Macrophage activation by CpG DNA requires toll-like receptor 9 and the adaptor protein MyD88. Gram-negative bacterial lipopolysaccharide also activates macrophages via a toll-like receptor pathway (TLR-4), but we and others have reported that lipopolysaccharide also stimulates tyrosine phosphorylation in macrophages. Herein we report that exposure of RAW 264.7 murine macrophages to CpG DNA (but not non-CpG DNA) provoked the rapid tyrosine phosphorylation of \( \text{vat1} \). PP1, a selective inhibitor of \( \text{src} \)-related tyrosine kinases, blocked both the CpG DNA-mediated tyrosine phosphorylation of \( \text{vat1} \) and the CpG DNA-mediated up-regulation of macrophage tumor necrosis factor secretion and inducible nitric-oxide synthase protein accumulation. Furthermore, we found that the inducible expression of any of three dominant interfering mutants of \( \text{vat1} \) (a truncated protein, \( \text{vatC} \); a form containing a point mutation in the regulatory tyrosine residue, \( \text{vatYF174} \); and a form with an in-frame deletion of six amino acids required for the guanine nucleotide exchange factor (GEF) activity of \( \text{vat1} \) for rac family GTPases, \( \text{vatGEFm1} \)) consistently inhibited CpG DNA-mediated up-regulation of tumor necrosis factor secretion and inducible nitric-oxide synthase protein accumulation in RAW-TT10 macrophages. Finally, we determined that CpG DNA-mediated up-regulation of NF-\( \kappa \)B activity (but not mitogen-activated protein kinase activation) was inhibited by preincubation with PP1 or by expression of the truncated \( \text{vatC} \) mutant. Taken together, our results indicate that the tyrosine phosphorylation of \( \text{vat1} \) by a \( \text{src} \)-related tyrosine kinase or kinases plays an important role in the macrophage response to CpG DNA.

Certain bacterial DNA sequences are recognized by the vertebrate immune system as foreign and directly activate B lymphocytes and macrophages (1–3). Unmethylated CpG dinucleotides in particular sequence contexts (CpG motifs, e.g. CACGTT for murine cells, GTCGTT for human cells) occur frequently in bacterial DNA but not in vertebrate DNA (3). Synthetic oligodeoxynucleotides containing these sequences (CpG DNA) stimulate macrophages to produce important inflammatory mediators such as tumor necrosis factor (TNF)\(^1 \) and nitric oxide (NO) (2–4). Although exposure of animals to CpG DNA also leads to a robust T helper cell 1 cytokine response in most cases, the effects of CpG DNA on T cells are indirect, resulting from the production of innate cytokines such as type I interferons, interleukin-12, and interleukin-18 (3).

We and others have implicated \( \text{hck} \) and other \( \text{src} \)-related tyrosine kinases (5–9) and substrates of these kinases, including \( \text{vat1} \) (10, 11), in the macrophage response to Gram-negative bacterial LPS. Although targeted disruption of three \( \text{src} \) family kinases (\( \text{hck} \), \( \text{lyn} \), \( \text{fgr} \)), alone or in combination, has failed to demonstrate an essential role for this pathway in macrophage activation by LPS (12), this may be due to compensation by other \( \text{src} \) family members. Recent studies with highly \( \text{src} \) family-selective tyrosine kinase inhibitors (13) provide additional evidence that these kinases are involved in macrophage responses to LPS and other stimuli (9, 14). However, little attention has been paid to the potential role of these tyrosine phosphorylation pathways in macrophage activation by bacterial DNA.

In the present study, we examined the ability of CpG DNA to provoke the tyrosine phosphorylation of \( \text{vat1} \) in macrophages and used complementary strategies to determine whether \( \text{vat1} \) phosphorylation played a critical role in CpG DNA-mediated macrophage activation.

