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CUTTING EDGE

# Cutting Edge: CpG Oligonucleotides Induce Splenic CD19$^+$ Dendritic Cells to Acquire Potent Indoleamine 2,3-Dioxygenase-Dependent T Cell Regulatory Functions via IFN Type 1 Signaling[1]

*Andrew L. Mellor,[2]*† Babak Baban,* Phillip R. Chandler,*† Anna Manlapat,* David J. Kahler,* and David H. Munn*‡*

***CpG oligodeoxynucleotides (CpG-ODNs) stimulate innate and adaptive immunity by binding to TLR9 molecules. Paradoxically, expression of the immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO) is induced following i.v. CpG-ODN administration to mice. CpG-ODNs induced selective IDO expression by a minor population of splenic CD19$^+$ dendritic cells (DCs) that did not express the plasmacytoid DC marker 120G8. Following CpG-ODN treatment, CD19$^+$ DCs acquired potent IDO-dependent T cell suppressive functions. Signaling through IFN type I receptors was essential for IDO up-regulation, and CpG-ODNs induced selective activation of STAT-1 in CD19$^+$ DCs. Thus, CpG-ODNs delivered systemically at relatively high doses elicited potent T cell regulatory responses by acting on a discrete, minor population of splenic DCs. The ability of CpG-ODNs to induce both stimulatory and regulatory responses offers novel opportunities for using them as immunomodulatory reagents but may complicate therapeutic use of CpG-ODNs to stimulate antitumor immunity in cancer patients. The Journal of Immunology,* 2005, 175: 5601–5605.***

Mammalian TLRs are an extended family of molecular pattern recognition receptors expressed by cells of the innate and adaptive immune systems. Microbial DNA and synthetic oligonucleotides containing unmethylated CpG motifs (CpG-oligodeoxynucleotides (CpG-ODNs)[3]) bind to TLR9 (1). As summarized in recent reviews, TLR9-dependent immunostimulatory properties of CpG-ODNs depend on their ability to promote immune cell maturation and effector functions, including cytokine secretion and enhanced T cell stimulatory functions of dendritic cells (DCs) (2–4).

Recently, we described a minor population of murine splenic DCs expressing CD19 that responded to CTLA4-Ig-mediated ligation of CD80/86 (B7) molecules by expressing the intracellular enzyme indoleamine 2,3-dioxygenase (IDO) (5). IDO catalyzes the first and rate limiting step of oxidative tryptophan catabolism, and functional IDO expression is linked mechanistically to suppression of T cell-mediated immunity in multiple systems (6). Following B7 ligation, splenic DCs acquired potent IDO-dependent regulatory functions that prevented proliferation of alloantigen-specific T cells in vitro and in vivo (7, 8). This response was highly selective as IDO up-regulation occurred exclusively in minor DC populations expressing B220, CD8$\alpha$, and CD19 following B7 ligation. However, acquired T cell regulatory functions of CD19$^+$ DCs were potent and dominant because the underlying T cell stimulatory functions of the majority of splenic (IDO$^-$) DCs were not evident until they were separated from IDO$^+$ DCs.

In the current study, we evaluated the effects of CpG-ODN-mediated ligation of TLR9 on the T cell stimulatory functions of splenic DCs. We found that systemic administration of CpG-ODNs induced selective IFN-$\alpha$-dependent activation of STAT-1 and IDO up-regulation in splenic CD19$^+$ DCs, which acquired potent IDO-dependent T cell regulatory functions as a consequence.

## Materials and Methods

### *Mice*

All mice were bred in a specific pathogen-free facility at MCG. BM3 TCR-transgenic mice, IDO-deficient (IDO-knockout (KO)), and IFN-type I receptor (IFNAR)-deficient (IFNAR-KO) mice were described previously (5, 7, 9). All procedures involving mice were reviewed and approved by the local Institutional Animal Care and Use Committee.

