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A GpC-Rich Oligonucleotide Acts on Plasmacytoid Dendritic Cells To Promote Immune Suppression

Claudia Volpi,* Francesca Fallarino,* Roberta Bianchi,* Ciriena Orabona,* Antonella De Luca,* Carmine Vacca,* Luigina Romani,* Bruno Gran,† Ursula Grohmann,* Paolo Puccetti,* and Maria L. Belladonna*

Short synthetic oligodeoxynucleotides (ODNs) rich in CpG or GpG motifs have been considered as potential modulators of immunity in clinical settings. In this study, we show that a synthetic GpC-ODN conferred highly suppressive activity on mouse splenic plasmacytoid dendritic cells, demonstrable in vivo in a skin test assay. The underlying mechanism involved signaling by noncanonical NF-κB family members and TGF-β–dependent expression of the immunoregulatory enzyme IDO. Unlike CpG-ODNs, the effects of GpC-ODN required TLR7/TRIF-mediated but not TLR9/MyD88-mediated events, as do sensing of viral ssRNA and the drug imiquimod. Induction of IDO by a GpC-containing ODN could also be demonstrated in human dendritic cells, allowing those cells to assist FOXP3+ T cell generation in vitro. Among potentially therapeutic ODNs, this study identifies GpC-rich sequences as novel activators of TLR7-mediated, IDO-dependent regulatory responses.  


One of the strategies used by mammalian cells in innate and adaptive immunity to sense pathogens implies selective recognition of unmethylated oligonucleotide sequences containing CpG motifs, particularly abundant in bacterial and viral but not mammalian genomic DNA (1). Surveillance mechanisms in the host involve, indeed, pattern recognition receptors occurring on the cell surface and in the cytoplasm, TLR7, TLR8, and TLR9, which are present in intracellular compartments, respond to single-stranded nucleic acids, activating innate and adaptive immune responses. TLR9, expressed by dendritic cells (DCs), B cells, and macrophages, not only reacts to pathogen-associated molecular patterns but also binds short synthetic CpG-containing oligodeoxynucleotides (ODNs), resulting in lymphocyte maturation, enhanced APC function, and the release of inflammatory cytokines and type I IFNs (2). These remarkable immunostimulatory properties of short CpG-ODNs, and mostly of those with phosphorothioate-stabilized backbone (3), have suggested a role for TLR9 in the induction of IDO, an enzyme that—dependent expression of the immunoregulatory enzyme IDO. Unlike CpG-ODNs, the effects of GpC-ODN required TLR7/TRIF-mediated but not TLR9/MyD88-mediated events, as do sensing of viral ssRNA and the drug imiquimod. Induction of IDO by a GpC-containing ODN could also be demonstrated in human dendritic cells, allowing those cells to assist FOXP3+ T cell generation in vitro. Among potentially therapeutic ODNs, this study identifies GpC-rich sequences as novel activators of TLR7-mediated, IDO-dependent regulatory responses. 

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Abbreviations used in this article: DC, dendritic cell; FL-DC, FLT3 ligand–cultured dendritic cell; 1-MT, 1-methyl-1-tryptophan; ODN, oligodeoxynucleotide; pDC, plasmacytoid dendritic cell; siRNA, small interfering RNA; TRIF, TIR domain–containing adapter inducing IFN-β.

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the protection of the lung against influenza A virus infections, mainly viaTLR7 signals (24).

In this study, we investigated the possible immunoregulatory effects of GpC-ODNs. We found that 1) a GpC-ODN selectively conferred suppressive properties on pDCs, contingent on functional IDO; 2) the induction of IDO by GpC depended on autocrine TGF-β and noncanonical NF-κB transcriptional activity; and 3) the downstream response culminating in IDO induction required TLR7/TIR domain-containing adapter inducing IFN-β (TRIF)-mediated, but not TLR9-mediated, signaling events.

