Shp2 Deficiency Impairs the Inflammatory Response Against *Haemophilus influenzae* by Regulating Macrophage Polarization

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Macrophages can polarize and differentiate to regulate initiation, development, and cessation of inflammation during pulmonary infection with nontypeable *Haemophilus influenzae* (NTHi). However, the underlying molecular mechanisms driving macrophage phenotypic differentiation are largely unclear. Our study investigated the role of Shp2, a Src homology 2 domain-containing phosphatase, in the regulation of pulmonary inflammation and bacterial clearance. Shp2 levels were increased upon NTHi stimulation. Selective inhibition of Shp2 in mice led to an attenuated inflammatory response by skewing macrophages toward alternatively activated macrophage (M2) polarization. Upon pulmonary NTHi infection, *Shp2*−/− mice, in which the gene encoding Shp2 in monocytes/macrophages was deleted, showed an impaired inflammatory response and decreased antibacterial ability, compared with wild-type controls. In vitro data demonstrated that Shp2 regulated activated macrophage (M1) gene expression via activation of p65–nuclear factor-κB signaling, independent of p38 and extracellular regulated kinase–mitogen-activated proteins kinase signaling pathways. Taken together, our study indicates that Shp2 is required to orchestrate macrophage function and regulate host innate immunity against pulmonary bacterial infection.

**Keywords.** *Haemophilus influenzae*; protein-tyrosine phosphatase Shp2; macrophage polarization; inflammatory response.

Intermittent or chronic infection with nontypeable *Haemophilus influenzae* (NTHi) is the most common cause of acute exacerbation of chronic obstructive pulmonary disease (COPD) and community-acquired pneumonia [1, 2]. NTHi, a kind of pleomorphic gram-negative bacillus, is one of the most common bacterial species colonizing the upper respiratory tract. NTHi usually causes otitis media in children and pneumonia in elderly individuals and people with chronic lung disease [3–5]. NTHi has multiple virulence factors, including lipooligosaccharide, catalase (encoded by *hktE*), peroxiredoxin-glutaredoxin (encoded by *pdgX*), and biofilm, which are involved in bacterial persistence and resistance to host clearance [6–9]. Previous studies have demonstrated that macrophages play a critical role in NTHi clearance [10, 11]. Macrophages are extensively explored for their remarkable phagocytosis of different groups of pathogens during the innate immune response and for their ability to phenotypically differentiate to regulate initiation, development, and cessation of inflammatory diseases [12–14].

Generally, polarized macrophages are classified into 2 groups: activated macrophages (M1 macrophages) and alternatively activated macrophages (M2 macrophages). M1 macrophages are proinflammatory and have a central role in host defense against infection, while M2 macrophages are associated with responses to anti-inflammatory reactions and tissue remodeling [15, 16]. M1 and M2 macrophages have distinct chemokine profiles. M1 macrophages are induced by lipopolysaccharides, Toll-like receptor ligands, and interferon γ. M1 macrophages release high levels of proinflammatory cytokines, such as interleukin 6 (IL-6), tumor necrosis factor α (TNF-α), inducible NO synthase (iNOS), and interleukin 12 (IL-12), promoting host immune response against invading bacteria. In contrast, M2 cells are typically stimulated by interleukin 4 (IL-4) and interleukin 13. They express high levels of arginase 1 (Arg1), chitinase 3–like protein 3 (Chi3l3, YM-1), and resistin-like α (Retnla, Fizz1) [16]. Previous studies have revealed that defective macrophages are associated with accelerated progression of airway inflammation during bacterial infection and COPD exacerbation [17–19]. However, the underlying molecular mechanisms driving macrophage phenotypic differentiation are still unclear during NTHi infection.

The Src homology 2 domain-containing phosphatases (Shps) comprise a small subfamily of nonreceptor protein-tyrosine phosphatases (PTPs) [20]. Shp2 has been shown to integrate multiple signaling events and play important roles in the pathogenesis of a wide variety of diseases, including chronic airway diseases, atherosclerosis, gastric carcinoma, and glioma [21–26].
In the present study, we revealed that Shp2 is required to host innate immunity against bacterial infection through the regulation macrophage polarization.

MATERIALS AND METHODS

Mice
Shp2<sup>flox/fox</sup> mice were crossed with LysM<sup>Cre/+</sup> mice to generate myeloid Shp2 conditional knockout mice [23, 27]. LysM<sup>Cre/+</sup> or Shp<sub>2</sub><sup>flox/fox</sup> (control) and LysM<sup>Cre/+</sup>:Shp<sub>2</sub><sup>flox/fox</sup> (Shp2<sup>−/−</sup>) mutant mice were used in the experiments. All animal experiments were in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee at Zhejiang University.