### *Abs and immunohistochemistry*

Details of Abs and protocols used to detect IDO, CD11c, CD19, IFN-$\alpha$, IFN-$\gamma$, and phospho-STAT-1 (P-STAT-1) by immunohistochemical, immunofluorescence, and cytospin staining were described previously (5, 8, 9).

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[3] Abbreviations used in this paper: CpG-ODNs, oligonucleotides containing (no) unmethylated CpG motifs; DC, dendritic cell; IDO, indoleamine 2,3-dioxygenase; KO, knockout; 1mT, 1-methyl-D-tryptophan; P-STAT-1, phosphorylated STAT-1; pDC, plasmacytoid DC; DOTAP, *N*-[1-(2,3 dioleoyloxy)propyl]-*N,N,N*-trimethylammonium methylsulfate; IFNAR, interferon type I receptor.



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120G8 mAb (10) was supplied by Schering-Plough with kind permission from Drs. G. Trinchieri (National Institute of Allergy and Infectious Diseases, National Institutes of Health) and C. Asselin-Paturel (Dardilly, France) and was biotinylated using EZ-Link NHS-LC-Biotin (no. 21336; Pierce).

*CpG-ODNs*

CpG-ODNs (CpG-B no.1826, TCCATGA**CG**TTCCTGA**CG**TT; control non-CpG-B no.2138, TCCATGA*GC*TTCCTGA*GC*TT) with fully phosporothioate backbones were purchased from Coley Pharmaceuticals. For in vivo treatment, mice were injected i.v. with relatively high doses of ODN (50 $\mu$g/mouse); in previous reports, doses up to 20 $\mu$g/mouse were used to induce IDO in lung (11). For in vitro treatment, DCs were incubated with CpG-ODNs at 12.5 $\mu$g/ml.

*Preparation of DCs and MLRs*

Procedures for isolating DCs and DC subsets from spleen and to evaluate their T cell stimulatory functions in vitro using responder T cells from BM3 TCR-transgenic mice were described previously (5, 8). In brief, freshly isolated spleens were injected with collagenase, and homogenous cell suspensions were prepared by disrupting tissues. Cells were stained with Abs and AutoMacs ($CD11c^+$; >80% enriched), or preparative flow cytometric methods (>98% pure) were used to select specific DC populations. DCs were mixed with BM3 responder T cells and T cell proliferation assessed after 72 h in a thymidine incorporation assay.

*Preparative and analytical flow cytometry*

Preparative cell sorts were performed on cells stained with fluorochrome-conjugated mAbs (sources as detailed above) using a Mo-Flo 4 way flow cytometer (DakoCytomation) equipped with 488 nm argon (for FITC, PE, PE-CY5) and 647 nm krypton (for allophycocyanin) lasers. Cells were gated based on forward and side scatter properties and on marker combinations to select cells of interest. Analytical flow cytometry to detect intracellular P-STAT-1 was performed by staining splenocytes from CpG-ODN-treated mice with CD11c and CD19 mAbs before fixing cells and staining with rabbit anti-mouse P-STAT-1$\alpha$ ($Tyr^{701}$) Ab using the manufacturer's protocol.

*Western blot analysis to detect P-STAT-1*

Cell lysates from freshly isolated splenocytes were gel electrophoresed and stained to detect P-STAT-1$\alpha$ and $\beta$-actin using Ab manufacturer's protocols, as described previously (12).

