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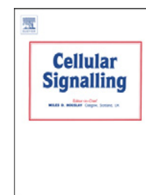

Enteropathogenic *Escherichia coli* Tir recruits cellular SHP-2 through ITIM motifs to suppress host immune response

Dapeng Yan [a,1], Heming Quan [b,1], Lin Wang [b], Feng Liu [a], Haipeng Liu [b], Jianxia Chen [b], Xuetao Cao [c], Baoxue Ge [a,b,*]

[a] Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences & Shanghai Jiaotong University School of Medicine, Shanghai, China
[b] Shanghai TB Key Laboratory, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China
[c] National Key Laboratory of Medical Immunology and Institute of Immunology, Second Military Medical University, Shanghai, China

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ABSTRACT

Immune responses to pathogens are regulated by immune receptors containing either an immunoreceptor tyrosine-based activation motif (ITAM) or an immunoreceptor tyrosine-based inhibitory motif (ITIM). The important diarrheal pathogen enteropathogenic *Escherichia coli* (EPEC) require delivery and insertion of the bacterial translocated intimin receptor (Tir) into the host plasma membrane for pedestal formation. The C-terminal region of Tir, encompassing Y483 and Y511, shares sequence similarity with cellular ITIMs. Here, we show that EPEC Tir suppresses the production of inflammatory cytokines by recruitment of SHP-2 and subsequent deubiquitination of TRAF6 in an ITIM dependent manner. Our findings revealed a novel mechanism by which the EPEC utilize its ITIM motifs to suppress and evade the host innate immune response, which could lead to the development of novel therapeutics to prevent bacterial infection.

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1. Introduction

Immune responses are usually positively regulated by receptors containing an immunoreceptor tyrosine-based activation motif (ITAM) or negatively regulated by receptors containing an immunoreceptor tyrosine-based inhibitory motif (ITIM) [1]. When the receptors are activated by their ligands, the tyrosine residues within their ITAMs will be rapidly phosphorylated, thereby forming the docking sites for several SH2-domain containing cellular proteins including Src family protein tyrosine kinases (PTK) such as Zap-70, Syk and other adaptor molecules to transduce a downstream signaling cascade by phosphorylating various effector molecules [2,3]. Many immune responses are mediated by ITAMs, such as phagocytosis, antibody-dependent cellular cytoxicity (ADCC), cytokine production, superoxide release and antigen presentation [4]. Conversely, activated immune responses are negatively regulated by ITIM-containing receptors [1,5]. Inhibitory activity of ITIM-containing receptors functions upon co-crosslinking with an ITAM-containing receptor and the inhibitory mechanism depends on the phosphorylation of the tyrosine in the ITIM by an ITAM-related PTK [6,7]. Tyrosine phosphorylation of ITIMs forms binding sites for SH2 containing cellular phosphatase such as protein tyrosine phosphatases SHP-1 or SHP-2. These phosphatases then decrease the phosphorylation of Syk, PLCγ, BLNK/SLP-76, VAV-1, Jak2, ZAP70, the γ-chain of the CD16 complex, or a series of downstream signals to inhibit the host immune response.

Interestingly, several bacterial proteins, such as Tir and CagA from *Helicobacter pylori*, contain regions that share sequence similarities with the host ITIMs [8]. Upon Src phosphorylation of these ITIM-like sequences, CagA recruits SHP-2 to stimulate tyrosine-phosphatase activity, which results in changes of the cell morphology [9]. The ITIM of the EPEC Tir is also shown to recruit the cellular inositol phosphatase SHIP2 to regulate actin-pedestal formation [10]. Recently, it has been shown that Tir interacts with SHP-1 and facilitates the recruitment of SHP-1 to TRAF6, thus inhibiting the ubiquitination of TRAF6 and subsequent expression of the proinflammatory cytokines. However, little is known about the role of the Tir and SHP-2 in the regulation of host immune responses. Therefore, we sought to explore whether the tyrosine residues within the Tir ITIM sequences play an important role in regulating host innate immune responses through SHP-2.

* Corresponding author at: Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences & Shanghai Jiaotong University School of Medicine, Shanghai, China. Tel.: +86 21 63859021.
E-mail address: gebaoxue@sibs.ac.cn (B. Ge).
[1] These authors contributed equally to this work.

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2. Methods

2.1. Cell culture and reagents

RAW264.7 cells and HEK293T cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Hyclone) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Gibco) and 100 U/ml penicillin and streptomycin. The HeLa cells and peritoneal macrophages were cultured in RPMI-1640 medium supplemented with 10% (v/v) FBS and 100 U/ml of penicillin and streptomycin. The following antibodies were used: rabbit anti-IκBα (9242), rabbit anti-p38 (9212), rabbit anti-phospho Erk1/2 (9101), rabbit anti-phospho p38 (9215) and rabbit anti-phospho Jnk (9251, all from Cell Signaling Technology); monoclonal mouse anti-TRAF6 (sc-8409) and rabbit anti-TRAF6 (sc-7221, both from Santa Cruz Biotechnology); rabbit anti-SHP-2 (ab10555, from Abcam); monoclonal mouse anti-Flag M2 Affinity Gel (A2220), rabbit anti-HA (H6908) and rabbit anti-Flag (F7425, all from Sigma); Protein G Sepharose™ 4 Fast Flow (GE Healthcare).

2.2. Plasmids and plasmid construction

The cDNA constructs encoding the EPEC (JPN15) Tir and EHEC (EDL933) Tir (from J. Leong at the University of Massachusetts Medical School) and the cDNA encoding EBV LMP2A (from R. Longnecker at Northwestern University) were cloned into the Flag-pcDNA3 vector. The cDNA encoding SHP-2 was cloned into either the HA–pcDNA3 or the Flag–pcDNA3 expression vector to generate the SHP-2 expression constructs. All the constructs were verified by DNA sequencing analysis.

