmacytoid dendritic cells (pDC) rapidly and massively produce type I IFN and other inflammatory cytokines in response to foreign nucleic acids, thereby indirectly influencing T-cell responses. Moreover, antigen (Ag)-presenting pDCs directly regulate T-cell differentiation. Depending on the immune environment, pDCs exhibit either tolerogenic or immunogenic properties. Here, we show that CpG-activated pDCs promote efficient Th17 differentiation. Indeed, Th17 responses are defective in mice selectively lacking MHCII on pDCs upon antigenic challenge. Importantly, in those mice, the frequency of Th17 cells infiltrating solid tumors is impaired. As a result, the recruitment of infiltrating leukocytes in tumors, including tumor-specific cytotoxic T lymphocytes (CTL), is altered and results in increased tumor growth. Importantly, following immunization with tumor Ag and CpG-B, MHCII-restricted Ag presentation by pDCs promotes the differentiation of antitumor Th17 cells that induce intratumor CTL recruitment and subsequent regression of established tumors. Our results highlight a new role for Ag presenting activated pDCs in promoting the development of Th17 cells and impacting on antitumor immunity. Cancer Res; 74(22); 1–11. ©2014 AACR.

Introduction
Plasmacytoid dendritic cells (pDC) exhibit important innate functions during infections, notably by producing large amount of type I IFN (IFNα; ref. 1). However, recent evidences directly involved these cells in adaptive immunity. pDCs upregulate MHC class II molecules (MHCII) upon inflammation (2) and function as antigen (Ag)-presenting cells (APC) in vivo in several mouse models of diseases to induce both T-cell-mediated immunity and tolerance. For instance, pDCs promote the initiation of myelin-induced Th17 responses and Experimental Autoimmune Encephalomyelitis (EAE; ref. 3). Furthermore, Ag targeting to pDCs via BST-2 in combination with Toll-like receptor (TLR) agonists provides an effective vaccination (4). On the other hand, Treg induction by pDCs was shown to delay cardiac allograft rejection, dampen asthmatic reactions to inhaled Ags, and protect against graft versus host disease (5–7). In addition, Ag targeting to pDCs via Siglec-H inhibits Th-cell–dependent autoimmunity (8). We recently formally demonstrated that MHCII-restricted presentation of myelin Ags by pDCs promotes Treg expansion and inhibits EAE (9).

The role of pDCs in antitumor immunity has been debated. In tumor microenvironment, pDCs exhibit a tolerogenic phenotype characterized by low costimulatory molecule expression and low IFNα production, and promote tumor growth possibly through Treg induction (10–13). In contrast, specific Ag delivery to pDCs using BST2 in combination with TLR agonists induced protective immunity against tumor growth (4). In humans, intranodal injections of pDCs activated and loaded with tumor Ag-associated peptides induced antitumor specific T-cell responses (14). However, because targeting Ag presentation in pDCs also increased their production of IFNα (15), those studies could not determine the contribution of Ag presentation by pDCs in impacting tumor T-cell immunity. In addition, TLR-triggered pDCs exhibit potent antitumor effects. Tumor regression has been correlated for some (i.e., R848 and CpG-A), but not all (CpG-B) TLR ligands, with IFNα production by pDCs (16). Thus, pDC-mediated antitumor potential may rely on other IFNα independent pDC functions, possibly via their ability to present tumor Ags.

Here, we investigated whether Ag-presenting pDC capacities could be modulated and exploited to enhance antitumor T-cell immunity. We show that upon immunization with an Ag and CpG-B, in vivo Ag-specific Th17 responses are significantly impaired in genetically modified mice lacking MHCII expression on pDCs. In contrast with what was shown in conventional DCs (cDC; ref. 17), MHCII deficiency does not impair innate pDC functions. Importantly, in mice further challenged with tumors, pDC-primed Th17 cells control tumor growth by promoting the recruitment of immune cells, including...
tumor-specific cytotoxic T lymphocytes (CTL), into the tumors. In addition, vaccination with an MHCIIRestricted tumor epitope of mice bearing established tumors significantly induces Th17 cells that promote intratumoral CTL recruitment and tumor growth inhibition, in a mechanism dependent on MHCI expression by pDCs. Thus, CpgG-activated pDCs contribute as APCs to the induction of antitumor Th17 cells that dramatically dampen established tumor growth, a property that may be of interest in the establishment of antitumor immunotherapies.

Materials and Methods

Mice

All mice [WT, H2-Aa−/−(18), pIII+/IV−/− (19), OT-I (20), OT-II Rag2−/− (21), μMT (22), μMT pIII+/IV−/− (9)] were of pure C57BL/6 background and maintained under specific-pathogen-free conditions. All animal husbandry and experiments were approved by the animal research committee of the University of Geneva (Geneva, Switzerland).