Materials and Methods

Mice and reagents

Female C57BL/6 mice were obtained from Charles River. Mice homozygous for the TLR9 (Tlr9-/-), Myd88 (Myd88-/-), or TRIF (Ticam1-/-) targeted mutation raised on the C57BL/6 background were generated as described (13) and bred at the animal facility of the University of Perugia. Mice homozygous for the TLR7 (Tlr7-/-) mutation were obtained from The Jackson Laboratory. The HY peptide (amino acid sequence, WMHHNMDLI) was synthesized and purified as described (25). Endotoxin-free GpC-ODN 1826 (5'-TCTGCGGGAATCTCCATTGTTCCAGGTCTT-3') and the negative control thereof (5'-TCTATGAGGCTCCATTTAAATTT-3'), both on a phosphorothioate backbone, were purchased from Invitrogen Life Technologies. In selected experiments (Fig. 1), a second ODN was used (1668; 5'-TCTATGAGGGCTCTTACCTGAGATC-3') after replacing the CpG motif with a GpC sequence (negative control ODN, 5'-TCTGCGGGAATCTCCATTGTTCCAGGTCTT-3') in order to determine the effects of the GpC-ODN relative to the CpG control. Endotoxins were removed, when measured by the Limulus assay (Sigma-Aldrich), which was negligible in all ODN preparations. Imiquimod (Sequoya Research Products, Bangor, UK) was used at the concentration of 40 ng/ml. All in vivo studies were done in compliance with national (Italian Parliament DL 116/92) and Perugia University Animal Care and Use Committee guidelines.

DC preparations and treatments

Splenic DCs were prepared and fractionated according to CD11c+CD8 expression using positive selection columns in combination with CD11c and CD8 MicroBeads (Miltenyi Biotec) and in the presence of EDTA to disrupt DC–T cell complexes, as previously described (26). The recovered cells were >99% CD11c+ (>98% MHC-I-A*, >98% B7-2*, >98% CD3+), and appeared to consist of 90–95% CD8+, 5–10% CD8’, and ~5% mPDCA-1+ cells. After cell fractionation, the recovered CD8+ cells were ~45% CD4+ and typically contained <0.5% contaminating CD8+ DCs, whereas the CD8’ fraction was made up of >95% CD8+ DCs. For positive selection of mPDCA-1+ pDCs, we fractionated CD11c+ cells using mPDCA-1 MicroBeads (Miltenyi Biotec). More than 95% of the mPDCA-1+ cells were stained by the 120G8 marker. pDCs were exposed for the indicated times to 1 μg/ml GpC-ODN and, after 18 h, L-kynurenine, the initial tryptophan catabolite, was measured in culture supernatants by HPLC, as described (26). The recovered cells were washed, and immediately used for in vitro or in vivo experiments. Nuclear localization of NF-κB subunits was assessed by immunoblotting using anti-p105/p50 (Cell Signaling) and anti-p65 Abs (Santa Cruz Biotechnology). Anti-nucleolin Ab (Santa Cruz Biotechnology) was used as a normalizer. IDO expression was investigated in cells cultured for 24 h with or without 1 μg/ml GpC-ODN (in specific experiments in the presence of neutralizing anti-TGF-β, 50 μg/ml, or an isotype control) by immunoblotting with rabbit anti-mouse IDO mAb raised in our laboratory (27). Anti–β-actin Ab (Sigma-Aldrich) was used as a normalizer.

Results

GpC-ODN confers tolerogenic activity on pDCs

Unmethylated synthetic ODNs affect immune responsiveness in different ways (29). A single base substitution of guanine for cytosine in the CpG motif converts this mostly stimulatory ODN into an autoimmune-suppressive GpG-ODN (21). We asked whether a guanine/cytosine inversion from CpG to GpC in ODN 1826 (hereafter referred to as GpC-ODN) would also affect the qualitative nature of ODN effects. Control and GpC-ODNs—on the presence of neutralizing anti-TGF-β, 50 μg/ml, or an isotype control—by immunoblotting with rabbit anti-mouse IDO mAb raised in our laboratory (27). Anti–β-actin Ab (Sigma-Aldrich) was used as a normalizer. For measurement of IDO functional activity, pDCs were stimulated with 1 μg/ml GpC-ODN and, after 18 h, t-kyurenine, the initial tryptophan catabolite, was measured in culture supernatants by HPLC, as described (28).

Human FLT3 ligand-cultured dendritic cell preparation and treatment

Human mature FLT3 ligand-cultured dendritic cells (FL-DCs) were obtained from PBMCs. Briefly, PBMCs were separated from whole blood of healthy donors by centrifugation through a density gradient of Ficoll-Paque (GE Healthcare). Whole blood was layered onto a sterile aqueous medium containing Ficoll at a predetermined density of 1.077 g/ml at 25°C. Gentle centrifugation at room temperature resulted in the separation of PBMCs at the blood–Ficoll interface, with the other WBCs and RBCs passing through the interface and accumulating at the bottom of the tube. Human FL-DCs were obtained from purified CD14+ monocytes from healthy donors by culturing cells in Iscove modified medium for 7 d in the presence of 50 ng/ml human FLTL3 (Peprotech). The resulting FL-DCs were washed and cultured for 18 h with 1 μg/ml of a GpC-ODN containing ODN 2006 (5'-TTGTCGTTTTTGGCTTTTGTGCTT-3') or negative control ODN 5'-TATATTGTGTATTTGATTAT-3'). FACS analysis revealed that FL-DCs were CD123+ CD11c+low BDC2+ and BDC4+. ODN-treated FL-DCs were cocultured with CD4+ T cells isolated from the same donor for 4 d, and FOXP3 expression was revealed by intracellular FACS analysis by means of a specific Alexa Fluor 488-labeled Ab (BioLegend).