2.3. Mouse strains and bacterial growth

C57BL/6J mice were bred in specific pathogen-free conditions at the Shanghai Research Center for Biomodel Organisms. All animal studies were approved by the Institutional Animal Care and Use Committee of the Shanghai Institutes for Biological Sciences (Chinese Academy of Sciences).

The site-directed point mutagenesis of the HA-tagged EPEC Tir was performed by PCR as described previously [11]. EPEC and its ΔTir strains were cultured at 37 °C in Luria–Bertani (LB) media supplemented with 100 μg/ml of ampicillin for 8 h. The pK184-based Tir plasmids were propagated in bacteria in media supplemented with 25 μg/ml of kanamycin. Before infection of host cells, EPEC were cultured in DMEM + 10 mM HEPES (pH 7.4) in 5% CO_2 for 15 h as previously shown [12].

2.4. RT-PCR analysis

The cells were incubated for 12 h without serum and were infected with bacteria for the indicated time. Total RNA was extracted with 1 ml of TRIzol reagent according to the manufacturer's instructions (Invitrogen). Next, 1 μg of total RNA was reverse transcribed

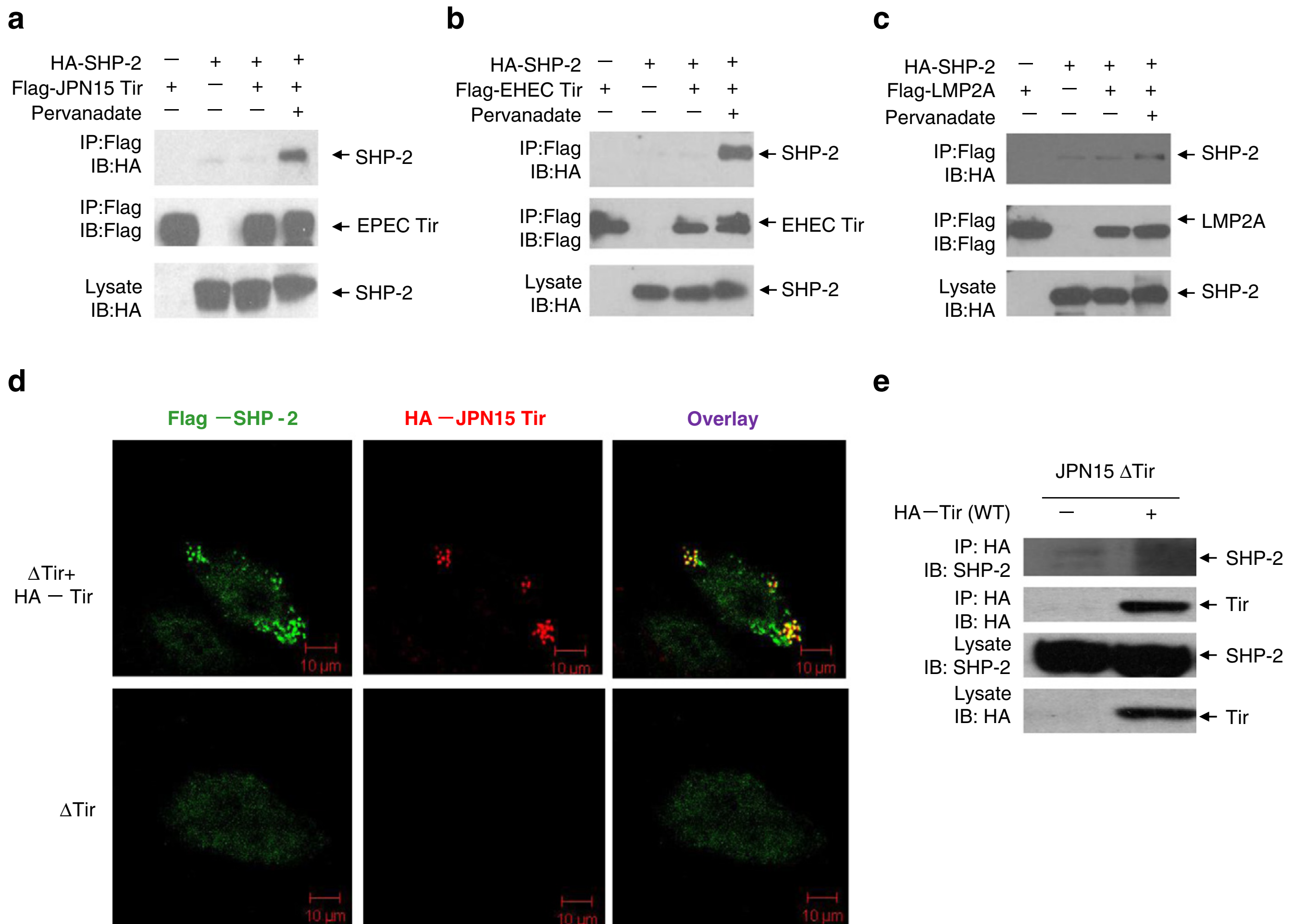

Fig. 1. Interaction of Tir with SHP-2. (a, b, c) Immunoprecipitation (IP) and immunoblot (IB) analysis of cell lysates from HEK293T cells transfected with the Flag- or HA-tagged EPEC Tir, EHEC Tir, LMP2A or SHP-2 performed using antibodies to either hemagglutinin (HA) or Flag. (d) Confocal view of HeLa cells transfected with Flag–SHP-2 and infected with the indicated bacterial strains. (e) Immunoassay of Raw264.7 cells infected with either the JPN15 ΔTir or the JPN15 (ΔTir + HA–Tir) strains. Data are representative of at least three independent experiments.

using the ReverTra Ace® qPCR RT Kit (Toyobo, FSQ-101) according to the manufacturer's instructions. A LightCycler (Roche, LC480) and a SYBR RT-PCR kit (Toyobo, QPK-212) were used for quantitative real-time RT-PCR analysis. Expression values were normalized to those obtained with the control gene *Gapdh* (encoding GAPDH).