**MATERIALS AND METHODS**

Reagents—Dulbecco’s modified Eagle’s medium was purchased from Mediatech Inc. (Herndon, VA). Penicillin/streptomycin and 1-glutamine were obtained from Invitrogen. Fetal bovine serum was purchased from HyClone Laboratories (Logan, UT). Nuclease-resistant phosphorothioate oligodeoxynucleotides (S-ODN) were purchased from Operon (Valencia, CA) and had no detectable endotoxin activity by Limulus assay. The sequences of the S-ODN used were 5′-TCCATGACGTTCCTGAC-3′.

1 The abbreviations used are: TNF, tumor necrosis factor; nCpG, non-CpG; TLR, toll-like receptor; LPS, lipopolysaccharide; GEF, guanine nucleotide exchange factor; iNOS, inducible nitric-oxide synthase; MAP, mitogen-activated protein; IFN-\( \gamma \), recombinant interferon-\( \gamma \); JNK, c-Jun NH\(_2\)-terminal kinase; ERK, extracellular signal-regulated kinase; ODN, oligodeoxynucleotide.
GTT-3'- (CpG DNA, ODN1826) and 5'TCCAGGACTTCCCTTCCCAGGTT3' (nCpG DNA, ODN1982). LPS purified from Escherichia coli strain O111:B4 and rIFNγ were obtained from Sigma. Zeocin and tetracycline were purchased from Invitrogen. PP1, a src family-selective tyrosine kinase inhibitor, was purchased from Calbiochem.

vav1 Constructs—We obtained c-myc epitope-tagged constructs encoding (a) vav1, a truncated form of vav1, comprising amino acids 538–845, including the C-terminal SH2 and SH3 domains; (b) vav1YF174, a tyrosine to phenylalanine point mutant at position 174; and (c) vav1GEFmt, a mutant containing an in-frame deletion of six amino acids in the GEF domain, as generous gifts from A. Weiss (University of California, San Francisco) (15, 16). The vav1 constructs were subcloned into the expression vector pTet/Zeo, a tetracycline opertator (tetO)-expressing vector (tetO) expression plasmid that also encodes resistance to the antibiotic Zeocin (D. Underhill, The Institute for Systems Biology, Seattle, WA).

Creation of Stably Transfected Cell Lines—RAW-TT10 cells (17) (D. Underhill and A. Aderem, The Institute for Systems Biology, Seattle, WA) are RAW 264.7 murine macrophages (ATCC, Manassas, VA) that have been engineered to express the tetracycline-controlled transactivator (tTA) (18, 19) from a tetracycline-regulated promoter. RAW-TT10 cells were transfected with the subcloned expression constructs using electroporation as described by Underhill et al. (20). In this tetracycline-regulatable “Tet-OFF” system, the promoter is quiescent in the presence of low doses of tetracycline; in the absence of tetracycline, there is a marked increase in the expression of cDNAs directed by the tetracycline operator (tetO)-promoter sequence (17). Cells expressing the indicated pTet/Zeo-based constructs were selected in the presence of Zeocin (1 mg/ml for 1 week and then 500 μg/ml). All stable cell lines were stored in liquid nitrogen and were cultured for less than 4 weeks prior to study.

Cells and Cell Culture—RAW 264.7 murine macrophages were obtained from ATCC. RAW 264.7 cells and RAW-TT10 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM l-glutamine, 50 units/ml penicillin G, and 50 μg/ml streptomycin. RAW-TT10/vav1C, RAW-TT10/vav1YF174, and RAW-TT10/vav1GEFmt cells were selected and maintained in medium containing both Zeocin (500–1000 μg/ml) and tetracycline (1–5 μg/ml). In the experimental conditions, RAW-TT10 subclones expressing the mutant forms of vav1 were grown in tetracycline 1 μg/ml (control) or in the absence of tetracycline (allowing construct expression) for 48–72 h prior to stimulation. Experiments were performed in 6-well (BD Biosciences) or 100-mm (Corning, Corning, NY) tissue culture dishes. The cells were grown to 60–70% confluence prior to stimulation. The cells were stimulated with either CpG DNA or nCpG DNA (range of concentrations, 0.1–12 μg/ml) alone or in the presence of rIFNγ. For studies of TNF secretion and inducible nitric-oxide synthase (iNOS) protein accumulation, cells were incubated for ~18 h before the cell supernatants were collected and/or cell lysates were prepared. For the study of vav1 tyrosine phosphorylation or MAP kinase activation, cells were stimulated for 10–40 min prior to preparation of the cell lysates and/or vav1 immunoprecipitates.

