Class I and III Phosphatidylinositol 3′-Kinase Play Distinct Roles in TLR Signaling Pathway

Cheng-Chin Kuo, Wen-Ting Lin, Chi-Ming Liang and Shu-Mei Liang

*J Immunol* 2006; 176:5943-5949; doi: 10.4049/jimmunol.176.10.5943

http://www.jimmunol.org/content/176/10/5943

---

**Why The JI?**

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

---

**References** This article cites 41 articles, 17 of which you can access for free at:
http://www.jimmunol.org/content/176/10/5943.full#ref-list-1

**Subscription** Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions** Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts** Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Class I and III Phosphatidylinositol 3′-Kinase Play Distinct Roles in TLR Signaling Pathway

Cheng-Chin Kuo,* Wen-Ting Lin,* Chi-Ming Liang, † and Shu-Mei Liang2*‡

PI3K involvement has been implicated in the TLR signal pathway. However, the precise roles of the different classes of PI3K in the pathway remain elusive. In this study, we have explored the functions of class I and class III PI3K in the TLR signal pathway using specific kinase mutants and PI3K lipid products. Our results reveal that class III PI3K specifically regulates CpG oligodeoxynucleotide (ODN)-induced cytokine and NO production as well as NF-κB activation, whereas class I PI3K regulates both CpG ODN- and LPS-induced IL-12 production and NF-κB activation. Additional studies of CpG ODN uptake with flow cytometric analysis show that class III PI3K, but not class I, regulates cellular CpG ODN uptake. Furthermore, experiments with MyD88-overexpressing fibroblast cells transfected with dominant-negative mutants of PI3K demonstrate that class III PI3K regulates CpG ODN-mediated signaling upstream of MyD88, while class I PI3K regulation is downstream of MyD88. These results suggest that class I and class III PI3K play distinct roles in not only the uptake of CpG ODN, but also responses elicited by CpG ODN and LPS. The Journal of Immunology, 2006, 176: 5943–5949.

Although NF-κB is one of the key factors that affect cytokine production, CpG DNA has been shown to activate not only NF-κB, but also other transcription factors that are important regulators for the expression of many proinflammatory cytokines. These transcription factors include activating transcription factor 2, CREB, C/EBP, etc. (16). In addition, CpG DNA also activates stress kinases such as p38 MAPK and PI3K. Stress kinase activation is essential for CpG-DNA-induced cytokine release of TNF-α and IL-12 (11). These kinases and NF-κB are also involved in TLR4 and TLR2 signal transduction pathways (18, 19). Recently, Strassheim et al. (20) have found that inhibition of PI3K with wortmannin prevents activation of not only NF-κB, but also p38 MAPK and ERK in TLR2-stimulated neutrophils, and suggested that the PI3K signaling cascade occupies a central role in TLR2-induced activation of neutrophils. Although the interaction between CpG DNA and TLR9 has been shown to be dependent on wortmannin-sensitive members of the PI3K family (21), it is unclear whether the PI3K signaling cascade also plays a central role in the cells activated via the TLR9 pathway.

PI3Ks belong to an evolutionarily conserved family of signal-transducing enzymes. Activation of PI3K by extracellular stimuli results in the phosphorylation of phosphoinositides on the 3 position of the inositol ring, leading to the transient accumulation of phospholipids in cell membranes (22). These lipid products serve as second messengers and/or signaling molecules to control many cellular events, such as mitogenic responses, cell differentiation, survival, cytoskeletal organization, vesicular trafficking, and phagocytosis (23–26). PI3Ks are classified into three classes on the basis of their structural characteristics and substrate specificities. Class I enzymes are heterodimers comprising a p110 catalytic subunit and a p85 or p101 regulatory subunit, and are activated by tyrosine kinase-based signaling pathways or heterotrimeric G protein-based signaling pathways. In vitro, class I PI3Ks phosphorylate phosphatidylinositol (PtdIns), PtdIns(4)P, and PtdIns(4,5)P2 to generate phosphatidylinositol-3-phosphorylation (PtdIns(3)P), PtdIns(3,4)P2, and PtdIns(3,4,5)P3. Class II enzymes are large enzymes (>200 kDa) characterized by a C2 domain in their C terminus. They phosphorylate PtdIns and PtdIns(4)P in vitro, but not PtdIns(4,5)P2 to produce PtdIns(3)P and PtdIns(3,4)P2. Class III...