Immunizations and T-cell transfer

Bone marrow (BM) chimeric mice were generated as described (9). Mice were immunized subcutaneously with OVAII peptide (ISQAVHAAHAEINEAGR; 2 × 106 moles; Invitrogen). In some experiments, cells were loaded with 0.5 μg/mL CpG-B 1668, 0.3 μg/mL CpG-A 1585, or 1 μg/mL imiquimod (Invivogen). In some experiments, cells were loaded with 10 μg/mL CpG-A 1585, (; 2.5 ng/mL; Polypeptide) and 5 × 105 EG7 thymoma cells, s.c.). Tumor size was measured with a caliper [L (length) × W (width)]. For tumor—filtrating lymphocytes (TIL) analysis, tumors were digested using Collagenase D (1 mg/mL) + DNase 1 (10 μg/mL; Roche) and TILs were enriched using lympholyte M (Cedarlane Laboratory) and restimulated for 18 hours with phorbol 12-myristate 13-acetate (PMA; 50 ng/mL; Sigma) and ionomycin (1 μg/mL; Sigma). When indicated, mice were depleted of CD8+ T cells using anti-CD8 mAbs (53-6.72), 100 μg i.p. at days 8, 11, and 14 after tumor challenge. In some experiments, EG7 tumor-bearing mice were adoptively transferred with OVA-specific CD8+ transgenic T cells (2 × 106) purified from LN and spleen of OT-I CD45.1 mice using magnetic sorting (Miltenyi Biotech).

In vitro generation of Th17 cells

OT-II naïve T cells were extracted from LN using CD4+ CD62L+ T cells isolation kit (Miltenyi). Cells were cultured for 3 days on anti-CD3 and anti-CD28 (BioXCell) coated plates in presence with IL6 (25 ng/mL) and TGFβ (2.5 ng/mL; eBioscience). Cells (1.106) were then injected i.v. into sublethally irradiated mice (500 Gy).

In vivo killing assay

CFSE labeled splenocytes were loaded (2.5 μmol/L CFSE) or not (0.5 μmol/L CFSE) with OVAIII peptide (SIINFEKL; 10 μg/mL; Polypeptide) and 5 × 105 each (ratio 1:1) was injected i.v. into tumor-bearing mice. Twenty-hours later, CFSE cells were analyzed in tumor dLN (TdLN) and nDLN. Specific in vivo killing was calculated as [1−(% CFSE 2.5 μmol/L TdLN/% CFSE 0.5 μmol/L TdLN)/ (% CFSE 2.5 μmol/L nDLN/% CFSE 0.5 μmol/L TdLN)] × 100.

Statistical analysis

Statistical significance was assessed by the Mann–Whitney test or by the two-way ANOVA test with Bonferroni correction for tumor growths using GraphPad software.* P < 0.05; ** P < 0.01; *** P < 0.001.

Results

pDCs induce Th17 cells upon Cpg-B activation

We investigated whether the lack of MHCIIR on pDCs has an impact on Th responses upon inflammatory antigenic challenge. MHCIIR expression is regulated by the master regulator CIITA, itself under the control of cell-specific promoters (Supplementary Fig. S1A; ref. 23). MHCIIR expression in pDCs strictly relies on pIII, whereas pII is expressed in macrophages and cDCs (23). We used mice deficient for pIII and pIV of CIITA (pIII+IV−/− mice; ref. 19). Because of the deletion of pIV that is expressed by

ELISAs

Cytokine production was measured in culture supernatants using ELISA Kit from eBioscience (TNFα, IL6, IL17, IL10, and TGFβ), BD (IFNγ), or PBL (IFNα).

qRT-PCR

Total RNA was isolated and RT-PCR was performed as described (9, 21). Primer sequences are provided in Supplementary Information.

Dendritic cell isolation from LN

LN cells were isolated (9, 21), depleted of lymphocytes using CD3 and CD19 antibodies and magnetic sorting (Miltenyi Biotech). pDCs (CD11cintB220 PDCA-1+) were sorted using a MoFlo Astrios (Beckman Coulter).