Phosphorylation and Immunoblotting—Adherent cells were lysed with extraction buffer (20 mM Tris, 100 mM NaCl, 50 μg/ml NaF, 1 mM Na3VO4, 0.2 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin). For quantitation of iNOS protein levels in cell lysates, the samples were electrophoresed on sodium dodecyl sulfate-polyacrylamide gels (SDS-PAGE), transferred to nitrocellulose membranes, blocked with 5% milk in Tris-buffered saline, and then reacted with the antibody to phospho-iNOS (Transduction Laboratories, Lexington, KY) or phospho-ERK (Upstate Biotechnology Inc., Lake Placid, NY) or phospho-JNK (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), or phospho-ERK (Upstate Biotechnology Inc., Lake Placid, NY).

For immunoprecipitations, lysates were cleared with normal rabbit serum (Sigma) prior to incubation with polyclonal rabbit antiserum specific for vav1 (Upstate Biotechnology Incorporated or Santa Cruz Biotechnology). Immune complexes were collected with protein A-Sepharose beads (Amersham Biosciences), subjected to SDS-PAGE, transferred to nitrocellulose membranes, and then reacted with the antibody to phospho-iNOS (Transduction Laboratories, Lexington, KY) or phospho-ERK (Upstate Biotechnology Inc., Lake Placid, NY) or phospho-JNK (Santa Cruz Biotechnology, Inc., Santa Cruz, CA).

vav1 protein tyrosine phosphorylation was detected by two methods.

Fig. 1. CpG DNA stimulated the tyrosine phosphorylation of vav1 in a time- and concentration-dependent manner in RAW 264.7 murine macrophages. Cells were cultured for the indicated interval (A) or for 15 min (B–D) in the presence of 6 μg/ml CpG DNA (A) or the indicated concentrations of CpG DNA (B–D). vav1 tyrosine phosphorylation was measured by either immunoblotting of vav1 immunoprecipitates with 4G10, a murine monoclonal antibody specific for phosphotyrosine residues (A, B, D), or by immunoblotting of whole cell lysates with a rabbit polyclonal antiserum specific for vav1 phosphorylated on residue 174 and a rabbit polyclonal antibody to vav1 for loading control (C). Stimulation with nCpG DNA for 15 min in a range of indicated concentrations failed to induce the tyrosine phosphorylation of vav1 (D).
CpG DNA Signaling via vav1 and src Family Kinases

**RESULTS**

CpG DNA Triggers the Tyrosine Phosphorylation of vav1 in RAW 264.7 Cells in a Time- and Concentration-dependent Manner—We and others have reported previously that LPS stimulates the tyrosine phosphorylation of vav1 in macrophages (10, 11) and have implicated hck and/or other src-related tyrosine kinases in this process (9, 11). In this study, we found that exposure of RAW 264.7 cells to CpG DNA (0.1–12.0 μg/ml) led to the rapid (detectable within 10–20 min; Fig. 1A) and dose-dependent (Fig. 1, B–D) phosphorylation of vav1 on tyrosine, as demonstrated by either immunoblotting of vav1 immunoprecipitates with 4G10, a murine monoclonal antibody specific for phosphotyrosine residues (Fig. 1, A, B, and D, representative experiments, n = 6) or by immunoblotting of whole cell lysates with a rabbit polyclonal antiserum specific for vav1 phosphorylated on tyrosine 174 (Fig. 1C is representative, n = 4). Exposure of RAW cells to control nCpG DNA (0.1–12.0 μg/ml) for 10–30 min failed to induce detectable vav1 phosphorylation (Fig. 1D is representative, n = 5).