Small interfering RNA synthesis and transfection, ELISA, and IDO functional analysis

Small interfering RNA (siRNA) sequences targeting Idol (sense, 5'-GGGCUUUUCUCCUGUCUCUt-3'; antisense, 5'-AGAGACGAGGAA-4AGCCCTt-3'), Chuk (sense, 5'-GAAUAAACAGGCUUCCUt-3'; antisense, 5'-GAAGACCUGUAUUUUCCt-3'), and Hdh (sense, 5'-GGUGCAUUAUAUUUCUAtt-3'; antisense, 5'-UUUAAGUAGAUGAUCCTgt-3') and control, scrambled sequences were synthesized as described (26). For transfection, siRNAs (6.7 μg) in 30 μl transfection buffer (20 mM HEPES, 150 mM NaCl; pH 7.4) were pipetted into a sterile Eppendorf tube. In a separate tube, 6.7 μg 1,2-dioleoyl-3-trimethylammonium-propane was mixed with 30 μl transfection buffer, and then both solutions were gently mixed. After incubation at room temperature for 20 min, the mixture was added to 1 ml complete medium containing 107 pDCs and incubated for 24 h at 37°C. In the meantime, GpC-ODN or negative control ODN was added 5 h after the specific silencing procedure. Cells were then recovered, washed, and immediately used for in vitro or in vivo experiments. Nuclear localization of NF-κB subunits was assessed by immunoblotting using anti-p105/p50 (Cell Signaling) and anti-p65 Abs (Santa Cruz Biotechnology). Anti-nucleolin Ab (Santa Cruz Biotechnology) was used as a normalizer. IDO expression was investigated in cells cultured for 24 h with or without 1 μg/ml GpC-ODN (in specific experiments in the presence of neutralizing anti-TGF-β, 50 μg/ml, or an isotype control) by immunoblotting with rabbit anti-mouse IDO mAb raised in our laboratory (27). Anti–β-actin Ab (Sigma-Aldrich) was used as a normalizer. For measurement of IDO functional activity, pDCs were stimulated with 1 μg/ml GpC-ODN and, after 18 h, t-kyurenine, the initial tryptophan catabolite, was measured in culture supernatants by HPLC, as described (28).
inversion was shared by another ODN (1668) in which the CpG sequence had likewise been replaced by a GpC motif (Fig. 1). Therefore, GpC-ODNs, used in vitro for conditioning pDCs, conferred strong suppressive activity on those cells, preventing the induction of skin test reactivity.

Tolerogenicity by GpC-ODN requires IDO

In previous studies, we have found that most molecules acting as tolerogenic stimuli in the skin test assay require functional IDO expression by DCs, used for sensitization either as a single population or as a minority fraction on cotransfer with immunogenic (IDO-negative) cells (31). To investigate a possible role for IDO in the suppressive activity of GpC-ODN–conditioned pDCs, we used immunogenic CD8– DCs admixed with 5% pDCs treated with GpC-ODN (or negative control ODN) for skin test sensitization. The conditioned pDCs were used either as such or after treatment with the IDO inhibitor 1-MT or after silencing of \( \text{Ido1} \) by specific siRNA (or control, scrambled siRNA) (Fig. 2A). The suppressive effect associated with coinjection of GpC-ODN–treated pDCs was negated by either maneuver, namely enzyme inhibition (via 1-MT treatment) or \( \text{Ido1} \) silencing. Moreover, immunoblot (at 24 h; Fig. 2B) and L-kynurenine level assessment (at 18 h; Fig. 2C) revealed increased IDO expression and function in pDCs exposed to GpC-ODN but not to negative control ODN. These data suggest that the suppressive effect of GpC-ODN in fostering a tolerogenic phenotype in pDCs was negated by either maneuver, namely enzyme inhibition (via 1-MT treatment) or \( \text{Ido1} \) silencing. Moreover, immunoblot (at 24 h; Fig. 2B) and L-kynurenine level assessment (at 18 h; Fig. 2C) revealed increased IDO expression and function in pDCs exposed to GpC-ODN but not to negative control ODN. These data suggest that the suppressive effect of GpC-ODN in fostering a tolerogenic phenotype in pDCs was contingent on IDO expression and function when GpC-ODN was used at concentrations at which CpG-ODN is instead proinflammatory (32). GpC was invariably tolerogenic when tested over a wide range of concentrations, that is, up to 10 \( \mu \text{g/ml} \) (data not shown).