†Address correspondence and reprint requests to Dr. Shu-Mei Liang, Institute of BioAgricultural Sciences, Academia Sinica, 128 Academia Road, Section 2, Taipei, Taiwan. E-mail address: smyang@gate.sinica.edu.tw

‡Abbreviations used in this paper: IKK, IκB kinase; ODN, oligodeoxynucleotide; PtdIns, phosphatidylinositol; PtdIns(3)P, phosphatidylinositol-3-phosphorylation.

Copyright © 2006 by The American Association of Immunologists, Inc.

0022-1767/06/$02.00
enzymes that are homologous to Vps34p of Saccharomyces cerevisiae have a substrate specificity restricted to PtdIns and produce PtdIns(3)P (26–28).

Although PI3Ks have important functions in cellular processes and the immune system, studies of the precise role of each PI3K in cellular biological and immune responses have been limited because of lack of specific inhibitors to individual PI3Ks. Accumulated evidence has shown that the PI3K pharmacological inhibitors, wortmannin and LY294002, can inhibit the TLR-mediated signaling (21, 29–32). However, neither wortmannin nor LY294002 differentiates between the different isoforms of PI3Ks. Therefore, the roles of individual PI3Ks in TLR-mediated signaling remain elusive. In the present study, we used kinase-dead mutants and synthetic phospholipids of PI3Ks to study the intracellular role of distinct PI3Ks in TLR9-mediated responses.

Materials and Methods

Reagents

Phosphostrisphosphate-modified CpG oligodeoxynucleotide (ODN) was synthesized by MWG Biotech. The sequences of ODN used on mouse cells were as follows: 5′-TCC ATG ACC CTC TGT ATG CT-3′. LPS, phosphatidylserine, and synthetic phosphatidylinositol phosphate product diC16PtdIns(3)P and diC16PtdIns(3,4,5)P3 were purchased from Sigma-Aldrich. Anti-mouse IL-6, IL-12, and TNF-α were purchased from BioSource International.

Cell culture and cell treatment

The 293T human embryonic kidney fibroblasts and mouse RAW264.7 macrophages were obtained from the American Type Culture Collection. Cells were cultured in DMEM supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin sulfate, 200 mM l-glutamine, and 50 µM 2-ME in a humidified atmosphere of 5% CO2 at 37°C. The medium was changed every 2 days for all experiments.

Plasmid constructs

Mouse TLR9 cDNA was generated by RT-PCR with total RNA of the mouse RAW264.7 cell line used as a template and the following oligonucleotides as primers: 5′-AAG CTT CTC GGT CCG CAG ACT-3′ and 5′-CTC GAG CTA TTC TGC TGT AGG TCC-3′. Because the primers incorporate HindIII and XhoI sites, the PCR product was cloned into HindIII- and XhoI-digested pcDNA3.1 (Invitrogen Life Technologies).

The human MyD88 cDNA was generated by RT-PCR with total RNA of the human THP-1 cell line used as a template and the following oligonucleotides as primers: 5′-GGG TCC ATG GCA GGA GGA CCC GGC-3′ and 5′-AAG CTT CTC AGG GCA GGA ACA AGG CCT-3′. Because the primers incorporate BamHI and HindIII sites, this PCR product was cloned into BamHI- and HindIII-digested pcDNA3.0. The plasmids derived from p110α of class IA PI3K, i.e., M-p110-Δkin-myc and M-p110α-myc, were provided by A. Klippel (Atugen, Berlin, Germany). M-p110α-myc is a constitutively active chimera that contains the iSH2 domain of modified Griess reagent (Sigma-Aldrich). Anti-mouse IL-6, IL-12, and TNF-α were purchased from BioSource International.