**PP1, a Selective Inhibitor of src-related Tyrosine Kinases, Potently Blocks CpG DNA-mediated TNF Secretion, iNOS**

reacted with the species-specific IgG peroxidase-linked conjugate secondary antibody (Amersham Biosciences), and proteins were then detected by enhanced chemiluminescence. The relative intensities of bands were compared using a Bio-Rad Model GS-700 densitometer.

Measurement of TNF Concentrations—Supernatants were collected and stored at −20°C. TNF protein levels were determined by a solid phase sandwich enzyme-linked immunosorbent assay specific for murine TNF as specified by the manufacturer (R & D Systems, Minneapolis, MN).

**NF-κB Activation: Transient Transfections and Luciferase Assay—**RAW 264.7 cells and RAW-TT10/vav1 cells were grown to ~80% confluency in 6-well plates. Cells were transfected with a mixture of NF-κB-luciferase and pRL-TK constructs using LipofectAMINE PLUS (Invitrogen). The transfected cells were pooled and split into 96-well plates at a volume of 200 μl (~1.25 × 10^4 cells/ml). RAW 264.7 cells were then preincubated with medium alone or PP1 at the indicated concentrations for 1 h prior to stimulation. RAW-TT10/vav1 cells were cultured in the presence or absence of tetracycline (1 μg/ml) for 72 h prior to transfection and maintained in the presence or absence of tetracycline after transfection. The cells were then stimulated with medium alone, CpG DNA (6 μg/ml), or LPS (1 μg/ml) for ~17 h. Luciferase activity in the cell extracts was analyzed according to the manufacturer’s protocol using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI). The luciferase activity was normalized using pRL-TK-luciferase activity in each sample.

**Fig. 2.** The src family-selective tyrosine kinase inhibitor, PP1, potently inhibited CpG DNA-mediated TNF secretion (A), iNOS protein accumulation (B), and vav1 tyrosine phosphorylation (C) in RAW 264.7 murine macrophages. Representative experiments are depicted: n = 8 (A), n = 4 (B), n = 5 (C). In the TNF experiments (A), cells were stimulated with the indicated concentrations of CpG DNA alone for 18 h in the presence or absence of PP1 (representative experiment depicted; performed in triplicate). Error bars represent standard deviation, n = 8. In the iNOS experiment depicted in B, cells were stimulated with medium alone (lanes 1, 6, and 10) or with rIFNγ 10 units/ml + nCpG DNA 6 μg/ml (lane 2) or rIFNγ 10 units/ml + CpG DNA 0.5 μg/ml (lanes 3, 7, and 11), 3 μg/ml (lanes 4, 8, and 12), or 12 μg/ml (lanes 5, 9, and 13) for 18 h. The blot was reprobed with an antibody recognizing total p38 MAP kinase protein as a loading control. For the vav1 phosphorylation experiment (C), cells were stimulated for 15 min with CpG DNA 0.5 μg/ml (lanes 1, 4, and 7), 3 μg/ml (lanes 2, 5, and 8), or 6 μg/ml (lanes 3, 6, and 9). Ponceau staining (prior to immunoblotting) confirmed equivalent protein loading for this blot (not shown).
Protein Accumulation, and vav1 Tyrosine Phosphorylation in RAW 264.7 Cells—The pyrazolopyrimidine PP1 is a potent and highly selective inhibitor of src-related tyrosine kinases (13) and lacks activity against other classes of tyrosine kinases (e.g., JAK kinases, ZAP-70/lyk kinases), although one recent report suggests that it may also be active against the abl kinase (21). We have reported previously that PP1 inhibits LPS-mediated activation of the src-related kinase hck and the subsequent tyrosine phosphorylation of vav1 (a target of hck and possibly other src-related kinases) in RAW 264.7 cells (9).