IDO induction by GpC-ODN requires TGF-\( \beta \)

In most experimental settings, IDO induction requires either type I/type II IFNs (28) or TGF-\( \beta \) (33). We investigated whether IDO induction by GpC-ODN is a direct or, rather, an indirect effect, requiring specific cytokine production. Purified pDCs were exposed to GpC-ODN or negative control ODN (1 \( \mu \text{g/ml} \), over-night), and supernatants were assayed for production of IFN-\( \alpha \), IFN-\( \gamma \), TGF-\( \beta \), IL-10, IL-6, and IL-23 (Fig. 3A). TGF-\( \beta \) was the only cytokine significantly induced by the GpC-ODN, and its production appeared to be necessary for IDO protein expression (Fig. 3B). Neutralization of TGF-\( \beta \) by specific Ab negated the tolerogenic effect conferred by GpC-ODN on pDCs, as measured in a skin test assay (Fig. 3C).

\( \text{GpC-ODN activates noncanonical NF-\( \kappa \text{B} \) in a TRIF-dependent manner} \)

Besides their potent stimulatory role in innate immunity, pDCs also provide suppressive function—detectable by skin test assay—once cells are primed with tolerogenic signals (including high-dose CpG-ODN, CTLA-4-Ig, CD200-Ig, 4-1BB ligand, CD40L, and

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**FIGURE 1.** GpC-ODN 1826 confers suppressive properties on pDCs. A mixture of CD8– DCs and 5% pDCs, both pulsed with the HY peptide, was transferred into recipient mice to be assayed for skin test reactivity at 2 wk. The pDC fraction was used as such or after in vitro treatment with 1 \( \mu \text{g/ml} \) GpC-ODN. pDC treatment with negative control ODN (nc-ODN) was also included. A portion of the minority pDC fraction was treated with GpC-ODN 1668 in place of GpC-ODN 1826. No effect was displayed by the negative control for GpC-ODN 1668 (data not shown in the figure). One experiment representative of three. *\( p < 0.005 \) (experimental versus control footpads).

**FIGURE 2.** GpC-ODN effects on pDCs involve IDO induction and activity. (A) CD8– DCs were admixed with a 5% fraction of differently treated pDCs, and the cell mixture was pulsed with the HY peptide and then injected into recipient mice to be assayed for skin test reactivity at 2 wk. 1-MT treatment, specific \( \text{Ido1} \) silencing, or negative control siRNA (nc siRNA) treatment was performed 5 h before stimulus addition. Control groups of pDCs stimulated with only GpC-ODN or negative control ODN (nc-ODN) are also included. One of three experiments. *\( p < 0.005 \) (experimental versus control footpads). (B) pDCs were exposed to GpC-ODN (1 \( \mu \text{g/ml} \)) or negative control ODN. IDO protein expression was assayed at 24 h by immunoblot analysis. Blots were stripped and reprobed with anti-\( \beta \)-actin Ab. The results are representative of several experiments. (C) L-kynurenine production was measured in culture supernatants of pDCs treated as in (B) by HPLC at 18 h. Means ± SD of three independent experiments. *\( p < 0.05 \).
GpC-ODN in pDCs (Fig. 4A). CD8+ DCs were admixed with 5% pDCs treated in vitro with siRNAs before GpC-ODN addition, and the cell mixture was pulsed with HY peptide and injected into recipient mice to be assayed for skin test reactivity at 2 wk. Anti-TGF-β or the isotype control (50 μg/ml) was present during GpC stimulation. One of three experiments. *p < 0.005 (experimental versus control footpads).