2.5. Transfection and RNA interference

The siRNA targeting endogenous SHP-2 were transfected into RAW264.7 cells using Amaxa program D-032 and the Cell Line Nucleofector Kit V with the Amaxa Nucleofector apparatus (Amaxa Biosystems) according to the manufacturer's instructions [13]. The SHP-2 targeting sequences were as following: #1 5′-GAGGGAAGAG CAAAUGUGUCAAGUA-3′; #2 5′-AAGUAUUCCUUGGUGGACCAGACAA-3′ [14]; the "nonsense" sequence (5′-UUCUCCGAACGUGUCACGUTT-3′) was used as a control siRNA.

2.6. Pervanadate treatment and immunoprecipitation

For immunoprecipitation, HEK293T cells were transiently transfected with the indicated expression plasmids using the calcium phosphate–DNA co-precipitation method. 48 h later, the cells were treated with pervanadate (0.1 mM sodium orthovanadate and 10 mM H_2O_2) for 30 min at 37 °C, washed with PBS, and subsequently lysed in lysis buffer (20 mM Tris at pH 7.5, 150 mM NaCl, 1% Triton X-100, sodium pyrophosphate, β-glycerophosphate, EDTA, Na_3VO_4, leupeptin) supplemented with 1% protease inhibitor cocktail (Sigma, P8340), 1 mM NaF and 1 mM Na_3VO_4. For the control sample, we omitted the pervanadate treatment. After 30 min on ice, the lysates were centrifuged for 15 min at 13,523 ×g at 4 °C to remove debris. The cell lysates were incubated with anti-Flag M2 Affinity Gel overnight at 4 °C. For the endogenous immunoprecipitation, the RAW264.7 cells were infected with either the JPN15 ΔTir strain or the JPN15 ΔTir + HA–Tir strain for 6 h. The cells were subsequently lysed, and the lysate was incubated with mouse anti-HA antibody and Protein G Sepharose™ 4 Fast Flow overnight at 4 °C. The Sepharose samples were centrifuged, washed three times with cell lysis buffer and boiled with SDS loading buffer for 5 min.

2.7. GST fusion proteins and precipitation assay

The Tir and SHP-2 encoding DNA sequences were amplified by PCR and were subcloned into the pGEX-4T1 vector (Amersham) and the pET28a (Novogen) vector, respectively. The site-directed point mutagenesis of GST–Tir was performed by PCR as described above. The His and GST fusion proteins were expressed in BL-21(DE3) bacteria (Invitrogen) or *Escherichia coli* (TKB1; Stratagene), respectively, according to the manufacturer's instructions. The RAW264.7 cells were lysed as described above. The lysates or purified His–SHP-2 protein were incubated for 4 h at 4 °C with equal amounts of the appropriate fusion protein coupled to glutathione beads. The beads were isolated by centrifugation, washed, boiled with SDS loading buffer for 5 min, and later analyzed by immunoblot.