To investigate the potential role of src-related tyrosine kinases in CpG DNA-mediated macrophage activation and vav1 tyrosine phosphorylation, we exposed RAW 264.7 cells to a range of concentrations of PP1 (1–20 μM) for 1 h prior to stimulation with CpG DNA ± rIFNγ as indicated (Fig. 2). CpG DNA has been reported previously to stimulate macrophage production of TNF and, in the presence of IFNγ, iNOS, and NO (2–4, 22). We first confirmed that exposure of RAW 264.7 cells to a range of concentrations (0.1–6.0 μg/ml) of CpG DNA (but not nCpG DNA) led to dose-dependent TNF secretion and, in the presence of low concentrations of rIFNγ (1–25 units/ml), iNOS protein accumulation (not shown). In the absence of rIFNγ, CpG DNA alone (0.1–12.0 μg/ml) failed to stimulate detectable iNOS accumulation (not shown).

Preincubation with PP1 (1–20 μM) led to marked inhibition of CpG DNA-mediated TNF (Fig. 2A, representative experiment performed in triplicate, n = 8) and iNOS production by RAW 264.7 cells (Fig. 2B, representative experiment, n = 5) and completely blocked CpG DNA-mediated vav1 tyrosine phosphorylation (Fig. 2C, representative experiment, n = 5). The PP1 IC_{50} for TNF and iNOS accumulation were <10 μM and ~10 μM, respectively. The PP1 IC_{50} for vav1 phosphorylation was less than 10 μM. PP1 (10 μM) inhibited CpG DNA-mediated TNF secretion by ~65% (range, 55–89%) and reduced CpG DNA-mediated iNOS protein accumulation by ~54%.

Inducible Expression of Any of Three Dominant Interfering Mutants of vav1 Inhibited CpG-mediated TNF Secretion and iNOS Protein Accumulation in RAW-TT10 Cells—To directly test the role of vav1 in macrophage activation by CpG DNA, we expressed a series of potentially dominant interfering mutants (Fig. 3A) in RAW 264.7 cells. Cells lines were established in the presence of tetracycline (1–5 μg/ml) to prevent construct expression prior to the time of the experimental conditions. All three mutant forms of vav1 were expressed in a tetracycline-regulatable fashion in RAW-TT10/vavC, RAW-TT10/vavYF174, and RAW-TT10/vavGEFmt cells (Fig. 3, B and C, is representative, n = 7).

Robust expression of all three mutant forms of vav1 was detected from cells grown in cell tissue culture medium without tetracycline for 48–72 h (compared with lysates from cells maintained in the presence of 1–5 μg/ml of tetracycline), as shown in representative immunoblots of cell lysates reacted with either a rabbit polyclonal antiserum specific for vav1 (to detect the full-length vav1 construct) or by immunoblotting with a murine monoclonal antibody specific for the c-myc epitope tag (for detection of the full-length vav1 constructs and vav1 GEFmt constructs (C)). The blot depicted in C was reprobed with an antibody recognizing total p38 MAP kinase protein as a loading control.

![Diagram of tetracycline regulatable vav1 constructs](https://example.com/diagram.png)

**Fig. 3. vav1 constructs (A) were expressed in a tetracycline-regulatable fashion (B and C) in stable cell lines derived from RAW-TT10 macrophages.** Three mutant forms of vav1 were expressed in RAW-TT10 cells in the absence (but not the presence) of tetracycline. Cells were cultured in the presence of tetracycline 1–5 μg/ml or in the absence of tetracycline for 72 h prior to the preparation of cell lysates. vav1 construct expression was confirmed by immunoblotting with a polyclonal rabbit antiserum specific for vav1 (for detection of the truncated vavC construct (B)) or by immunoblotting with a murine monoclonal antibody specific for the c-myc epitope tag (for detection of the full-length vav1YF174 and vav1 GEFmt constructs (C)).
FIG. 4. Tetracycline-regulated expression of each of three vav1 mutants (A–C) in RAW-TT10 macrophages inhibited CpG DNA-mediated TNF secretion. Cells were cultured in the presence of tetracycline 5 μg/ml (control) or absence of tetracycline (allowing construct expression) for 72 h prior to stimulation with the indicated concentrations of rIFNγ + CpG DNA for 18 h prior to collection of cell supernatants.
In this study, we demonstrated that exposure of RAW 264.7 murine macrophages to CpG DNA induces rapid tyrosine phosphorylation of vav1. We provided two types of evidence that this event plays an important role in macrophage responses to CpG DNA. First, we demonstrated that a selective inhibitor of src-related tyrosine kinases, PP1 (13), potently inhibits CpG DNA-mediated TNF secretion and iNOS protein up-regulation in macrophages at concentrations comparable with those needed to block CpG DNA-mediated vav1 tyrosine phosphorylation. Furthermore, we show that the tetracycline-regulatable expression of any of three dominant interfering mutants of vav1 in stable subclones of RAW-TT10 macrophages inhibits both TNF secretion and iNOS protein accumulation in response to the challenge with CpG DNA. These results indicate that the phosphorylation of vav1 by one or more src-related kinases plays a key role in the macrophage response to CpG DNA.