The TRIF requirement for GpC-ODN activity was further investigated in vivo by a skin test assay, showing that appropriately conditioned pDCs from Ticam1−/− (but neither wild-type nor Myd88−/−) mice would allow the induction of skin test reactivity by cotransferred CD8+ DCs (Fig. 4C). Therefore, GpC-ODN did not activate the proinflammatory, canonical pathway of NF-κB, and, in fact, rendered pDCs tolerogenic, through TRIF and non-canonical NF-κB–dependent transcriptional expression of Idol. GpC-ODN effects on pDCs are TLR7-dependent and TLR9-independent

Among various TLRs, TLR9 is an intracellular receptor recognizing microbial DNA, whose ligation by ODNs containing CpG or GpG motifs has been found to trigger MyD88-mediated signaling that may either activate or suppress immune responses, depending on a specific setting (13, 22). The observation that GpC-ODN signaling in our experimental paradigm required TRIF as do TLR7 ligands (34) prompted us to investigate any role of TLR7 in GpC-ODN effects on pDCs are TLR7-dependent and TLR9-independent

FIGURE 3. TGF-β is required for GpC to confer suppressive properties on pDCs. (A) pDCs were exposed to GpC-ODN (1 μg/ml) or negative control ODN (nc-ODN). Specific cytokine production (indicated) was measured by ELISA in 18-h culture supernatants. Data are means ± SD of three independent experiments. *p < 0.05. (B) pDCs were exposed to GpC-ODN (1 μg/ml) or negative control ODN in the presence of anti-TGF-β (50 μg/ml) or an isotype control. IDO protein expression was assayed at 24 h by immunoblot analysis. One experiment representative of three. (C) CD8+ DCs were admixed with 5% fraction of differently treated pDCs, and the cell mixture was pulsed with the HY peptide and then injected into recipient mice to be assayed for skin test reactivity at 2 wk. Anti-TGF-β or the isotype control (50 μg/ml) was present during GpC stimulation. One of three experiments. *p < 0.005 (experimental versus control footpads).
tion of imiquimod, a reference activating ligand of TLR7. Under the same experimental conditions, however, the copresence of GpC-ODN and imiquimod allowed for the production of TGF-β (Fig. 5D).

**IDO induction and promotion of human regulatory T cell responses by GpC**

We investigated the possible effects of GpC conditioning on human FL-DCs by analyzing IDO expression and FL-DC ability to promote a regulatory phenotype in naive T cells. Purified FL-DCs were exposed to modified GpC-ODN 2006 (1 μg/ml, overnight) and were then used in immunoblotting experiments (Fig. 6A). Unlike the negative control ODN, the human TLR9-recognizing sequence containing a GpC motif strongly induced IDO expression. When the GpC-conditioned FL-DCs were cocultured in vitro with human purified CD4+ T cells for 4 d, the recovered T cells manifested strong FOXP3 induction—a marker of regulatory T cell activity—on FACS analysis (Fig. 6B).

**Discussion**

Over the past few years, unmethylated synthetic DNA sequences containing CpG motifs have been advocated as potential vaccine adjuvants capable of evoking innate and adaptive anti-microbial as well as anti-tumor responses through ligation of TLR9, an intracellular receptor that—expressed by sentinel cells such as pDCs and macrophages—is involved in immune surveillance (29). However, some studies have documented that CpG-ODNs may also lead to IDO-dependent activation of suppressive pathways as a physiological means of restoring the homeostatic balance and avoiding hyperinflammatory responses (13, 16, 32, 35). Thus CpG-ODNs are endowed with dual activity, and their adjuvant effects might, at least in part, be blunted by the activation of suppressive mechanisms, rendering their overall immunotherapeutic efficacy either suboptimal or unpredictable.

Besides CpG-rich stimulatory sequences, nonstimulatory and/or inhibitory TLR9 ligands have also been searched, reflecting the need for identifying TLR9-targeting sequences capable of opposing hyperinflammation and autoimmunity. Studies have revealed a complex relationship between ODN structure, TLR9 binding, and functional activity of several oligonucleotides. Structurally different sequences can differently modulate TLR9 cleavage—strictly required for ODN binding to C-terminal TLR9 and activation—and thus bias functional outcomes of TLR9 ligation (12, 36). In particular, an ODN containing GpC motifs was found to act as a nonstimulatory ligand because it failed to bind C-terminal TLR9 efficiently (20). In contrast, an ODN containing CpG inhibitory sequences was an effective antagonist of CpG responses by virtue of a competitive mechanism on C-terminal TLR9 (12, 20).