## Results and Discussion

*CpG-ODNs induce selective IDO up-regulation in splenic $CD19^+$ DCs*

B6 mice were injected i.v. with relatively high doses (50 $\mu$g/mouse) of CpG-ODN (no. 1826; CpG-B) or non-CpG-ODN (no. 2138) with a near-identical DNA sequence containing no CpG motifs, and IDO expression was assessed 24 h later by immunohistochemical analysis of spleen. In CpG-ODN-treated mice, $IDO^+$ cells were dispersed throughout splenic red pulp, but not in lymphoid follicles (Fig. 1*A*), and displayed a distinctive plasmacytoid-like morphology (data not shown). Similar patterns of IDO expression were observed in mice exposed to soluble CTLA4 (CTLA4-Ig), which ligates B7 molecules (5, 8). As in these previous studies, few $IDO^+$ cells were present in spleen of untreated mice, and treatment with non-CpG-ODN did not increase the number of $IDO^+$ cells (Fig. 1*B*). IDO up-regulation in spleen was not detected when mice were treated with lower amounts of CpG-ODNs injected i.v. (no. 1826, $\leq$40 $\mu$g) or when CpG-ODNs (no. 1826; 50 $\mu$g) were injected into the peritoneum (data not shown). Two-color immunofluorescence staining revealed that many $IDO^+$ cells coexpressed CD19 (Fig. 1*C*). In contrast, $CD19^+$ cells located in lymphoid follicles (i.e., B cells) did not coexpress IDO. No increases in numbers of $IDO^+$ cells were observed in other lymphoid tissues from mice exposed to CpG-ODNs (data not shown).

To further characterize $IDO^+$ cells, splenic DC populations were purified from untreated B6 mice by rapid preparative flow cytometry (Mo-Flo, > 98% pure) using gating criteria (Fig. 1*D*) for CD11c and CD19 expression, as described previously (5, 8). Purified DCs were incubated with CpG-ODN no. 1826 (10 $\mu$g/ml), and 24 h later, cytospins were stained with anti-IDO Ab. Most (>90%) purified $CD11c^{high}CD19^+$ DCs expressed IDO, while few (<5%) cells expressed IDO in purified $CD11c^{high}CD19^-$ and $CD11c^{low}$ DC populations (Fig. 1*D*). Non-CpG-ODN (no. 2138) did not induce IDO expression in any purified DC subset (data not shown). Collectively, these data revealed that CpG-ODNs induced selective IDO up-regulation in a minor subset of $CD19^+$ DCs located in splenic red pulp.