Tumor experiments

Mice were immunized subcutaneously with Cpg-B (5 μmoles) and OVAIII (10 μg) either before (7 days) or after (between 7 days and 10 days) tumor cell transplantation (5 × 103 EG7 thymoma cells, s.c.). Tumor size was measured with a caliper [L (length) × W (width)]. For tumor—filtrating lymphocytes (TIL) analysis, tumors were digested with collagenase D (1 mg/mL) + DNase 1 (10 μg/mL; Roche) and TILs were enriched using lympholyte M (Cedarlane Laboratory) and restimulated for 18 hours with phorbol 12-myristate 13-acetate (PMA; 50 ng/mL; Sigma) and ionomycin (1 μg/mL; Sigma). When indicated, mice were depleted of CD8+ T cells using anti-CD8 mAbs (53-6.72), 100 μg i.p. at days 8, 11, and 14 after tumor challenge. In some experiments, EG7 tumor-bearing mice were adoptively transferred with OVA-specific CD8+ transgenic T cells (2 × 106) purified from LN and spleen of OT-I CD45.1 mice using magnetic sorting (Miltenyi Biotech).

In vitro cell differentiation and treatments

pDCs were differentiated from BM with FLt3-L (100 ng/mL; Peprotech; ref. 9), sorted as CD11c+PDCA-1+ pDCs, using a MoFlow Astros (Beckman Coulter) and treated with 0.3 μmol/L. Cpg-B 1668, 0.3 μmol/L Cpg-A 1585, or 1 μg/mL imiquimod (In vivogen). In some experiments, cells were loaded with 10 μg/mL of OVAIII peptide.

Flow cytometry

Siglec-H (eBio440c), CD8α (53.6.7), TNFα (MP6-XT22), Foxp3 (FJK-16s), CD45.1 (A20), CD3 (145-2C11), and PDCA-1 (eBio927) antibodies were from eBioscience, CD11c (n418), B220 (RA3-6B2), CD19 (6D5), F4/80 (BM8), and CD11b (M1/70) antibodies from Biologend and 1-Ab (AF6-120.1), CD4 (RM4.5), IL17 (TC11-18H10), and IFNγ (XMGI.2) antibodies from BD. H-2kb SIINFEKL pentamer was purchased from Proimmune.
cortical thymic epithelial cells, pIII+IV−/− mice are devoid of CD4+ T cells (24). Consequently, and as described before (9), we restored the CD4+ T-cell population by generating BM chimeric mice using irradiated WT recipients. Furthermore, because pIII is expressed in B cells, we have used BM precursors derived from B-cell deficient (µMT) mice that were backcrossed with pIII+IV−/− mice. In both cases (µMT:WT and µMT pIII+IV−/−:WT), BM chimeric mice lack B cells, the only difference being presence (µMT:WT), or absence (µMT pIII+IV−/−:WT) of MHCII on pDCs (Supplementary Fig. S1B). In contrast with pDCs, cDCs express normal MHCII levels in naïve mice, as well as the frequency of Foxp3+ cells, as well as the frequency of CD11b+ DCs (25). Consequently, and as described before (9), we used OVA-specific OT-II T cells to promote Th17 responses. Con- sistently, CpG-B–treated, OVA-loaded pDCs were found competent to induce IL17 production by OT-II cells in vitro (Fig. 1D). We further demonstrated that impaired Th17 responses observed in CpG-B context resulted from the selective abrogation of MHCII expression by pDCs. Indeed, levels of MHCII expression by other APCs (including CD8+ DCs and macrophages) were rigorously similar in µMT:WT and µMTpIII+IV:WT chimeric mice, first in steady-state before immunization, and then equally upregulated 12 hours after CpG-B treatment (Supplementary Fig. S3). CD11b+ DCs already expressed very high levels of MHCII in naïve condition, and did not further upregulate MHCII after CpG-B treatment, CD11b+ DCs from µMT:WT and µMTpIII+IV:WT mice; however, expressing similar MHCII levels (not shown). Altogether, our results demonstrated that impaired Ag-specific Th17 observed in µMTpIII+IV:WT compared with µMT:WT immunized in the context of CpG-B resulted from the selective abrogation of MHCII expression by pDCs.

**Innate pDC functions are not affected by the absence of MHCII**

In cDCs, MHCII molecules potentiate TLR-triggered innate functions (17). We tested both in vitro and in vivo whether pDC innate functions could be affected by the loss of MHCII, and explain altered Th17 responses in mice lacking MHCII on pDCs. We first analyzed TLR-triggered responses in pDCs derived in vitro from BM of WT, pIII+IV−/−, and H2-Aα−/− mice. Imiquimod and CpG-B treatments induced a significant MHCII upregulation by WT, but not H2-Aα−/− and pIII+IV−/− pDCs (Fig. 2A). However, WT, pIII+IV−/−, and H2-Aα−/− pDCs produced equal levels of IL6, TNFα, and IFNα in response to both TLR ligands after 6 and 24 hours (Fig. 2B and C). Intracellular TNFα staining showed similar results (Fig. 2D). Accordingly, WT, H2-Aα−/−, and pIII+IV−/− pDCs expressed comparable levels of Tnfα, Il6, Il1β, and Ifna4 mRNAs after imiquimod or CpG-B stimulation (Supplementary Fig. S4A). As described (25), pDCs produce low IFN levels after imiquimod or CpG-B treatment (Fig. 2B and C and Supplementary Fig. S4A). In contrast, expressions of Ifna4 and Ifnb mRNA were similarly strongly induced after CpG-A treatment in both MHCII-sufficient and MHCII-deficient pDCs (Supplementary Fig. S4B).