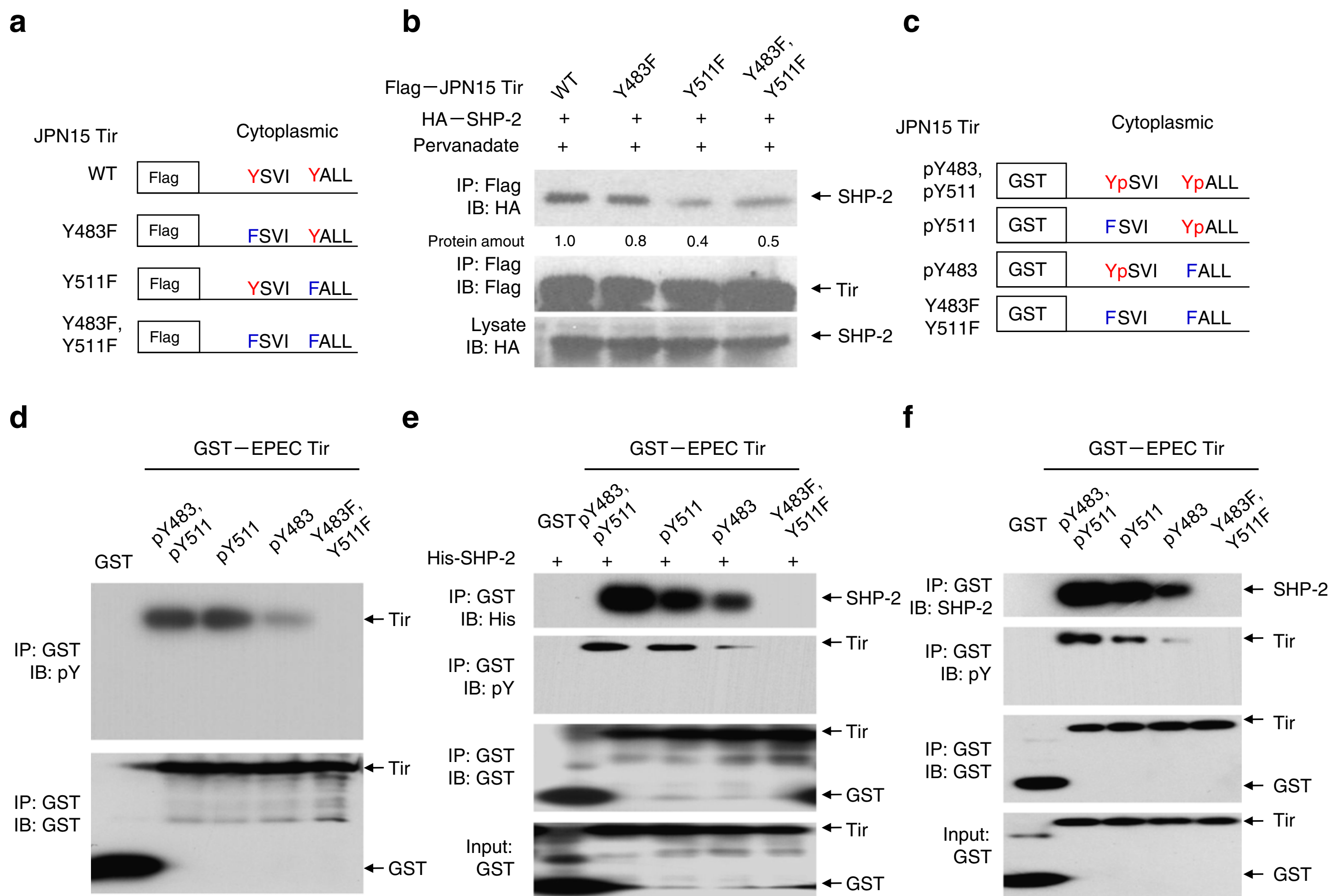

Fig. 2. ITIM phosphorylation-dependent interaction of the Tir with SHP-2. (a) A schematic of Tir ITIM mutants. (b) Immunoassay of cell lysates from HEK293T cells expressing SHP-2 and various Tir ITIM mutants; (c) GST fusion mutants of Tir. p-Y and Yp indicate phosphorylated tyrosine. (d) Tyrosine phosphorylation detection of GST fusion Tir or its ITIM mutants purified from TKB1 bacteria. (e, f) GST precipitation assay of GST–Tir or its ITIM mutants with purified His–SHP-2 (e) or endogenous SHP-2 from Raw264.7 cells (f). Data are representative of at least three independent experiments.

2.8. Cell staining and confocal microscopy

The indicated plasmids were transfected into HeLa cells with Lipofectamine™ 2000 (11668-019, Invitrogen) according to the manufacturer's instructions. 48 h after transfection, the cells were infected with the JPN15 ΔTir + HA–Tir strain or the JPN15 ΔTir strain for 4 h and then fixed with 4% formaldehyde for 30 min at 25 °C. The cells were stained and visualized under a Leica confocal microscope equipped with analytical software as described previously.

3. Results

3.1. EPEC Tir interacts with SHP-2

The EPEC Tir contains two ITIM-like regions at its C-terminus [8]. Our previous results have demonstrated that EPEC Tir interacts with SHP-1 [15]. The cellular ITIM-containing proteins usually recruit SHPs to inhibit immune responses [16,17]. To examine whether the EPEC Tir interacts with SHP-2, Flag-tagged EPEC Tir and hemagglutinin (HA)-tagged SHP-2 proteins were co-transfected into HEK293T cells for co-immunoprecipitate assay. The EPEC Tir and SHP-2 were found to co-immunoprecipitate in the presence of the phosphatase inhibitor pervanadate (Fig. 1a), indicating that EPEC Tir interacts with SHP-2. Two other ITIM-containing proteins, the EHEC Tir and Epstein–Barr virus (EBV) LMP2A, were also found to immunoprecipitate with SHP-2 (Fig. 1b,c), which suggest that the interaction between pathogen proteins and SHP-2 is a general mechanism. Next we examined the intracellular localization of EPEC Tir and SHP-2 by confocal microscopy in the context of EPEC infection. HeLa cells expressing Flag–SHP-2 were infected with either the JPN15 ΔTir strain, a mutant strain of JPN15/pMAR7 (an Ampicillin resistant (Amp^r) derivative of EPEC O127:H6 strain E2348/69) in which the Tir is deleted,