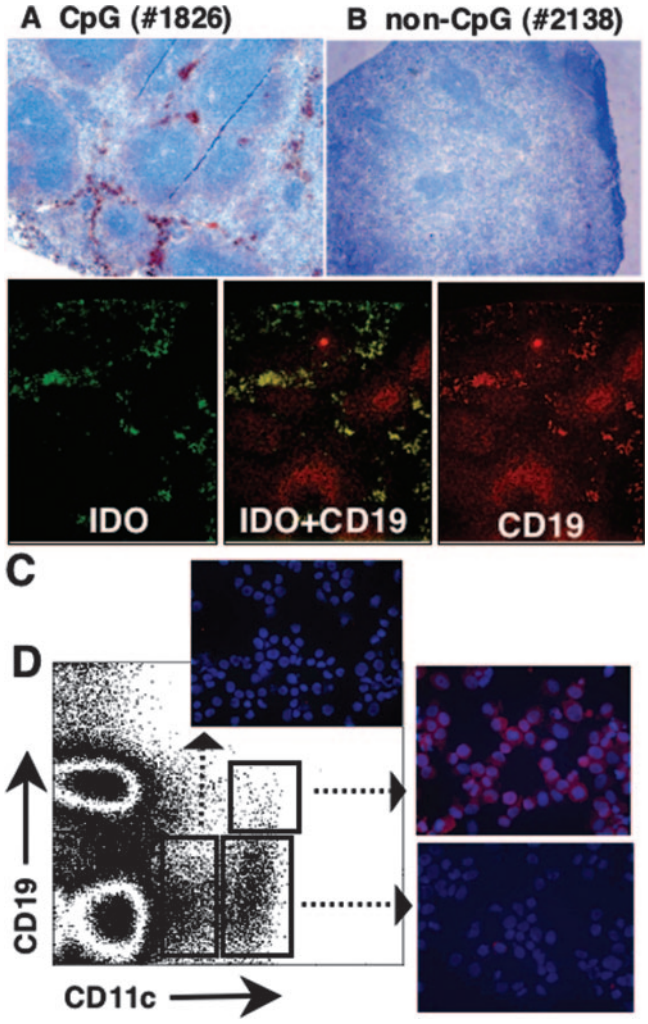


**FIGURE 1.** CpG-ODNs induce IDO expression exclusively in splenic $CD19^+$ DCs. *A–C*, Mice (B6) were injected (50 $\mu$g, i.v.) with CpG-ODN (no. 1826; *A* and *C*) or control non-CpG-ODN (no. 2138; *B*), as described in *Materials and Methods*. Mice were sacrificed 24 h later, and spleen tissue sections were stained with anti-IDO Ab and H&E counterstained (*A* and *B*) or with anti-IDO Ab and anti-CD19 mAb and stained cells visualized by immunofluorescence (*C*). *D*, Splenic DC populations from B6 mice were purified by preparative flow cytometry based on their CD11c and CD19 staining profiles as shown. DCs were incubated with CpG-ODN (no. 1826; 10 $\mu$g/ml) for 24 h, and cytospins were stained with a Hoechst nuclei dye (blue) and cells stained with anti-IDO Ab were visualized by immunofluorescence (red). Original magnifications, ×100 (*A–C*); ×400 (*D*).

IDO up-regulation in DCs following CpG-ODN administration appears paradoxical because CpG-ODNs have potent immunostimulatory properties (4), and induced IDO expression correlates with potent inhibition of T cell-mediated adaptive immunity in experimental models of tumor growth, tissue

transplantation, autoimmune disease, and pregnancy (6, 13). One potential resolution of this paradox is that high doses of CpG-ODNs (≥50 μg/mouse) administered i.v. were needed to up-regulate IDO in spleen. Recently, Raz and colleagues (11) reported that CpG-ODNs suppressed symptoms of asthma in an experimental mouse model due to induction of IDO. In this study, 20 μg/mouse of CpG-ODNs were injected i.v., which induced IDO in lung DCs and epithelial cells. However, this lower dosing regimen did not induce IDO activity in spleen. Our findings were consistent with these outcomes because 50 μg/mouse of CpG-ODN no. 1826 was the minimum dose required to up-regulate IDO in spleen. It is unclear why injection of 50 μg/mouse of CpG-ODN into the peritoneum did not induce IDO in spleen; presumably, this is due to different pharmacokinetics of CpG-ODNs administered directly into blood or into the peritoneum. Highly selective IDO induction in splenic $CD19^+$ DC populations was also observed in our previous studies using CTLA4-Ig as a B7 ligand (5, 8).

### *CpG-ODNs induce $CD19^+$ DCs to mediate IDO-dependent T cell suppression*

To test whether CpG-ODN (i.v.) administration affected T cell stimulatory functions of splenic DCs, we purified specific DC populations from spleen of CpG-ODN-treated mice and assessed their ability to stimulate T cell proliferation in vitro, as described previously (5, 8). Splenic $CD11c^+$ (AutoMacs enriched) DCs from B6 (wild-type) mice exposed to CpG-ODNs (no. 1826) did not stimulate T cell proliferation (Fig. 2*A*).