Weinstein et al. (5) first reported that macrophage activation by Gram-negative bacterial LPS provoked tyrosine phosphorylation and that an inhibitor of tyrosine kinases (herbimycin A) could block the production of inflammatory mediators by macrophages exposed to LPS. Subsequently, we (6, 8, 9, 11) and others (7, 10, 13) have provided biochemical and pharmacological evidence that tyrosine kinases of the src family are activated in response to LPS and play key roles in macrophage activation. We have also previously identified vav1 as a substrate of hck and perhaps other src family kinases in macrophages (9, 11). The role of src-related kinases in LPS-mediated macrophage activation remains controversial because targeted disruption of up to three src-related kinases (hck, lyn, fgr) in mice failed to ablate LPS signaling (12) and because the recently discovered mammalian toll-like receptors (TLRs) signal via a cascade that does not include tyrosine kinases (23–25). However, the recent availability of highly src family-selective pyrazolopyrimidine tyrosine kinase inhibitors, such as PP1 and PP2 (9, 13), has provided additional support for the role of these kinases in macrophage responses to LPS. For example, we found that PP1 potently inhibited the LPS-mediated up-regulation of TNF and iNOS production in macrophages at concentrations comparable with those needed to block the activation of hck and the phosphorylation of vav1 (9).

vav1, first identified as a proto-oncogene expressed in hematopoietic cells, is a 95-kDa protein containing a src homology 2 domain, two src homology 3 domains, a pleckstrin homology domain, and a dbl homology domain found in proteins that serve as GEFs for small GTPases (26). vav1 functions in part as a GEF for rac1 (27) and possibly also for other rho family GTPases. The GEF activity of vav1 is required for many (26, 28), but not all (15), of the functions of this protein in lymphocytes. We (11) and others (10) have reported previously that vav1 undergoes rapid tyrosine phosphorylation in response to macrophage activation by LPS. In addition, we observed that vav1 was physically associated with the src-related tyrosine kinase hck (11) in macrophages and that both broadly active and src-family-selective tyrosine kinase inhibitors blocked LPS-stimulated hck activation and vav1 tyrosine phosphorylation at concentrations that also inhibited macrophage production of TNF and iNOS (8, 9). Phosphorylation of vav1 on tyrosine 174 is necessary to activate the GEF activity of the protein (27), and the structural basis for this effect (relief of autoinhibition of the dbl homology domain of vav1) has recently been elucidated (29).
Fig. 6. Pre-incubation of RAW 264.7 cells with PP1 or expression of the truncated vavC mutant inhibits CpG DNA-mediated up-regulation of NF-κB activity but not MAP kinase activation. A, preincubation of RAW 264.7 murine macrophages with PP1 for 1 h prior to stimulation inhibited CpG DNA-mediated activation of NF-κB, as demonstrated by reporter gene luciferase assay. Cells were stimulated with medium alone or CpG (6 μg/ml) for 17 h before harvest and dual-luciferase assay. B, expression of the truncated vavC mutant in RAW-TT10/vavC cells cultured in the absence of tetracycline resulted in diminished up-regulation of NF-κB activity in response to stimulation with CpG DNA or LPS. C, preincubation of RAW 264.7 cells with PP1 (10–20 μM) failed to inhibit CpG DNA-mediated increases in the activity of ERK, p38, or JNK MAP kinases (40-min stimulations). Lanes 1, 3, and 5 = stimulation with medium alone; lanes 2, 4, and 6 = stimulation with CpG DNA 6 μg/ml (representative experiment, n = 5). D, expression of the truncated vavC mutant in RAW-TT10/vavC cells cultured in the absence of tetracycline failed to inhibit CpG DNA-mediated increases in the activity of ERK, p38, or JNK MAP kinases (40-min stimulations) (representative experiment, n = 7). Ponceau staining (prior to immunoblotting) confirmed equivalent loading for the gels shown in C and D (not shown), and the blots were also reprobed with an antibody recognizing total p38 MAP kinase as a loading control.
In the present study, we found that the tetracycline-regulatable expression of any of three different mutant forms of vav1 (vavC, vavYP174, or vavGEFnt) in stable cell lines derived from RAW-TT10 macrophages substantially inhibited iNOS protein accumulation and TNF secretion in response to CpG DNA challenge. Our findings suggest that the function of vav1 in this macrophage signaling pathway depends upon its GEF activity, unlike its role in T lymphocytes. Weiss and colleagues (16) report that overexpression of the truncated vavC construct inhibited the activation of Jurkat T cells but that expression of wild-type vav1, vavYP174, or vavGEFnt was sufficient to augment T-cell activation and nuclear factor of activated T-cell activity (15, 16).