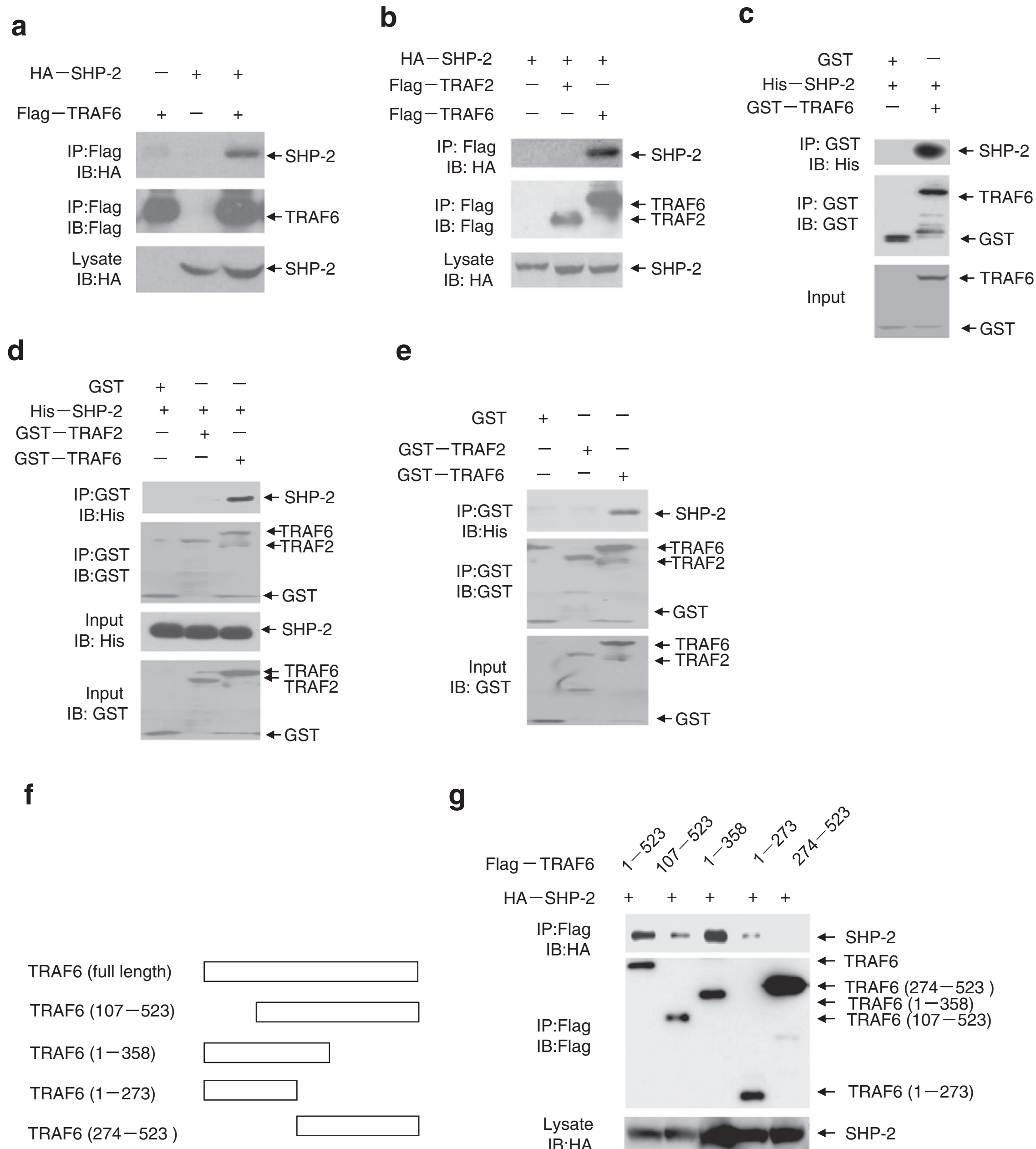

Fig. 3. SHP-2 interacts with TRAF6. (a, b) IP and IB of cell lysates from HEK293T cells expressing the indicated vectors. (c) In vitro GST precipitation assay of TRAF6 and SHP-2. (d) In vitro GST precipitation assay of TRAF2 and SHP-2. (e) Precipitation of endogenous SHP-2 from RAW264.7 cells with GST–TRAF2. (f) A schematic of TRAF6 deletion mutants. (g) Immunoassay of cell lysates from HEK293T cells expressing SHP-2 and various TRAF6 deletion mutants. Data are representative of at least three independent experiments.

or the JPN15 (ΔTir + HA–Tir) strain, a JPN15 ΔTir strain transformed with an HA-tagged Tir encoded by the pK184 vector to examine the intracellular localization of the EPEC Tir and SHP-2. The HA–Tir and Flag–SHP-2 were shown to significantly co-localize with each other under the immunofluorescence confocal microscopy (Fig. 1d). The interaction between the EPEC Tir and endogenous SHP-2 was further examined in RAW264.7 cells that had been infected with either the JPN15ΔTir strain or the JPN15 (ΔTir + HA–Tir) strain with co-immunoprecipitate assay, as expected, the Tir was found to associate with endogenous SHP-2 (Fig. 1e). Thus, all the data presented here demonstrated that SHP-2 can interact with Tir.

3.2. Tir-SHP-2 interaction requires ITIM phosphorylation

Signal transduction by ITIM containing proteins of immune cells depends on the phosphorylation of its two ITIMs, and subsequent recruitment of SHP-2 [8]. To investigate whether ITIM motifs of microbial proteins need to be phosphorylated for its interaction with SHP-2, we generated Tir mutants in which the tyrosine residues of two ITIM motifs were replaced with phenylalanine individually and together (Fig. 2a) and transfect them together with SHP-2 in HEK293T cells. The data showed that substitution of Tyr483 resulted in a little less binding of EPEC Tir to SHP-2, while substitution of