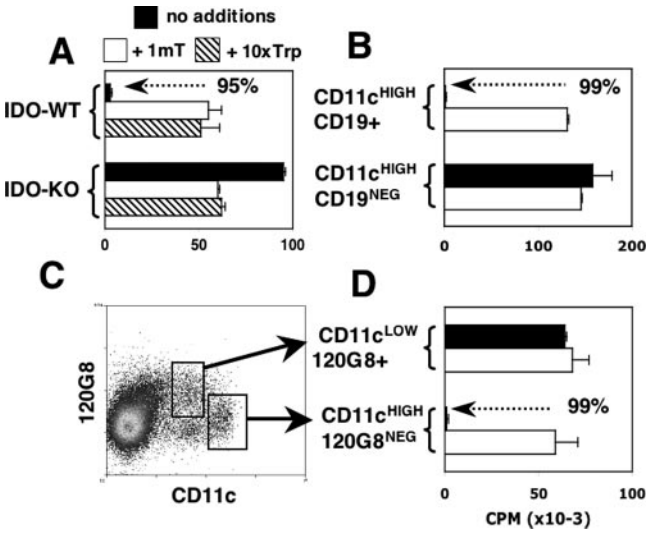


**FIGURE 2.** $CD19^+$ DCs acquire potent IDO-dependent, T cell-suppressive functions following CpG-ODN treatment. *A*, B6 (wild-type (WT)) and IDO-KO mice were treated with CpG-ODN, and 24 h later, splenic $CD11c^+$ DCs were isolated by AutoMACS and used as stimulators in MLRs with H-$2K^b$-specific responder T cells from BM3 TCR-transgenic mice with (□) or without (■) addition of IDO inhibitor, 1mT (100 μM), or excess tryptophan (▨) as described in *Materials and Methods*. *B–D*, Purified DC subsets were isolated by preparative flow cytometry based on CD11c and CD19 (*B*) and CD11c and 120G8 (*C* and *D*) expression and used as stimulators in MLRs. Mean DC yields were as follows, expressed as percentage of total splenic ($CD11c^+$) DCs; $CD11c^{high}CD19^+$ (~10%), $CD11c^{high}CD19^-$ (~40%); $CD11c^{low}120G8^-$ (~25%), $CD11c^{high}120G8^+$ (~25%). *C*, Gating criteria for CD11c and 120G8 staining to purify DC subsets used in MLRs shown in *D*. *A*, *B*, and *D* indicate (percentage) suppression of T cell proliferation due to IDO activity expressed relative to control MLRs containing 1mT.

However, addition of IDO inhibitor 1-methyl-D-tryptophan (1mT) (100 μM) or excess tryptophan (10× Trp, 250 μM final) to MLRs restored potent T cell stimulatory functions DCs from CpG-ODN-treated mice. In contrast, DCs from IDO-KO (B6) mice treated with CpG-ODNs stimulated robust T cell responses that were not enhanced by addition of 1mT or excess tryptophan.

Consistent with previous studies on DCs from CTLA4-Ig-treated mice (5, 8), preliminary studies revealed that purified $CD11c^{high}$, but not $CD11c^{low}$, DCs from CpG-ODN-treated mice mediated IDO-dependent T cell suppression (data not shown). Therefore, we tested whether purified populations of splenic DCs expressing CD19 and plasmacytoid DCs (pDCs) expressing the marker 120G8 (10) mediated IDO-dependent T cell suppression following CpG-ODN administration. We purified $CD11c^{high}CD19^+$ DCs and $CD11c^{high}CD19^-$ DCs from CpG-ODN-treated B6 mice using gating criteria described previously (5). Purified $CD11c^{high}CD19^+$ DCs did not stimulate T cell proliferation, unless 1mT was added, while $CD11c^{high}CD19^-$ DCs stimulated robust T cell proliferation, which was not enhanced in the presence of 1mT (Fig. 2*B*). In separate experiments, we purified pDCs expressing 120G8 and relatively low levels of CD11c ($CD11c^{low}120G8^+$) and compared their ability to stimulate T cell proliferation with copurified non-pDCs, defined as $CD11c^{high}120G8^-$ (Fig. 2*C*). $CD11c^{high}120G8^-$ DCs from CpG-ODN-treated mice did not stimulate T cell proliferation, unless 1mT was added to MLRs, which restored robust T cell stimulatory functions (Fig. 2*D*). In contrast, copurified pDCs ($CD11c^{low}120G8^+$) stimulated robust T cell responses, which were not further enhanced by adding 1mT. In each case, purified DC populations that suppressed T cell proliferation following in vivo CpG-ODN exposure were minor DC subsets (~15–25% of total splenic $CD11c^+$ DCs). However, as in previous studies with DCs from CTLA4-Ig-treated mice (5, 8), T cell regulatory functions of these minor DC populations predominated over the T cell stimulatory functions of the majority of splenic DCs (e.g., $CD11c^{high}CD19^-$ DCs and pDCs), which manifested only when they were separated from DCs that mediated IDO-dependent T cell suppression (Fig. 2 and data not shown). Thus, IDO-dependent suppression was mediated exclusively by a discrete, minor population of splenic $CD19^+$ DCs that could be distinguished from pDCs expressing 120G8 following systemic exposure to CpG-ODNs.