Bacterial Culture
NTHi (ATCC49247) was harvested after overnight incubation in brain heart infusion broth supplemented with nicotinamide adenine dinucleotide (3.5 μg/mL) and hemin (5 μg/mL) [28]. Then, 100 μL of NTHi culture was inoculated into 10 mL of fresh broth culture and subjected to shaking at 37°C for another 5 hours to reach middle-to-late log phase. Bacteria were quantified according to the OD<sub>600</sub>-based bacterial growth curve and bacterial colony-forming units (CFUs) assay.

Cell Culture
Bone marrow–derived macrophages (BMDMs) were established as previously described [13, 14]. In brief, femurs were flushed with Roswell Park Memorial Institute (RPMI) 1640 medium, using a 21-gauge needle. Cells were grown in RPMI 1640 medium containing 10% fetal calf serum (FCS) and 10 ng/mL macrophage colony-stimulating factor (M-CSF; Peprotech, Rock Hill) in 5% CO2 at 37°C for 7 days.

Bacterial Killing Ability in Macrophages
The bacterial killing capability of macrophages was evaluated using an in vitro assay.

A total of 2 × 10<sup>5</sup> BMDMs in a 12-well tissue culture plate were infected with bacteria (multiplicity of infection [MOI], 20) for 1 hour at 37°C. After this period, monolayers were washed with RPMI 1640 medium. Fresh RPMI 1640 medium containing 10% FCS, M-CSF, and 50 μg/mL gentamicin was added for 1 hour to rapidly kill extracellular NTHi. Incubation was then continued for 1 hour, 3 hours, or 5 hours after the addition of 1 μg/mL gentamicin, the minimum concentration required to inhibit extracellular bacteria [29, 30]. At the indicated time points, cells were lysed with 0.1% Triton X-100 to release live intracellular bacteria, which were plated and quantified [13]. To evaluate the antibacterial ability of macrophages, a mean killing index (MKI) was calculated as the mean number of CFUs before the addition of gentamicin minus the number of CFUs at 1 hour, 3 hours, or 5 hours after the addition of gentamicin for wild-type (WT) and Shp2<sup>−/−</sup> cells.

Lung Infection Model
A pulmonary infection model was established in pathogen-free, 8-week-old female C57BL/6 mice. In brief, 50 μL of NTHi (1 × 10<sup>7</sup> CFUs) was instilled intratracheally in lightly anesthetized mice. The application of phenylhydrazonopyrazolone sulfonate 1 (PHPS1; Santa Cruz Biotechnology) was performed by intraperitoneal injection at concentrations of 3 mg/kg dissolved in saline with 0.5% dimethyl sulfoxide 0.5 hours before NTHi stimulation. Mice were euthanized 12 hours after NTHi infection, and whole lungs and bronchoalveolar lavage (BAL) fluid were collected. The same procedure was used for Shp2<sup>−/−</sup> mice to establish the NTHi infection model.

Histopathological Changes
The whole lung of mice was fixed with 10% paraformaldehyde and embedded with paraffin. Four-micrometer sections were sliced for hematoxylin staining. Morphometric analysis was conducted under an automatic photomicroscope (Leica).

Bacterial Assay in BAL Fluid
BAL fluid was collected by lavaging the lungs with 1 mL of sterile phosphate-buffered saline (PBS) for 5 times. The fluid was

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Figure 1. Nontypeable Haemophilus influenzae (NTHi) induced increased Src homology 2 domain-containing phosphatase 2 (Shp2) expression in bone marrow–derived macrophages (BMDMs) and lung tissue in a mouse infection model. BMDMs from wild-type (WT) mice were incubated with bacteria (multiplicity of infection, 5) for 8 hours. Messenger RNA (mRNA; A) and protein (B) expression of Shp2 in BMDMs was detected. WT mice were infected by intratracheal instillation of NTHi (1 × 10<sup>7</sup> colony-forming units) for the indicated times. The expression of the gene encoding Shp2 was assessed in lung tissues (C). There were 3–5 mice per group. *P < .05. Abbreviation: PBS, phosphate-buffered saline.
serially diluted 1:10 in PBS and plated on chocolate agar plates to determine the number of CFUs.

Cell Counting and Measurement of Cytokines and Nitrite Levels in BAL Fluid
After collecting BAL fluid, erythrocytes were removed using lysis buffer (eBioscience). A total of $2 \times 10^5$ cells were loaded onto a slide by cytospin (Statspin) and stained with Giemsa stain for neutrophil counting. Cell-free BAL fluid underwent enzyme-linked immunosorbent assays (ELISAs) to measure the concentration of keratinocyte-derived chemokine (KC), macrophage inflammatory protein 2 (MIP-2), TNF-$\alpha$, and IL-6 (R&D Systems) according to the manufacturer’s protocol. Nitrite, the stable oxidized derivative of NO, was determined by the Griess reagent kit (Molecular Probes) according to the manufacturer’s instructions [13].