In vivo, LN WT pDCs (Fig. 2E) expressed steady state levels of MHCII, whereas pIII+IV−/− pDCs were MHCII negative (Fig. 2F). WT and pIII+IV−/− pDCs similarly increased the expression levels of Il1β, Il6, Tnfα, and Ifnb mRNAs upon CpG-B injection (Fig. 2G), demonstrating that MHCII deficiency does not impair TLR-mediated pDC innate responses in vivo.
Altogether, our data demonstrate that TLR9-activated pDCs act as APCs to induce Th17 cell differentiation.

**Tumor Ag presentation by CpG-activated pDCs induces Th17 cells that control subsequent tumor challenge by recruiting immune cells in solid tumors**

To determine whether pDC-induced Th17 responses could be exploited in antitumor T-cell immunity, we immunized μMT:WT and μMT pIII+IV−/−:WT mice with OVA11 + CpG-B and further challenged mice with OVA-expressing tumors (EG7) in the same flank. Mice lacking MHCI on pDCs exhibited a significant increase in tumor growth (Fig. 3A), suggesting that OVA11 peptide presentation by CpG-activated pDCs controls tumor growth. Similar results were observed when BM chimeric mice were immunized with OVA protein instead of OVA11 peptide (Supplementary Fig. S5A), further confirming that pDCs function as bona fide APCs in our model. In addition, we observed a significant reduction in absolute CD45hi TIL numbers in μMT pIII+IV−/−:WT compared with μMT:WT mice (Fig. 3B). Although frequencies of CD4+ T cells, CD8+ T cells, cDCs, and pDCs infiltrating solid tumors were identical whether pDCs express MHCI or not, the dramatic reduction of TILs in the absence of MHCI on pDCs led to a significant decrease in absolute numbers of those cells (Fig. 3C–H).

To investigate whether tumor growth was controlled by Th17 cells primed by CpG-activated pDCs, we further analyzed effector function of tumor-infiltrating CD4+ T cells. We observed a significant decrease in frequencies of both IL17+ and IL17+IFNγ+ CD4+ T cells (Fig. 4A, C, and E) in μMT pIII+IV−/−:WT mice compared with μMT:WT mice. On the contrary, the IFNγ+ CD4+ T cells (Fig. 4A and D) and Foxp3+ CD4+ T-cell frequencies remained unchanged (Fig. 4B and F), whereas absolute numbers of all these populations were decreased in μMT pIII+IV−/−:WT mice, reflecting the drastic reduction in total TILs (Fig. 4C–F). Consistent with impaired IL17+ and IL17+IFNγ+ CD4+ T-cell frequencies, the production of IFNγ and IL17 by TILs was significantly decreased in mice lacking MHCI on pDCs (Fig. 4G). In contrast, IL10 and TGFβ productions were not affected, further confirming that Ag presentation by CpG-activated pDCs did not lead to Treg development (Fig. 4G).

Consistent with a role of tumor-specific Th17 cells in the inhibition of tumor growth, and as described in the mouse B16 melanoma tumor model (20), we observed a significant reduction in tumor size (Fig. 4H), as well as increased intratumor...
IL17 cytokine levels (Fig. 4I), when in vitro differentiated Th17, but not Th0 cells (Supplementary Fig. S6A and S6B), were adoptively transferred in mice bearing established tumors.