Cell culture and cell treatment

The 293T human embryonic kidney fibroblasts and mouse RAW264.7 macrophages were obtained from the American Type Culture Collection. Cells were cultured in DMEM supplemented with 10% heat-inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin sulfate, 200 mM l-glutamine, and 50 µM 2-ME in a humidified atmosphere of 5% CO2 at 37°C. The medium was changed every 2 days for all experiments.

Plasmid constructs

Mouse TLR9 cDNA was generated by RT-PCR with total RNA of the mouse RAW264.7 cell line used as a template and the following oligonucleotides as primers: 5′-AAG CTT CTC GGT CCG CAG ACT-3′ and 5′-CTC GAG CTA TTC TGC TGT AGG TCC-3′. Because the primers incorporate HindIII and XhoI sites, the PCR product was cloned into HindIII- and XhoI-digested pcDNA3.1 (Invitrogen Life Technologies).

The human MyD88 cDNA was generated by RT-PCR with total RNA of the human THP-1 cell line used as a template and the following oligonucleotides as primers: 5′-GGG TCC ATG GCA GGA GGA CCC GGC-3′ and 5′-AAG CTT CTC AGG GCA GGA ACA AGG CCT-3′. Because the primers incorporate BamHI and HindIII sites, this PCR product was cloned into BamHI- and HindIII-digested pcDNA3.0. The plasmids derived from p110α of class IA PI3K, i.e., M-p110-Δkin-myc and M-p110α-myc, were provided by A. Klippel (Atugen, Berlin, Germany). M-p110α-myc is a constitutively active chimera that contains the iSH2 domain of modified Griess reagent (Sigma-Aldrich). Anti-mouse IL-6, IL-12, and TNF-α were purchased from BioSource International.

Cytokine-specific ELISA

Microtiter plates (96-well) were coated with anti-mouse IL-6, IL-12, and TNF-α (BioSource International) in PBS at 4°C overnight. After the plates were blocked and washed, supernatants from stimulated cells (1 × 106 cells/ml) were added and the plates were incubated for 1 h at room temperature. The plates were then washed and treated with biotinylated anti-cytokine, followed by streptavidin-HRP (BioSource International). A standard curve generated using rIL-6, rIL-12, and rTNF-α was used to determine cytokine concentration.

NO assay

NO production in the supernatant samples was quantified using the Griess method to measure nitrite, a stable breakdown product of NO. Samples of 50 µl were transferred to a 96-well microtiter plate, followed by the addition of 50 µl of modified Griess reagent (Sigma-Aldrich). After 15-min incubation at room temperature, nitrite concentration was measured at 540 nm on a microtiter plate reader. Nitrite concentrations were calculated by comparison with a standard curve for sodium nitrite.

Synthetic lipid product treatments

Synthetic phosphatidylinositol phosphate (0.1 mg/ml) and phosphatidylserine (0.1 mg/ml) were solubilized in a mixture of chloroform-methanol, 1:1 (v/v), dried under N2. The dried pellet was dispersed by sonication for 15 min at 20°C in buffer containing 25 mM HEPES (pH 7.4) and 1 mM EDTA. Samples were centrifuged at 6000 g for 15 min at 4°C, and the supernatant was added into cells incubated in a stimulated or unstimulated medium (28).

CpG ODN uptake

In brief, rhodamine-CpG ODN (MWG Biotech)-stimulated cells were washed with PBS and fixed with 1× Cytofix/Cytoperm buffer (BD Pharmingen) for 20 min at room temperature. Stained cells were washed, and the rhodamine-CpG ODN uptake (1.5 × 104 cells) was quantified by flow cytometry (Beckman Coulter).