In a recent study, Fallarino et al. (14) reported that splenic pDCs, defined as $CD11c^+B220^+120G8^+$, suppressed T cell-mediated delayed-type hypersensitivity responses following CD200R ligation due to selective IDO induction in this DC subset. In the current study, systemic CpG-ODN exposure did not inhibit the robust T cell stimulatory functions of pDCs, defined as $CD11c^{low}120G8^+$. The distinct outcomes from the two studies might arise for several reasons, including use of different mouse strains, different ligands to induce IDO (which may act on distinct DC populations), and use of different methods to isolate DC populations and to assess their T cell stimulatory functions. Because IDO-dependent T cell suppressive functions of splenic $CD19^+$ DCs were both potent and dominant (as well as exclusive) properties of these DCs, immunoregulatory outcomes could emerge when minor cohorts of $CD19^+$ DCs are present among much larger cohorts of other DCs with T cell stimulatory functions.

*IFN-α signaling is essential for STAT-1 activation and IDO up-regulation in CD19$^+$ DCs*

To examine the mechanism of IDO up-regulation following CpG-ODN treatment, we injected CpG-ODNs into mice with defective IFN type I receptors (IFNAR-KO) and control BALB/c mice and assessed IDO expression in spleen as before. As in B6 mice, CpG-ODNs (no. 1826) induced IDO expression in spleen of BALB/c mice (Fig. 3*A*). In contrast, CpG-ODNs did not induce IDO up-regulation in spleens of IFNAR-KO mice (Fig. 3*B*). IFN-α signaling was also essential for STAT-1 activation because P-STAT-1 was not induced in splenocytes from IFNAR-KO (B6 background) mice exposed to CpG-ODNs, while STAT-1 activation was detected in splenocytes from treated IFN-γ R-KO (129/SvJ background) mice (Fig. 3*C*). This response was highly selective as P-STAT-1 was detected exclusively in CD19$^+$ DCs following flow cytometric analyses (Fig. 3*D*).