**FIGURE 5.** TLR7 involvement in the GpC-ODN effect on pDCs for IDO induction and activity. (A) Wild-type CD8- DCs were admixed with 5% pDCs from Tlr7−/− or Tlr9−/− mice stimulated with GpC-ODN or negative control ODN (nc-ODN), and the cell mixture was pulsed with the HY peptide and then injected into recipient mice to be assayed for skin test reactivity at 2 wk. One of three experiments. *p < 0.005 (experimental versus control footpads). (B) Wild-type or Tlr7−/− pDCs were exposed to GpC-ODN or negative control ODN, both at 1 μg/ml. IDO protein expression was assayed at 24 h by immunoblot analysis. Blots were stripped and reprobed with anti-β-actin Ab. The results are representative of several experiments. (C) L-kynurenine production was measured by HPLC at 18 h in culture supernatants of pDCs treated as in (B). Means ± SD of three independent experiments. *p < 0.05. (D) pDCs were cultured in the copresence of equimolar concentrations of GpC-ODN and imiquimod to be assayed for cytokine production (indicated). Means ± SD of three independent experiments. *p < 0.05–0.01.

**FIGURE 6.** Conditioning of human FL-DCs by a GpC–containing ODN. (A) FL-DCs were exposed to modified GpC-ODN 2006 (1 μg/ml) or negative control ODN (nc-ODN) overnight. IDO protein expression was assayed at 24 h by immunoblot analysis. Blots were stripped and reprobed with anti-β-actin Ab. The results are representative of three experiments. (B) GpC-ODN–stimulated or negative control ODN-stimulated FL-DCs were cocultured with CD4+ T cells for 4 d, and FOXP3 expression was assessed by cytfluorimetric analysis. Numbers represent percentages of FOXP3+ cells.
In this study, we demonstrate that a single base pair switch, converting the CpG into a GpC motif, affects TLR binding (20) and the qualitative type of the associated immune response. In particular, we found that GpC-ODN, sensed by pDCs as an exquisitely tolerogenic signal, invariably activated the suppressive program of noncanonical NF-κB-dependent transcriptional expression of the IDO-encoding gene (31). We also found that noncanonical NF-κB activation would not result in our setting from TLR9 ligation and MyD88-dependent transduction, as is the case for CpG, but from TLR7/TRIF-dependent events.

Among TLRs, TLR3 is typically associated with the TRIF transduction pathway, yet TLR3 is poorly expressed by pDCs (37). Sustained IDO induction depends on noncanonical NF-κB activation (19). Because TRIF-mediated signaling may involve noncanonical NF-κB family members (38, 39), we hypothesized that a TLR7/TRIF axis could be at work in IDO induction by GpC-ODN. Our data revealed that GpC-ODN acts as a tolerogenic signal for pDCs, which are also capable of initiating IDO-dependent long-term suppression independent of IDO’s catalytic function (40). Notably, imiquimod is also a TLR7 ligand, but in contrast to GpC-ODN in our setting, it is considered to be an immunostimulant molecule when used at therapeutic concentrations (41). Nevertheless, contrasting results have been reported in the literature as resulting from TLR7 ligation, which either inhibited Th17 responses via induction of IL-10 (42) or triggered Th17-type responses in humans (43). It is possible that different ODN sequences activate distinct transduction pathways upon TLR7 ligation, leading to opposite functional outcomes. Also, it is well documented that TLR7 activation by ssRNA and small molecules such as imidazoquinolines is mainly MyD88 dependent. In response to the imidazoquinolines, neither MyD88- nor TLR7-deficient mice show any inflammatory cytokine production by macrophages, proliferation of splenocytes, or maturation of DCs (44). Therefore, our finding that GpC-induced signaling events are abolished in pDCs from both TRIF- and TLR7-deficient mice points to a previously undescribed pathway of TLR7 signaling.

Physiologically, TLR7 recognizes and responds to viral ssRNA through a signal transduction pathway leading to both induction of type I IFNs—typically involved in virus elimination—and differentiation of DCs (45). Imiquimod, resiquimod (R-848), and loxoribine are RNA homologs widely used as TLR7 agonists to mimic viral infection and thus evoke functional responses in DCs, resulting in immune activation. Abnormal TLR7-mediated immune activation has been advocated in the pathogenesis of systemic lupus erythematosus (14). Remarkably, the GpC-ODN used in this study acted as an exquisitely tolerogenic agonist of TLR7, initiating signaling events that culminated in tolerance induction. Therefore, TLR7 appears to be capable of mediating opposite functional effects, depending on the ligand nature and experimental setting. These observations may pave the way to the use of TLR7 as a new potential target of chemically modified ODNs for induction of immunosuppression in negative vaccination strategies that would target autoimmune conditions.

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Disclosures
The authors have no financial conflicts of interest.

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