**CpG-activated pDC primed Th17 responses promote CTL recruitment in tumors**

Because tumor rejection is mainly mediated by CTLs, we investigated whether tumor-specific CD8$^+$ T cells were affected by the loss of MHCII expression by pDCs. Although the percentage of IFN$\gamma$+ CD8$^+$ T cells was slightly increased in EG7 tumors from μMT pIII−/IV−:WT compared with μMT:WT, frequencies of OVA-specific (pentamer$^+$) CD8$^+$ T cells were similar (Fig. 5A–D). Absolute numbers of IFN$\gamma$ producing and pentamer$^+$ CD8$^+$ T cells were lower in μMT pHII−/IV−:WT.
mice compared with μMT:WT mice (Fig. 5C–D). In dLN, percentages (Fig. 5E–F) and absolute numbers (not shown) of pentamer CD8+ T cells were, however, identical in both groups, suggesting an impaired recruitment of those cells in tumors. Moreover, CTL cytotoxic activity was similar whether pDCs express MHCII or not in an in vivo killing assay using OVAII-loaded CFSE-stained target cells (Fig. 5G and H). These results suggest that increased tumor-specific Th17 cells induced by CpG-activated pDCs did not alter priming of CTL and Th1 cells in dLN but impaired the recruitment of those cells in solid tumors. Accordingly, total cells isolated from tumor dLN migrated less efficiently toward EG7 tumor supernatant extracted from μMT pIIIIV−/−:WT compared with μMT:WT chimeras (Supplementary Fig. S7A), although relative frequencies of LN cell subsets were not affected (not shown). In addition, the IL17-induced chemokines CCL-2 and CXCL-2 were found significantly decreased in tumor supernatants of μMT pIIIIV−/−:WT compared with μMT:WT chimeras (Supplementary Fig. S7B). Altogether, our data demonstrated that CpG-activated pDCs promoted antitumor Th17 cells, intratumoral recruitment of immune cells, including CTLs, and resulted in tumor growth inhibition.

**Ag-presenting functions of CpG-activated pDCs can be exploited for antitumor immunotherapies**

To determine whether CpG-activated pDC ability to promote antitumor Th17 cells could be used as a therapeutic strategy, we next transferred CpG-activated OVAII-loaded WT or pIIIIV−/− pDCs in EG7 tumor-bearing mice. Unfortunately, we could not observe any regression of tumors from mice injected with MHCII sufficient pDCs (not shown). Similarly, the vaccination of mice bearing established tumors with OVAII + CpG-B had no beneficial effect on tumor growth when performed in the flank in which the tumor developed (not shown), suggesting that pDC ability to prime effector T cells might have been altered by the tumor microenvironment. However, contralateral OVAII + CpG-B vaccination led to a significant reduction in tumor size (Fig. 6A). In addition, we observed similar results when tumor-bearing mice were immunized with OVA protein + CpG-B (Supplementary Fig. S3B). Importantly, tumors from OVAII + CpG-B vaccinated mice exhibited a dramatic increase in infiltrating CD45+IL17+ cells, with a selective enrichment of Th17 cells (Fig. 6B–E). In agreement, IL17-producing CD8+ T cells were significantly increased (Fig. 6F). These data show that OVAII + CpG-B vaccination of tumor-bearing mice promotes Th17 cells that induce intratumor cell recruitment and tumor growth inhibition. Consistent with our previous observations, vaccination did not affect tumor-specific CTL frequencies (Fig. 6G–I). However, as a consequence of enhanced cell infiltration, absolute numbers of Th1, Th17, pentamer CD8+ and IFNγ-producing CD8+ T cells were significantly increased (Fig. 6C, D, and G–I).
tumor growth inhibition, whereas, in contrast, no protective effect was observed in µMT plII+IV−/−:WT, in which pDCs are MHCII deficient (Fig. 6J). Altogether, these results demonstrate that OVAⅱ+CpG-B-mediated tumor regression, and that this effect was dependent on pDC Ag-presenting functions.

Antitumor adaptive immune responses against EG7 tumors are mainly CTL-dependent (27–29). To determine whether Th17-mediated control of tumor growth was CTL dependent, CD8+ T cells were depleted in WT tumor-bearing mice vaccinated or not with OVAⅱ+CpG-B. As expected, CD8 depletion led to a significant increase in tumor size (Fig. 7A). Importantly, we observed a total abrogation of OVAⅱ+CpG-B vaccine efficacy in the absence of CD8+ T cells (Fig. 7A), suggesting that OVAⅱ-mediated Th17 cells control tumor growth in a CD8-dependent manner. Most of the cancer therapies and vaccines developed so far have been directed to target specific CD8+ T cells (30). Thus, we next wondered whether having a concomitant antitumor Th17 and CD8 T-cell responses improve tumor rejection. For that we adoptively transferred OT-I cells into WT EG7 tumor-bearing mice that we vaccinated or not with OVAⅱ+CpG-B. We injected suboptimal OT-I amounts (2×10⁶), which only conferred a partial and time-limited control of tumor growth when transferred alone (Fig. 7B). OVAⅱ+CpG-B vaccination increased OT-I recruitment into tumors (Fig. 7C), and consequently significantly ameliorated and prolonged OT-I–mediated tumor growth inhibition (Fig. 7B). Accordingly, OVAⅱ+CpG-B vaccination dramatically improved OT-I–mediated tumor rejection in µMT:WT chimeras (Fig. 7D). In striking contrast, OVAⅱ+CpG-B vaccination had no effect on tumor growth in OT-I transferred µMT plII+IV−/−:WT (Fig. 7D), highlighting the importance of Ag presentation by pDCs in this model.