Macrophage responses to bacterial products are coordinated primarily by TLRs (23, 25) and the key adaptor protein, MyD88 (24, 30–32). There are 10 TLRs in mice and man, and ligands for more than half of the known TLRs (most appear to have multiple ligands) have been identified (23), including Gram-negative bacterial LPS, recognized by TLR-4; bacterial lipoproteins, recognized by TLR-2; flagellin, recognized by TLR-5; double-stranded RNA, recognized by TLR-3; and bacterial DNA (CpG motifs), recognized by TLR-9 (25). After interacting with CpG DNA, TLR-9 recruits MyD88 via an interaction between the C-terminal Toll/interleukin-1 receptor domains, leading to downstream signals that include the activation of interleukin-1 receptor-associated kinases 1 (IRAK-1) and 4 (IRAK-4) and tumor necrosis factor α-receptor-associated factor 6 (TRAF-6), leading to up-regulation of NF-κB activity as well as the activation of p38 and c-Jun NH2-terminal kinase (JNK) MAP kinase pathways. TLR-9-mediated responses to CpG DNA absolutely require MyD88 (32), whereas both MyD88-dependent and -independent signaling has been observed for TLR-4 and TLR-3 ligands (33). Downstream mediators of the response to CpG DNA include the ERK, JNK, and p38 MAP kinases, which are activated at least in part via TRAF-6 (30, 31). We found that an inhibitor of src-related tyrosine kinases (PP1) or the expression of dominant-interfering mutants of vav1 blocked the up-regulation of NF-κB activity by CpG DNA in RAW 264.7 cells but failed to inhibit CpG DNA-mediated MAP kinase activation in these cells.

Our data indicate that one or more src-related tyrosine kinases and a target of these kinases, vav1, also play a key role in the up-regulation of inflammatory mediator production in macrophages exposed to bacterial DNA. Future studies in our laboratory aim to define the step(s) at which these tyrosine phosphorylation pathways diverge from the TLR-9/MyD88 pathway in macrophages stimulated with CpG DNA, to identify the specific src-related kinase(s) that are activated by CpG DNA, and to delineate the mechanism(s) by which vav1 serves to up-regulate TNF and iNOS production in macrophages exposed to bacterial DNA and other bacterial products.

REFERENCES

Role of \textit{vav1} and \textit{src}-related Tyrosine Kinases in Macrophage Activation by CpG DNA

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