Statistical analysis

All values are given as means ± SD. Data analysis involved one-way ANOVA with subsequent Scheffe test.

Results

PI3Ks play important roles in CpG ODN-mediated responses

Previous reports suggested that PI3K might play a critical role in regulating CpG ODN-mediated NF-κB activation and IL-12 production (21). In this study, we showed that the PI3K inhibitor wortmannin impaired CpG ODN-induced NF-κB activation (p < 0.005) and IL-12 production (p < 0.001) in mouse RAW264.7 cells. In addition, we also found that wortmannin interfered with CpG ODN-induced production (p < 0.001) of NO, TNF-α, and IL-6 (Fig. 1). These results support the proposal that wortmannin-sensitive PI3K plays a critical role in CpG ODN-mediated responses, including NF-κB activation and production of cytokines and NO.

Role of class I and class III PI3Ks in CpG ODN- or LPS-stimulated responses

The isoform-specific kinase-dead mutants of class I PI3K (M-p110-Δkin-myc) (33, 34) and class III PI3K (hVPS34kd) (35) were used to further examine the role of specific classes of PI3K in CpG ODN-mediated responses. Production of NO, IL-6, and TNF-α mediated by CpG ODN was reduced by transient and stable expression of hVPS34kd in RAW264.7 cells, but not expression of M-p110-Δkin-myc (Figs. 2 and 3A). Interestingly, NF-κB activation induced by CpG ODN was impaired by both class I and class III PI3K kinase-dead mutants transiently expressed in RAW264.7 cells (Fig. 3B).

Because PI3K has been reported to play an important role in LPS-stimulated responses (29, 30, 32), we also examined the physiological functions of class I and III PI3Ks in LPS-mediated responses. As shown in Fig. 3, A and B, class I kinase-defective
mutation impaired LPS-stimulated NF-κB activation as well as NO, IL-6, and TNF-α production. In addition, it also enhanced the production of IL-12 induced by LPS or CpG ODN (p < 0.001) (Fig. 3C). However, the LPS-mediated activation of NF-κB and cytokine production was not substantially altered in RAW264.7 cells overexpressing the kinase-dead mutant hVPS34kd or in stably expressed hVPS34kd (RAW264.7-hVPS34kd). Taken together, these results suggest that although both class I and class III PI3K are involved in the CpG ODN-mediated activation of NF-κB, only class III PI3K is involved in the CpG ODN-mediated induction of cytokines and NO. Class I PI3K plays an important role in LPS-stimulated responses, while class III PI3K does not.

diC16PtdIns(3,4,5)P3 reversed the class III PI3K dominant mutant-induced inhibition of CpG ODN-mediated responses

To further confirm the specific role of class III PI3K in CpG ODN-mediated responses, we examined the effect of the class III PI3K product, diC16PtdIns(3,4,5)P3, on cytokine production, NO production, and NF-κB activation induced by CpG ODN. Cells stably expressing dominant-negative class I or class III PI3K were treated with diC16PtdIns(3,4,5)P3 in a liposome form (28) for 4 h, followed by CpG ODN stimulation. diC16PtdIns(3,4,5)P3 substantially reversed the inhibitory effect of RAW264.7-hVPS34kd on CpG ODN-mediated responses, including production of NO, IL-6, IL-12, and TNF-α as well as NF-κB activation in a dose-dependent manner (p < 0.005) (Fig. 4). This effect was specific because diC16PtdIns(3,4,5)P3 did not alter the inhibition of CpG ODN-induced NF-κB activation caused by the class I PI3K kinase-defective mutant (Fig. 4B). These results indicate that the class III PI3K lipid product plays a crucial role in the CpG ODN-mediated production of NO and cytokines as well as NF-κB activation.

diC16PtdIns(3,4,5)P3 reversed the class I PI3K dominant mutant-induced inhibition of CpG ODN-mediated responses