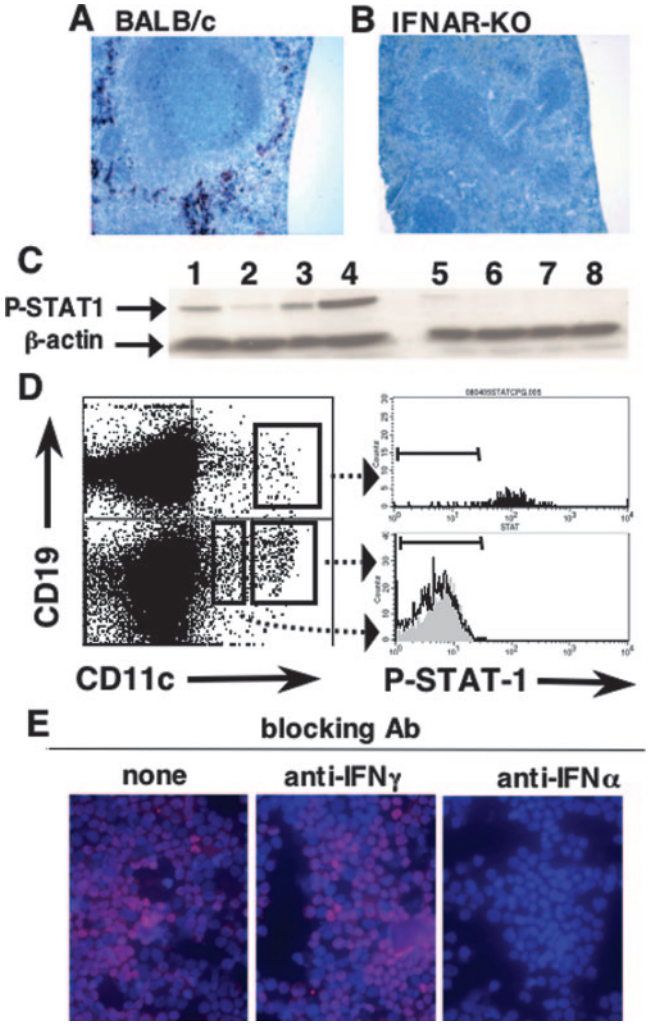


**FIGURE 3.** IFN-α induces STAT-1 activation and IDO up-regulation in CD19$^+$ DCs following CpG-ODN exposure. *A* and *B*, IDO expression in spleen of BALB/c (*A*) and IFNAR-deficient (*B*) mice was assessed by immunohistochemical analysis following CpG-ODN (no. 1826) administration (50 μg, i.v.). *C*, Cell lysates of splenocytes from BALB/c (*lanes 1* and *5*), IFNAR-KO (*lanes 2* and *6*), 129/SvJ (*lanes 3* and *7*), and IFN-γ R-KO (*lanes 4* and *8*) mice treated with CpG- (*lanes 1–4*) or non-CpG-ODNs (*lanes 4–8*) were analyzed by Western blot to detect P-STAT-1 and β-actin. *D*, Splenocytes from B6 mice treated with CpG-ODNs were stained with CD11c, CD19, and anti-P-STAT-1 mAbs and analyzed by flow cytometry. A two-color plot shows gates used to assess P-STAT-1 expression on gated CD19$^+$ and CD19$^-$ (▨, CD11c$^{low}$; line, CD11c$^{high}$) DCs in histograms. Bars on histograms indicate P-STAT-1 staining profile (>95% of cells) for DCs from untreated mice. *E*, AutoMacs-enriched CD11c$^+$ DCs from BALB/c mice were incubated with CpG-ODNs (10 μg/ml) in the absence or in the presence of blocking anti-IFN-γ or anti-IFN-α mAbs as indicated. After 5 h, cytospins were stained with Hoechst nuclear dye (blue) and anti-P-STAT-1 Ab, as described in *Materials and Methods*. Original magnifications, ×100 (*A* and *B*); ×400 (*E*).

Signaling requirements for STAT-1 activation were analyzed further by incubating CD11c$^+$ DCs (AutoMACS enriched) from untreated BALB/c mice with CpG-ODNs (10 μg/ml) for 5 h and staining cytospins to detect P-STAT-1. Activated P-STAT-1 was detected in ~50% of DCs and had translocated to nuclei of ~30% of DCs (Fig. 3*E*). This response was not observed when CD11c$^+$ DCs were incubated with non-CpG-ODNs at the same concentration (data not shown). Addition of anti-IFN-γ mAb to cultures had no effect on STAT-1 activation induced by CpG-ODNs. However, STAT-1 activation was blocked completely when anti-IFN-α mAb was present. Collectively, these data revealed that IFN-α, but not IFN-γ, signaling was essential for STAT-1 activation and IDO up-regulation in CD19$^+$ DCs following CpG-ODN treatment.