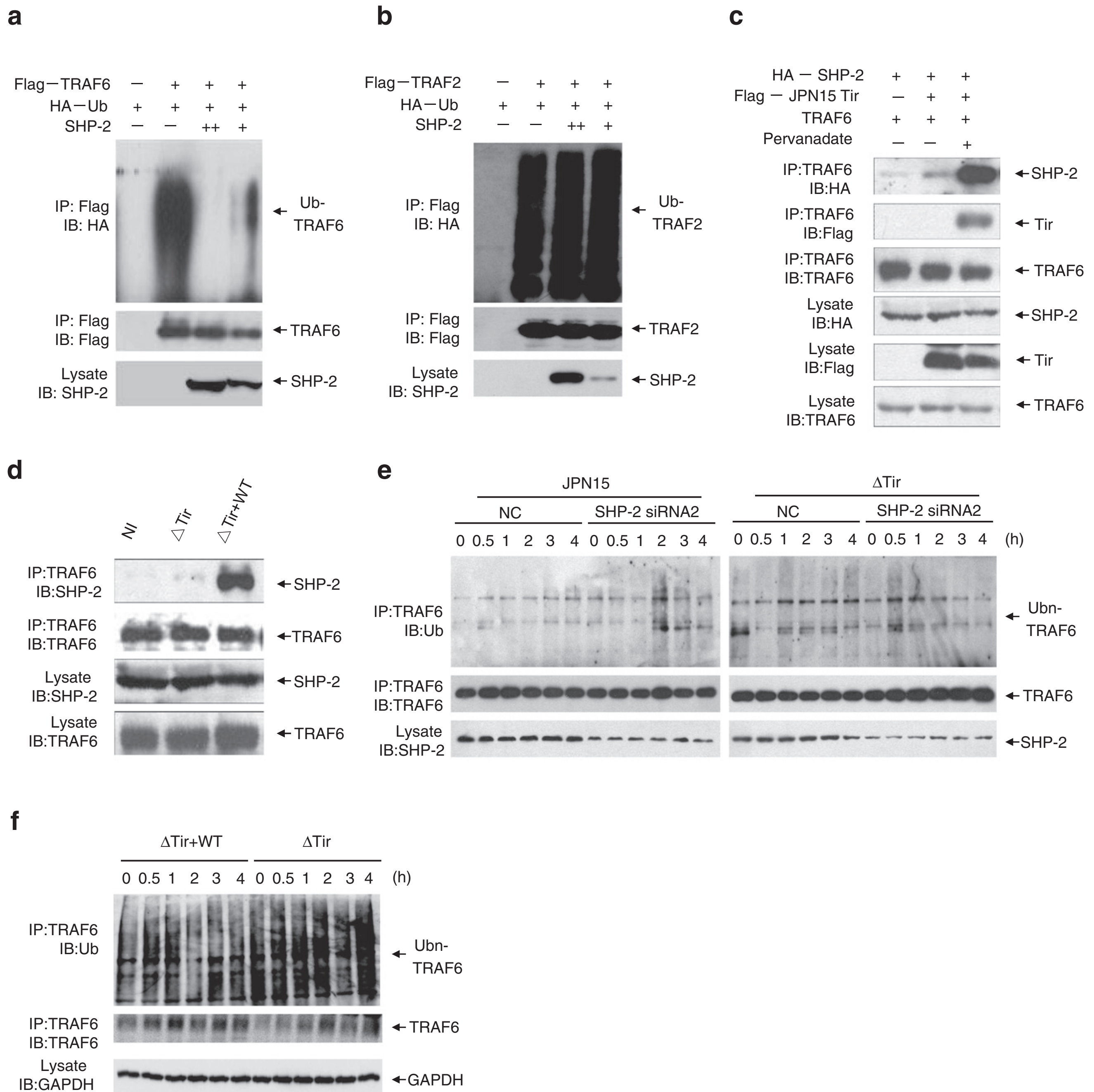

Fig. 4. The Tir facilitates binding of TRAF6 to SHP-2 and prevents TRAF6 ubiquitination. (a, b) Immunoassay of HEK293T cell lysates expressing the indicated vectors. ++, double amount. (c) Immunoassay of HEK293T cells expressing the indicated vectors. (d) Immunoassay of primary peritoneal macrophage cells infected with the indicated strains. (e) IP and IB of cell lysates from siRNA-treated RAW264.7 cells infected for indicated times with either the JPN15 or the JPN15 ΔTir strains. (f) IP and IB of cell lysates from primary peritoneal macrophage cells infected for indicated times with the indicated strains. Data are representative of at least three independent experiments.

Tyr511 resulted in much less binding (Fig. 2b), suggesting that the interaction of EPEC Tir with SHP-2 is dependent on the phosphorylation of ITIM of Tir, especially Tyr511. Next, we used *E. coli* with an inducible tyrosine kinase gene and induced the bacteria to express either phosphorylated recombinant wild-type EPEC Tir or its point mutants, then purified the recombinant proteins as glutathione S-transferase (GST) fusion proteins to determine whether ITIM phosphorylation was required for direct interaction between Tir and SHP-2. Using these purified recombinant proteins in an in vitro glutathione S-transferase (GST) precipitation assay, We found that only the EPEC Tir protein that possessed phosphorylated tyrosine-containing ITIMs could associate with purified recombinant His–SHP-2 or endogenous SHP-2 from RAW264.7 cells and that mutating the tyrosine residues in the ITIMs eliminated this association (Fig. 2d-f), especially Tyr511. Indeed, substitution of Tyr483 resulted in a little less binding of EPEC Tir to SHP-2, while substitution of Tyr511 resulted in much less binding and substitution of both ITIMs almost eliminated the binding, suggesting that the interaction of EPEC Tir with SHP-2 is dependent on the phosphorylation of ITIM of Tir, especially Tyr511. Taken together, these results suggest that the direct interaction of Tir with SHP-2 is dependent on its tyrosine phosphorylation of ITIMs.

3.3. SHP-2 can directly interact with TRAF6

To find out which signaling pathways SHP-2 down-regulates to inhibit cytokine production during EPEC infection, we investigated whether SHP-2 interact with TRAF6, an important adaptor protein of TLR4 signaling pathway [18–20]. Flag–TRAF6 and HA–SHP-2 were co-transfected into HEK293T cells, we found that TRAF6 immunoprecipitated together with SHP-2 (Fig. 3a), suggesting that SHP-2 interacts with TRAF6. In addition, we also examined the interaction of SHP-2 with other TRAFs and the results showed that TRAF6, but not TRAF2, immunoprecipitated with SHP-2 (Fig. 3b). To exam whether SHP-2 can directly interact with TRAF6, we therefore performed pull-down assays using the recombinant His–SHP-2 fusion protein and GST–TRAF6 fusion protein. The anti-GST pull-down assay showed that GST–TRAF6 immunoprecipitated with purified recombinant His–SHP-2 (Fig. 3c), but GST cannot, indicating that SHP-2 can directly interact with TRAF6. Similarly, TRAF2 didn't interact with SHP-2 directly (Fig. 3d,e). Collectively, these results demonstrated that TRAF6 interacts with SHP-2 directly. We made deletion mutants of TRAF6 (Fig. 3f) and assessed whether these mutants retained their ability to interact with SHP-2 to map the region of TRAF6 protein that binds to SHP-2. The TRAF6 fragments containing amino acid residues 107–523 and 1–273 decreased the ability to bind to SHP-2, while residues 274–523 almost abolished this binding. In fact, SHP-2 bound only to the region of TRAF6 corresponding to amino acid residues 1–358 (Fig. 3g). Thus, residues 107–358 are essential for the binding of TRAF6 and SHP-2.

3.4. Tir enhances the binding of SHP-2 with TRAF6 to decrease TRAF6 ubiquitination

It is known that TRAF6 activation depends on its auto-ubiquitination and that deubiquitination of TRAF6 blocks downstream signaling events [21–23]. To detect if SHP-2 interferes with the autoubiquitination of TRAF6, we transfected Flag-tagged TRAF6 together with HA-tagged