Altogether, our results demonstrate that antitumor vaccination using MHCII-restricted epitopes in the presence of CpG-B induce tumor antigen presentation by pDCs and subsequent antitumor Th17 responses. pDC-mediated Th17 cells consequently potentize CTL-mediated tumor immunotherapies by increasing intratumor CTL recruitment.

**Discussion**

pDCs are potent sensors of nucleic acid and become activated to produce large amounts of IFNγ (31). Those cells, despite low Ag uptake capacity, also function as APCs in several conditions and, depending on their activation status and the cytokine environment, promote either T-cell tolerance (3, 4) or effective T-cell immunity (5–9). In tumors, it has been demonstrated that IFNγ displays antitumoral effects through antiproliferative and proapoptotic functions, thus inhibiting tumor cells survival and angiogenesis (32, 33). Moreover, IFNγ

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**Figure 6:** MHCII-mediated pDC-dependent vaccination with CpG-B + OVAⅱ inhibits established tumors. A–I, EG7 cells were implanted s.c. in WT mice. Ten days later, mice were immunized (VAX) or not (CT) s.c. with OVAⅱ+CpG-B in a contralateral manner. A, tumor growth was measured every 1 to 2 days. B–I, at day 5 after immunization, TILs were analyzed. B, histograms show absolute numbers of CD45hi TILs. C–E, and G–I, percentages and absolute numbers of CD4+ T cells (C), IFNγ+ CD4+ T cells (D), IL17+ CD4+ T cells (E), CD8+ T cells (G), Pent+ CD8+ T cells (H), and IFNγ+ CD8+ T cells (I). F, IL17 was measured in culture supernatants. B–I, results show the mean and SEM derived from 5 mice and are representative of three independent experiments. J, EG7 cells were implanted s.c. in µMT:WT and µMT plII+IV−/−:WT mice. Ten days later, mice were immunized s.c. with OVAⅱ+CpG-B in a contralateral manner. Tumor growth was measured every 1 to 2 days. Results show the mean and SEM derived from 5 mice and are representative of three independent experiments.
limits tumor progression by enhancing CD8α+ DCs mediated cross-presentation of tumor Ags and subsequent CTL priming (34, 35). Consequently, IFNIs are used in the treatment of various cancers (32). However, pDC ability to produce IFNI is impaired in tumor microenvironment, therefore converting these cells into tolerogenic pDCs and leading to immunosuppression in several different cancers (for review, see ref. 33). In addition, in breast tumors in particular, pDCs induce Treg expansion in IDO and/or ICOS-dependent mechanisms (10–12). Accordingly, pDC infiltration in tumors has been correlated with poor clinical outcome (36, 37). Thus, promoting pDC activation in tumor context would restore IFNI production, inhibit Treg expansion, and represent an effective approach to induce antitumoral immune response. In agreement, CpG-A–activated, IFNI-producing pDCs injected directly into tumors initiate an effective and systemic antitumor immunity through the orchestration of an immune cascade involving the sequential activation of natural killer (NK) cells, cDCs, and CD8+ T cells (38). Moreover, TLR7 ligands or CpG-B intratumoral injection inhibit murine mammary tumor growth in a pDC-dependent manner (16). Interestingly, however, protection was linked to restoration of IFNI occurring after TLR7 ligand, but not after CpG-B, administration. Thus, although the role of pDC-mediated IFNI in antitumor immunity has been convincingly demonstrated, it remains unknown whether those cells could directly contribute to antitumoral T-cell immunity as APCs, and in particular after being activated by CpG-B.

Here, using mice lacking MHCII expression by pDCs, we show that upon CpG-B activation, pDCs drive Th17 cell differentiation through MHCII-dependent Ag presentation. For experimental reasons, our mouse model lacks peripheral B cells, which might not be neutral. However, we compared MHCII+ and MHCII− pDCs in a B-cell-deficient background, and investigated the selective impact of MHCII-mediated Ag presentation by pDCs on CD4+ T-cell responses and tumor growth.

It has been previously demonstrated that TLR triggered pDCs indirectly impact T-cell responses by activating other APCs (1). Our results, together with previous studies, highlight a direct role for pDCs as APCs in the modulation of T-helper activation and outcome. Our data further establish that, in contrast with what was described for cDCs, innate pDC functions are not altered by the loss of MHCII. Because MHCII machinery is differentially regulated in pDCs and cDCs (39, 40), regulation of TLR-triggered inflammatory responses may also be different in those cells, and, in contrast with cDCs (17), MHCII may not promote TLR signaling in pDCs. Altogether, our data demonstrate that loss of MHCII expression by pDCs leads to impaired Th17 responses without affecting pDC innate functions and that CpG-activated pDCs function as efficient pro-Th17 bona fide APCs.