The outcomes reported above implied that IFN-α production occurred before selective STAT-1 activation in CD19$^+$ DCs after CpG-ODN treatment. Based on previous studies (15–18), pDCs are a likely source of IFN-α after CpG-ODN treatment. Purified CD11c$^{high}$ and CD11c$^{low}$ DCs secreted IFN-α rapidly after culture with CpG-ODNs (10 μg/ml, 5 h; data not shown). Because highly purified CD19$^+$CD11c$^{high}$ DCs up-regulated IDO after culture with CpG-ODNs (Fig. 1*D*), these DCs may also express sufficient IFN-α to induce STAT-1 activation and IDO up-regulation following TLR9 ligation (at least in vitro). Thus, IFN-α from pDCs may signal STAT-1 activation and subsequent IDO up-regulation in CD19$^+$ DCs via a paracrine mechanism, although the possibility that CD19$^+$ DCs also produce IFN-α following CpG-ODN treatment cannot be excluded completely. Recent reports show that a cationic lipid *N*-[1-(2,3 dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methylsulfate enhanced TLR9-mediated IFN-α production by DCs (17, 18). Because DOTAP was not required to induce IDO after CpG-ODN treatment, relatively low amounts of IFN-α may be sufficient to signal IDO up-regulation by CD19$^+$ DCs. These issues notwithstanding, the key point is that rapid IFN-α production by DCs in response to TLR-9 ligation, whether autocrine or paracrine, is a critical mechanistic event that causes selective IDO induction in CD19$^+$ DCs.

Microbial DNA containing unmethylated CpG motifs stimulates immune cell maturation and IFN-α production in humans and mice (2, 4, 19). Classically, both events have been considered proinflammatory. However, it is not unusual in the immune system for proinflammatory stimuli to also elicit counterregulatory, anti-inflammatory responses that follow in short sequence. In mice, microbial infections, bacterial DNA containing CpG motifs, and synthetic CpG-ODNs induce a unique subset of splenic (B220$^+$,CD11c$^{low}$,120G8$^+$) pDCs to produce IFN-α (10, 15, 16). In these (as in many other) previous studies, CD19 was used as an exclusion marker to remove B cells during purification of DC populations. Hence, to our knowledge, the effects of CpG-ODNs on CD19$^+$ (CD11c$^{high}$120G8$^-$) DCs identified in the current study,

which accounted for <20% of all splenic DCs, have not been described previously. $CD19^+$ DCs were the principal DC population to activate STAT-1, up-regulate IDO, and acquire potent IDO-dependent T cell-suppressive functions following i.v. administration of relatively high doses of CpG-ODNs to B6 mice. These responses by $CD19^+$ DCs were dependent on IFN-$\alpha$ signaling, suggesting that $CD19^+$ DCs may be specialized to respond to CpG-ODN-mediated TLR9 ligation by producing IFN-$\alpha$ themselves or by responding to IFN-$\alpha$ produced by pDCs in response to TLR9 ligation. The different anatomic locations of $IDO^+CD19^+$ DCs in splenic red pulp and pDCs, which reside in T cell areas of secondary lymphoid tissues (10), testifies to the distinctive characteristics of these DC subsets, even though they share some morphologic (plasmacytoid) and phenotypic ($B220^+$) characteristics. By ligating B7 molecules, CTLA4-Ig induced IFN-$\alpha$-dependent STAT-1 activation and IDO up-regulation in a closely related (if not identical) population of splenic $CD19^+$ DCs that coexpressed B220 and/or CD8$\alpha$ (5, 8). Thus, splenic $CD19^+$ DCs are specialized to respond to IFN-$\alpha$ induced after TLR9 and B7 ligation by acquiring potent IDO-dependent T cell regulatory functions.

The potent immunostimulatory effects of CpG-ODNs have been well characterized, and CpG-ODNs are currently being evaluated clinically as vaccine adjuvants to enhance T cell-mediated antitumor immunity in cancer patients. However, CpG-ODNs also inhibited Th2-mediated inflammation in experimentally induced asthma (11). The inducible IDO-dependent T cell regulatory functions of splenic $CD19^+$ DCs may explain some of the counterinflammatory effects of DNA and ODNs containing CpG motifs. However, the remarkable dichotomy of functional effects induced by CpG-ODNs emphasizes the critical need to identify underlying biological mechanisms that determine responses to CpG-ODNs as immunomodulatory reagents.

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## Disclosures

The authors have no financial conflict of interest.

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