We also evaluated further the role of class I PI3K in CpG ODN-mediated responses using the class I PI3K product diC16PtdIns(3,4,5)P3. Fig. 5A shows that diC16PtdIns(3,4,5)P3 not only increased CpG ODN-stimulated NF-κB luciferase activity in RAW264.7 cells, but also recovered NF-κB activities in RAW264.7-p110Δkin cells. Similarly, the increased level of CpG ODN-induced IL-12 production in RAW264.7-p110Δkin cells was suppressed by diC16PtdIns(3,4,5)P3 (as shown in Fig. 5B). Interestingly, diC16PtdIns(3,4,5)P3 also increased NF-κB activities in RAW264.7-hVPS34kd, but had no substantial effect on CpG ODN-mediated production of NO, IL-6, and TNF-α in RAW264.7, RAW264.7-hVPS34kd, and RAW264.7-p110Δkin cells (data not shown). These data indicate that the class I PI3K
lipid product primarily regulates CpG ODN-mediated NF-κB activation and IL-12 production.

Class III PI3K functions upstream and class I PI3K functions downstream of MyD88

Because both PI3K class I and III were involved in CpG ODN-mediated NF-κB activation, we attempt to delineate the sequence of action of these two classes of PI3K in the TLR signaling pathway. MyD88 is known as an important signal adaptor in the TLR signaling pathway. To determine whether PI3Ks exert their functions upstream or downstream of MyD88, 293T cells were transiently transfected with various concentrations of PI3K kinase-defective plasmids (hVPS34kd or M-p110-Δkin-myc) plus p5xNF-κB luciferase reporter plasmid and various doses of hVPS34kd or M-p110-Δkin-myc plasmids. As seen in Fig. 6, M-p110-Δkin-myc impaired the NF-κB luciferase activity induced by MyD88 overexpression in a dose-dependent manner, but hVPS34kd had no substantial effect on MyD88-induced NF-κB activation. These results suggest that class III PI3K regulates CpG ODN-mediated signaling upstream of MyD88, while class I PI3K regulation is downstream.

Class III PI3K is involved in CpG ODN uptake in RAW264.7 cells

Recently, wortmannin was reported to impair the CpG ODN uptake of mice bone marrow-derived dendritic cells (21). In this study, we confirmed that wortmannin inhibited rhodamine-CpG ODN uptake in RAW264.7 cells by flow cytometric analysis (Fig. 7A). Because we had demonstrated previously that hVPS34kd interfered with CpG ODN-mediated responses upstream of MyD88, we next investigated whether class III PI3K was involved in CpG ODN uptake. The level of rohdamine-CpG ODN uptake was significantly decreased in wild-type class III PI3K (hVPS34kd) cells compared with control RAW264.7 cells (Fig. 7B). In contrast, constitutively active class I PI3K kinase plasmid (M-p110Δkin-myc) and its respective inactive version (M-p110-Δkin-myc) (33, 34) had no substantial effect on CpG ODN uptake.

We then assessed the effect of class I and class III PI3K products on CpG ODN uptake to further elucidate which PI3Ks are important for the uptake of CpG ODN. The inhibition of CpG ODN uptake in RAW264.7-hVPS34kd cells was recovered by the class III product diC16PtdIns(3)P, but not by the product diC16PtdIns(3,4,5)P3 (Fig. 7C). Taken together, these results strongly suggest that class III PI3K plays an important role in CpG ODN uptake.