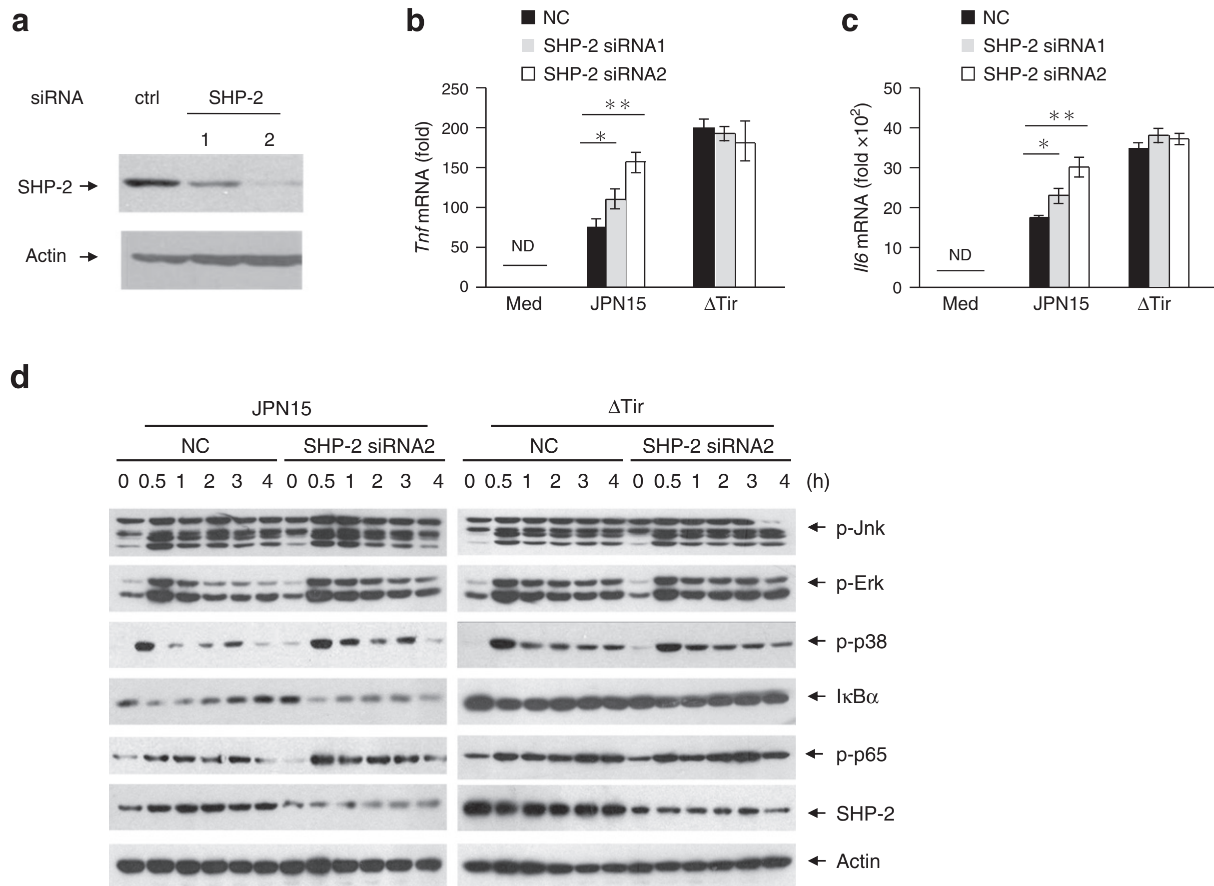

Fig. 5. Inhibition of cytokine production via SHP-2. (a) Immunoblot of lysates isolated from RAW264.7 cells transfected with either the control siRNA or SHP-2 siRNA. (b, c) Quantitative RT-PCR of *Tnf* and *Il6* mRNA in siRNA-treated Raw264.7 cells after infection with either the JPN15 or the JPN15 ΔTir strains. (d) IB of cell lysates from siRNA-treated RAW264.7 cells after infected for indicated times with either the JPN15 or the JPN15 ΔTir strains. $^{*}P < 0.05$ (Student's *t*-test). $^{**}P < 0.01$ (Student's *t*-test). Data are representative of at least three independent experiments (mean and s.e.m. in b, c, e and f).

ubiquitin into HEK293T cells and then analyzed autoubiquitination of TRAF6 by western blotting. We found that ectopic expression of SHP-2 in HEK293T cells significantly decreased the ubiquitination of TRAF6 in a dose dependent manner (Fig. 4a) but not of TRAF2 (Fig. 4b). Further, overexpression of the EPEC Tir in HEK293T cells enhanced the interaction of TRAF6 and SHP-2 (Fig. 4c). Furthermore, we found that the enhanced interaction of SHP-2 and TRAF6 in primary peritoneal macrophage cells exclusively occurred when cells were infected with JPN15 (ΔTir + HA–Tir) strains, but not JPN15 ΔTir strains (Fig. 4d), suggesting that Tir could enhance the interaction of SHP-2 with TRAF6 in bacterial infectious conditions. Next, we detected the effect of SHP-2 on TRAF6 ubiquitination for endogenous proteins. As expected, Ctrl siRNA-transfected RAW264.7 cells infected with JPN15 strain showed much less ubiquitination than that of JPN15 ΔTir strain, suggesting that Tir can inhibit the bacterial infection induced TRAF6 ubiquitination. However, transfection of RAW264.7 cells with SHP-2 siRNA substantially enhanced the ubiquitination of TRAF6 upon JPN15 infection, especially 3 h after infection, whereas there is no significant difference on the ubiquitination of TRAF6 when infected with JPN15 ΔTir strains (Fig. 4e). Similarly, JPN15 (ΔTir + HA–Tir) strains inhibited the ubiquitination of TRAF6 compared to JPN15 ΔTir strains (Fig. 4f).