CD45.1 cells (2 × 10⁶) and immunized or not in a contralateral manner with OVAα + CpG-B. Tumor growth was measured every 1 to 2 days. Results show the mean and SEM derived from 8 mice and are representative of two independent experiments. CD45.1+ CD8+ cells (%). Here, using mice lacking MHCII expression by pDCs, we show that upon CpG-B activation, pDCs drive Th17 cell differentiation through MHCII-dependent Ag presentation. For experimental reasons, our mouse model lacks peripheral B cells, which might not be neutral. However, we compared MHCII+ and MHCII− pDCs in a B-cell-deficient background, and investigated the selective impact of MHCII-mediated Ag presentation by pDCs on CD4+ T-cell responses and tumor growth.

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Figure 7: MHCII-mediated pDC-dependent beneficial effect of OVAα vaccine to OT-1 transfer in inducing tumor regression. A–C, EG7 cells were implanted s.c. in WT mice. A, 8 days later, mice were injected or not with depletion anti-CD8 mAbs (days 8, 11, 14) and immunized or not in a contralateral manner with OVAα + CpG-B (day 8). Tumor growth was measured every 1 to 2 days. Results show the mean and SEM derived from 6 mice and are representative of two independent experiments. B and C, 8 days later, mice were transferred or not with OT-1 CD45.1 cells (2 × 10⁶) and immunized or not in a contralateral manner with OVAα + CpG-B. B, tumor growth was measured every 1 to 2 days. Results show the mean and SEM derived from 6 mice and are representative of two independent experiments. C, OT-I cell frequencies (CD45.1+ cells) were analyzed in tumors 18 days after T-cell transfer. Representative flow-cytometric profiles and histograms represent a pool of two experiments with 4 mice/group. D, EG7 cells were implanted s.c. in μMT:WT and μMT pIII−IV−/−:WT mice. Eight days later, mice were transferred with OT-I limits tumor progression by enhancing CD8α+ DCs mediated cross-presentation of tumor Ags and subsequent CTL priming (34, 35). Consequently, IFNIs are used in the treatment of various cancers (32). However, pDC ability to produce IFNI is impaired in tumor microenvironment, therefore converting these cells into tolerogenic pDCs and leading to immunosuppression in several different cancers (for review, see ref. 33). In addition, in breast tumors in particular, pDCs induce Treg expansion in IDO and/or ICOS-dependent mechanisms (10–12). Accordingly, pDC infiltration in tumors has been correlated with poor clinical outcome (36, 37). Thus, promoting pDC activation in tumor context would restore IFNI production, inhibit Treg expansion, and represent an effective approach to induce antitumoral immune response. In agreement, CpG-A–activated, IFNI-producing pDCs injected directly into tumors initiate an effective and systemic antitumor immunity through the orchestration of an immune cascade involving the sequential activation of natural killer (NK) cells, cDCs, and CD8+ T cells (38). Moreover, TLR7 ligands or CpG-B intratumoral injection inhibit murine mammary tumor growth in a pDC-dependent manner (16). Interestingly, however, protection was linked to restoration of IFNI occurring after TLR7 ligand, but not after CpG-B, administration. Thus, although the role of pDC-mediated IFNI in antitumor immunity has been convincingly demonstrated, it remains unknown whether those cells could directly contribute to antitumoral T-cell immunity as APCs, and in particular after being activated by CpG-B.

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It has been previously demonstrated that TLR triggered pDCs indirectly impact T-cell responses by activating other APCs (1). Our results, together with previous studies, highlight a direct role for pDCs as APCs in the modulation of T-helper activation and outcome. Our data further establish that, in contrast with what was described for cDCs, innate pDC functions are not altered by the loss of MHCII. Because MHCII machinery is differentially regulated in pDCs and cDCs (39, 40), regulation of TLR-triggered inflammatory responses may also be different in those cells, and, in contrast with cDCs (17), MHCII may not promote TLR signaling in pDCs. Altogether, our data demonstrate that loss of MHCII expression by pDCs leads to impaired Th17 responses without affecting pDC innate functions and that CpG-activated pDCs function as efficient pro-Th17 bona fide APCs.