Discussion

The activation of PI3Ks by many microbial and viral stimuli such as LPS, peptidoglycan, and CpG DNA/ODN plays an important role in regulating cellular defense and immune response, including cytokine production, phagocytosis, and apoptosis (26, 32). Much evidence has shown that PI3K inhibitors such as wortmannin and LY294002 interfere with TLR-mediated responses (21, 29–32). However, few studies have addressed the classes of PI3Ks responsible for these responses. The isofom-specific kinase-defective mutants and synthetic lipid products of class I and class III PI3K have previously been used to identify the roles of PI3Ks played in physiological signaling (28, 33–35). In the present study, we used both approaches to demonstrate that class I and class III PI3K play distinct roles in CpG ODN-driven responses. Our results indicate that class I PI3K mainly affects CpG ODN-mediated NF-κB activation and IL-12 production, while class III PI3K is involved in NF-κB activation and production of IL-6, IL-12, TNF-α, as well as NO (Figs. 1–3). In addition, because only the kinase-dead mutant of class I PI3K (M-p110-Δkin-myc), but not that of class III PI3K (hVPS34kd) inhibited NF-κB activation induced by MyD88 overexpression (as shown in Fig. 6), class III PI3K most likely regulates CpG ODN-mediated signaling upstream and class I downstream of MyD88. Class I and class III PI3K thus exhibit different modes of action in the cells.

Although CpG DNA/CpG ODN induces IL-12, it also activates PI3K, which in turns down-regulates IL-12 to a certain level to prevent excessive innate immune responses (36, 37). In this study, we showed that this down-regulation was mainly via class I PI3K,
because in kinase-dead mutants of class I PI3K (RAW-p110\(\text{kin}\)) cells, the down-regulation mechanism was blocked and the level of CpG ODN-induced IL-12 increased as compared with that of wild-type RAW cells (Fig. 3C). Addition of class I PI3K product, diC16PtdIns(3,4,5)P\(_3\), restored the down-regulation mechanism and brought the level of CpG ODN-induced IL-12 back to that observed in wild-type RAW cells (Fig. 5B). In view of the observation that diC16PtdIns(3,4,5)P\(_3\) did not cause the level of CpG ODN-induced IL-12 to decline in the wild-type RAW (Fig. 5B), we propose that after long duration (20 h) of CpG ODN stimulation, the inhibitory effect of endogenous diC16PtdIns(3,4,5)P\(_3\) in wild-type RAW cells might have reached the plateau, which results in little, if any, responses to the additional diC16PtdIns(3,4,5)P\(_3\).

It is noteworthy that CpG DNA has been shown to activate not only NF-\(\kappa\)B, but also other transcription factors that are important regulators for the expression of many proinflammatory cytokines (16). In addition, CpG DNA also activates stress kinases such as p38 MAPK, whose activation is essential for CpG-DNA-induced cytokine release of TNF-\(\alpha\) and IL-12 (11). Our observation that the isoform-specific kinase-dead mutant of class I PI3K M-p110-\(\Delta\)kin-myct did not impair cytokine production in cells treated with CpG-ODN seems to suggest that activation of other key factors by CpG ODN compensates the inhibition of NF-\(\kappa\)B by M-p110-\(\Delta\)kin-myct. An alternative explanation is that M-p110-\(\Delta\)kin-myct does not impair cytokine production induced by CpG-ODN via other kinase pathways such as p38 MAPK.

FIGURE 4. Class III PI3K product (diC16PtdIns(3)P) reverses the decline of NO, IL-6, IL-12, and TNF-\(\alpha\) production of CpG ODN-treated cells expressing hVSP34kd. A, Cells were stimulated with CpG ODN for 20 h in the presence of various doses of diC16PtdIns(3)P. NO, IL-6, IL-12, and TNF-\(\alpha\) levels in the culture supernatants were measured by ELISA. B, Cells were transfected with p5xNF-\(\kappa\)B luciferase overnight and then stimulated with CpG ODN (1.5 \(\mu\)M) for 8 h in the presence of various concentrations of diC16PtdIns(3)P. NF-\(\kappa\)B luciferase activities were then measured. Data represent the mean \(\pm\) SD of three experiments. \(*, p < 0.005\) for the increase induced by diC16PtdIns(3)P vs vehicle alone.