3.5. Tir inhibits EPEC infection induced cytokine production via SHP-2

Since Tir interacts with SHP-2, we next investigated whether Tir inhibits the cytokine production through SHP-2. Non-targeting control small interfering RNA (Ctrl siRNA) or two pairs of SHP-2 siRNA were introduced into RAW264.7 cells. Western blotting assay was performed to determine the SHP-2 knockdown efficiency, and the data showed that SHP-2-specific siRNA could significantly decrease the expression of SHP-2 (Fig. 5a). As expected, Ctrl siRNA-transfected RAW264.7 cells infected with JPN15 strain produced much less *Tnf* or *Il-6* than that of JPN15 ΔTir strain, suggesting that Tir can inhibit the bacterial infection induced host immune response. However, transfection of RAW264.7 cells with SHP-2 siRNA substantially enhanced the expression of *Tnf* or *Il-6* upon JPN15 infection, whereas there is no significant difference on the expression of *Tnf* or *Il-6* when infected with JPN15 ΔTir strains (Fig. 5b,c). To dissect the signal pathways inhibited by Tir-SHP-2 axis. The RAW264.7 cells were transfected with either Ctrl siRNA or SHP-2 siRNA 2. 48 h later, the cells were infected with either the JPN15 or JPN15 ΔTir strain, and the activation of the MAP kinase and NF-κB signaling pathways was analyzed to examine whether SHP-2 mediated Tir inhibited MAP kinase and NF-κB pathways. When infected with JPN15 strain, the infection of Ctrl siRNA treated RAW264.7 cells induced the phosphorylation of Jnk, Erk, p38, and the degradation of the upstream NF-κB regulator IκBα. Importantly, these effects were more striking in cells treated with SHP-2 siRNA, especially at 3 h post-infection, when the insertion of the Tir protein into the host cell membrane is probably at its peak. However, 48 h after RAW264.7 cells were transfected with either Ctrl siRNA or SHP-2 siRNA, there is no significant difference on the signal pathways when infected with JPN15 ΔTir strains (Fig. 5d). Thus, all the results demonstrated that SHP-2 is required for the EPEC Tir-mediated inhibition of cytokine production and signal transduction (Fig. 6).

4. Discussion

EPEC infections usually cause unexpected low inflammation, suggesting that the pathogen may develop some strategies to interfere with the host inflammatory pathways [24]. During infection, T3SS delivery system of EPEC transfers numerous 'effector' proteins directly into host cells to interfere with different cellular functions [25]. EPEC Tir is the only effector shown to be essential for disease development [26,27]. However, relatively little is known about how the Tir regulates the innate inflammatory responses. As has been reported before, some pathogen proteins also contain ITIM motifs [8]. Our previous results have demonstrated that Tir from EPEC could interact with the cellular tyrosine phosphatase SHP-1 in an ITIM phosphorylation-dependent manner. The association of the Tir with SHP-1 facilitated the recruitment of SHP-1 to TRAF6 and inhibited the ubiquitination of TRAF6 and downstream cytokine production [15]. The cytoplasmic tyrosine phosphatases, SHP-1 and SHP-2, are implicated and play the same role in the control of immune response in many cases [28]. Here, we identify SHP-2 as another host protein that interacts with Tir to suppress the host cytokine production, and determine that binding of SHP-2 with Tir is dependent on ITIM phosphorylation. Furthermore, interaction of SHP-2 with Tir increases the recruitment of SHP-2 to TRAF6, and "knockdown" of SHP-2 by RNAi decreases the Tir-mediated inhibitory signaling and inhibition of cytokine production. Thus, in addition to SHP-1 [15], SHP-2 emerges as another novel molecule in the regulation of bacterial protein mediated inhibitory signaling. However, the manner in which the complex of SHP-1, SHP-2 and Tir coordinates to negatively regulate cytokine production needs further investigation.

ITIM-like motifs are a notable feature of the Tir protein and are shown here to be essential for its interaction with SHP-2, and thus, its ability to inhibit host cytokine production. Other microbial proteins also contain ITIM motifs, and we have shown that at least two other ITIM-containing proteins, EHEC Tir and EBV LMP2A, co-immunoprecipitate with SHP-2. Thus, the utilization of cellular ITIM-like motifs by microbial proteins to interfere with host immune responses could be a general mechanism by which microbial pathogens establish successful infection.

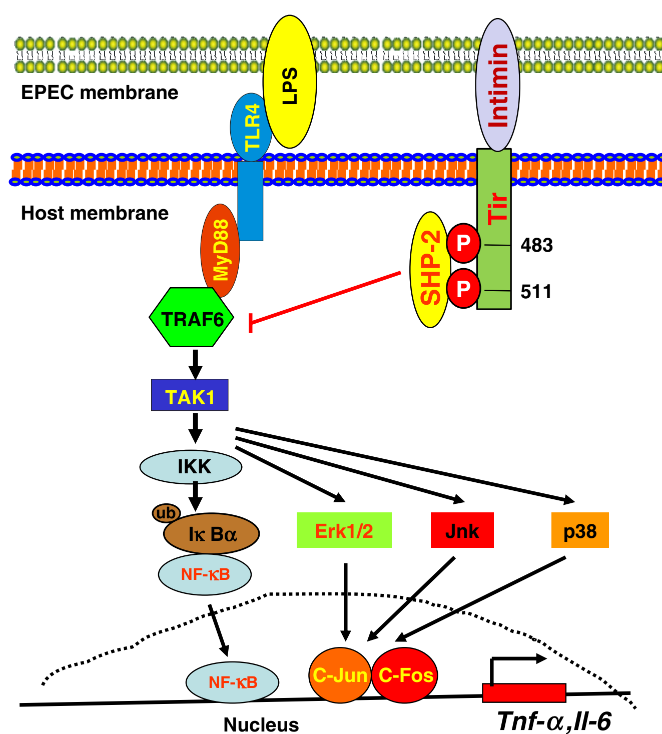

Fig. 6. Diagram depicting the regulation of EPEC Tir signaling by SHP-2. When EPEC interacts with macrophages, LPS activates TLR4–TRAF6–TAK1–MAPK and NF-κB pathway. This leads to translocation of transcription factors into nucleus and transcription of cytokines. After having been injected into host cells, Tir is phosphorylated at tyrosine residues within the ITIM motif, which leads to the recruitment of SHP-2 and subsequently inhibition of TRAF6 autoubiquitination and downstream signaling pathway.

Contributions

D.Y., H.Q., X.C. and B.G. designed this study. D.Y. and H.Q. performed experiments, assisted by L.W., F.L., H.L., J.C., D.Y. and B.G. analyzed the

data and wrote the manuscript. All authors discussed the results and commented on the manuscript.

Competing financial interests

The authors declare no competing financial interests.

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