CD45.1 cells (2 × 10⁶) and immunized or not in a contralateral manner with OVAα + CpG-B. Tumor growth was measured every 1 to 2 days. Results show the mean and SEM derived from 8 mice and are representative of two independent experiments.
Tissue infiltrating pDC numbers were correlated with the presence of Th17 cells in mucosa and skin of GvHD patients (41, 42) and in mouse tumors (43, 44). pDC depletion during EAE leads to impaired encephalitogenic Th17 responses and decreased clinical scores (3). In vitro, pDCs activated with TLR7 or TLR9 ligands induce Th17 cell differentiation from either naive or memory T cells (45, 46). In addition, TGFβ-treated pDCs transferred into collagen-induced arthritis mice promote Th17 cells and exacerbate the disease (47). Depleting antibody experiments demonstrated that production of some cytokines by pDCs, namely TGFβ, IL6, IL1β, IL23, IFNγ, and TNFα depending on the studies, was involved in Th17 cell differentiation (44, 46, 47). We clearly determined that, in addition to pDC-derived cytokines that are preserved in our model, pDC Ag-presenting capacities are implicated in Th17 induction. However, mechanisms accounting for CpG-B-activated pDC ability to drive Th17 cells as APCs remain to be elucidated. Future experiments, including careful analysis of protein expression and dynamic interactions with T cells, will decipher intrinsic pDC properties, which allow them to promote distinct T-cell responses (Th1, Th17, Treg) depending on inflammatory contexts.

Subsequent tumor cell challenge showed that pDC-primed Th17 cells control tumor growth. Moreover, our data suggest that pDC-induced Th17 and Th1/17 cells promote inflammation and intratumor recruitment of immune cells, including Ag-specific CTLs, resulting in an increased antitumor immunity. Th17 cells implication in tumor immunity remains controversial. For instance, IL17 production favors angiogenesis and tumor cell survival (48, 49). Conversely, Th17 might improve tumor rejection by recruiting other immune cells such as DCs, Th, CTLs, and NK cells in the tumor (50–52). Accordingly, the IL17-induced CCL-2 and CXCL-2 chemokines, were decreased in tumor supernatants from mice lacking MHCI on pDCs. Decrease in CCL-2 and CXCL-2 chemokines, which induce the recruitment of lymphocytes, myeloid cells (53–55), and neutrophils (56), respectively, may explain impaired recruitment of several immune cell populations into tumors in mice lacking MHCI on pDCs. Altogether, our data demonstrated that MHCI+ pDC-derived, tumor-specific Th17 cells increase overall immune tumor infiltrate, including CTLs that would then mediate tumor rejection. Finally, and in agreement with our results showing a decrease in IFNγ IL17+ Th cells in absence of MHCI on CpG-activated pDCs, production of IFNγ by Th17 cells was shown to be essential for Th17-dependent tumor rejection (26).

Tumor microenvironment maintains pDCs in a partially activated phenotype that would prevent their ability to drive effective antitumor T-cell responses (10). Apparently, in tumor-bearing mice, immunization with a tumor Ag in presence of CpG-B in an ipsilateral manner, or transfer of CpG-B activated and tumor Ag-loaded pDCs, failed to inhibit tumor growth. However, tumor Ag immunization at a distal site leads to induction of tumor-specific Th17 cells, massive recruitment of immune cells in tumors, and inhibition of tumor growth. Importantly, those protective effects are abrogated in mice lacking MHCI on pDCs, demonstrating requirement of pDC Ag-presenting functions for effective tumor regression. Interestingly, efficacy of therapeutic vaccines was more potent than in a prevention setting. One probable explanation is that when the vaccine is administered to tumor-bearing mice, tumor-specific CTLs have already been primed, and as soon as some vaccine-derived Th17 cells are induced, and infiltrate solid tumors to promote intratumor recruitment of already primed endogenous tumor-specific CTLs. In contrast, in the case of preventive vaccine, Th17 cells are primed in the absence of any MHCI tumor Ags and consequently attract CTLs only once they have been generated, days after tumor challenge. OVA, vaccine-induced protection is abrogated in CD8-depleted mice, providing direct evidence that pDC-mediated Th17-induced tumor rejection relies on tumor-specific CTLs.

Notably, intratumoral specific CTL recruitment, as well as CTL-mediated tumor rejection, is significantly enhanced by concomitant vaccination with MHCI-restricted tumor Ag and CpG-B. Importantly, this effect is exclusively dependent on tumor Ag presentation by pDCs to CD4+ T cells and subsequent induction of antitumor Th17 responses. Thus, strategies aiming at enhancing tumor-specific CTL priming, such as MHCI tumor epitope vaccines, could be synergized by promoting CTL recruitment into tumors via induction of pDC-dependent tumor-specific Th17.

Here, we demonstrate that Ag-presenting activated pDCs induce potent Ag-specific Th17 cells, suggesting that pDCs could be used not only as inflammatory cytokines producers, but also as efficient APCs, to improve tumor vaccine efficacy. Those results pave the way for future manipulation to restore the antitumor T-cell responses, notably by combining targeting on both innate and adaptive pDCs functions.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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