FIGURE 5. The effect of class I PI3K product (diC16PtdIns(3,4,5)P\(_3\)) on CpG ODN-mediated IL-12 production and NF-\(\kappa\)B activation. Cells were pre-fed with diC16PtdIns(3,4,5)P\(_3\), followed by 1.5 \(\mu\)M CpG ODN stimulation for 8 h (A) or 20 h (B). A, NF-\(\kappa\)B luciferase activities were then measured. B, IL-12 levels in the culture supernatants were measured by ELISA. Data represent the mean \(\pm\) SD of three experiments. \(*, p < 0.005\).

FIGURE 6. Class I PI3K kinase-dead mutant impaired NF-\(\kappa\)B activation induced by wild-type MyD88 overexpression. In addition to the NF-\(\kappa\)B-driven luciferase gene, wild-type MyD88-overexpressing 293T cells were transfected with kinase-deficient plasmid hVSP34kd or M-p110-\(\Delta\)kin-myct. After 24-h transfection, NF-\(\kappa\)B luciferase activities were measured. Data represent the mean \(\pm\) SD of three experiments.
RAW264.7 cells were incubated with 1.5 μM Rhodamine-CpG ODN uptake was measured by flow cytometry (0–60 min). Directed endosome trafficking to lysosomes also requires PtdIns(3)P generation (35, 40). Because uptake of CpG ODN involves endosomes, we propose that PtdIns(3)P might play a critical role in CpG ODN uptake. Our findings that cells fed with class III PI3K lipid product (diC16PtdIns(3)P) increased CpG ODN uptake and reversed the inhibition of CpG ODN uptake induced by hVPS34kd (Fig. 7C) are consistent with this proposal. To what extent this increase in uptake of CpG ODN is due to the effect of diC16PtdIns(3)P on endosomes, however, remains to be clarified.

Our results show that the dominant-negative mutant and synthetic lipid product of class III PI3K affected the CpG ODN-mediated production of IL-6, TNF-α, and NO (Figs. 3 and 4). LPS-driven cytokines and NO production as well as NF-κB activation, however, were not altered by the dominant-negative mutant and synthetic lipid product of class III PI3K (Fig. 3). The different effect of class I and class III PI3K on LPS- and CpG ODN-mediated responses correlates well with the report that LPS and CpG ODN trigger signaling via two distinct cellular locations (41). Nonetheless, it will be interesting to elucidate how class III PI3K specifically regulates the uptake and signaling of CpG ODN, but not those of LPS.

Although PI3K inhibitor was reported previously to impair the LPS-induced production of NO, IL-6, and TNF-α (21, 29–32), it was not shown whether class I or class III PI3K plays any important role. Our findings, that the dominant-negative mutant of class I PI3K, but not class III PI3K, decreased the LPS-mediated activation of NF-κB and cytokine production (Fig. 3), indicate that class I, but not class III PI3K is involved in signaling of the LPS pathway. It is noteworthy that the dominant-negative mutant and lipid product of class I PI3K had no effect on CpG ODN-induced production of NO, IL-6, and TNF-α (Figs. 2 and 4). How class I PI3K selectively affects LPS- but not CpG ODN-mediated NO, IL-6, and TNF-α is currently under study.

Because PI3Ks participate in several functions in the immune system, it is important to clarify the precise role of individual PI3K enzymes in immune signaling. In the present study, we clearly demonstrate that class I and class III PI3Ks play distinct roles in TLR signaling pathways. Selective activation or inhibition of these PI3Ks might be useful for certain immunological or therapeutic applications.

**Acknowledgments**

We thank Dr. Angela Wandinger-Mess for providing plasmids hVSP34 and hVSP34kd. We also thank Dr. Anke Klippel for providing plasmids M-p110*–myc and M-p110–Δkin–myc.

**Disclosures**

The authors have no financial conflict of interest.

**References**


16: 3516–3521.