RNA Isolation and Quantitative Polymerase Chain Reaction (PCR) Analysis
RNA was extracted from lung tissue and BMDMs, using Trizol (Invitrogen). SYBR Green PCR Master Mix (Toyobo) was used to detect messenger RNA levels. The sequences for the primers were as follows: Shp2, 5-GAAACGGTCATTCAGCCACT-3 (forward) and 5-GCAGCCAAGGAGTCATCTTC-3 (reverse); IL-6, 5-AGTTGCCTTCTTGGGACTGA-3 (forward) and 5-TCCACGATTTCAGAGAACAC-3 (reverse); TNF-$\alpha$, 5-CTG GGACAGTGACCTGGACT-3 (forward) and 5-GCACCTCAGGGAGTAGTCACC-3 (reverse); Arg-1, 5-GAGAAGATGGAAGTGTCAG-3 (forward) and 5-CAGAGTGTCAG-3 (forward); Ym1, 5-GGATGCGCTACCTGGGAAA-3 (forward) and 5-AGAAAGGTCACTCAGGATAA-3 (reverse); Fizz1, 5-CCC TCCACTGTAACGAAG-3 (forward)

Figure 2. Inhibition of Src homology 2 domain-containing phosphatase 2 led to decreased pulmonary inflammation. Phenylhydrazonopyrazolone sulfonate 1 (PHPS1)–pretreated wild-type (WT) mice were infected with instillation of nontypeable Haemophilus influenzae (NTHi; $1 \times 10^7$ colony-forming units). After 12 hours, mice were euthanized for the evaluation of lung inflammation. A, Hematoxylin and eosin staining of lung tissue (100 $\times$ original magnification). B, The expression levels of inflammatory cytokines such as interleukin 6 (IL-6), tumor necrosis factor $\alpha$ (TNF-$\alpha$), keratinocyte-derived chemokine (KC), and macrophage inflammatory protein 2 (MIP-2) were measured by quantitative polymerase chain reaction. C, The levels of the inflammatory cytokines IL-6, TNF-$\alpha$, KC, and MIP-2 in bronchoalveolar lavage fluid were also detected by enzyme-linked immunosorbent assays. There were 3–5 mice per group. *P < .05. Abbreviations: DMSO, dimethyl sulfoxide; mRNA, messenger RNA; PBS, phosphate-buffered saline.
and 5-GTGGTCCAGTCAACGAGTAA-3 (reverse); and iNOS, 5- CTGCAGCACTTGGATCAGGAACCTG-3 (forward) and 5-GGAGTAGCCTGTGTGCACCTGGAA-3 (reverse). β-actin (5-GTATCCTGACCCTGAAGTACC-3 [forward] and 5-TGAAGGTCTCAAACATGATCT-3 [reverse]) was amplified as an endogenous control gene. After normalization of target gene RNA with that of the gene encoding β-actin, expression of each target gene was measured as the fold increased expression above the control, calculated by a corrected ΔΔ cycle threshold method with a Sequence Detection Software (Applied Biosystems) [31].

Western Blot
Proteins were extracted from BMDMs by using radioimmuno-precipitation assay buffer supplemented with protease inhibitors. The protein concentration was measured by the BCA Protein Assay Kit, and samples were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and immunoblotting, using antibodies against the following proteins: β-actin, p38, p-p38, ERK, p-ERK, p-65, p-p65 (Cell signaling), and Shp2 (Santa Cruz Biotechnology).

Luciferase Reporter Assay
Nuclear factor κB (NF-κB)-driven reporter activity and IL-6 promoter activity were measured in Shp2−/− or WT macrophages in response to NTHi stimulation. Briefly, cells were seeded in 6-well plates (2 × 10^5 cells/well) overnight and transfected with luciferase reporter plasmids and subjected to NTHi infection (MOI, 5) 40 hours later. After 6 hours of bacterial stimulation, luciferase activities in cell extracts were measured with dual-luciferase reporter assays (Promega) as described previously [13, 32].

Statistical Analysis
All data, unless otherwise indicated, are presented as mean values ± standard errors of the mean. The statistical significance of differences between 2 groups was analyzed with the Student t test. For the CFU assay in mouse models, the Wilcoxon rank sum test was performed, and results are shown as median values and interquartile ranges. A P value of ≤ 0.05 was considered statistically significant. All calculations were performed using the Prism software program for Windows (GraphPad Software).

RESULTS
NTHi Induced Shp2 Overexpression in BMDMs and Lung Tissue in a Mouse Infection Model
The tyrosine phosphatase Shp2 has been shown to modulate the lung inflammatory response [21–23]. Cell and lung infection models were established to investigate the possible involvement of Shp2 in acute lung inflammation. After NTHi infection, a remarkable elevation in the level of Shp2 expression was found in BMDMs at both the transcriptional and protein levels (Figure 1A and 1B). Consistently, the level of Shp2 expression was also elevated in lung tissues 12 hours after infection (Figure 1C).

Inhibition of Shp2 Led to Decreased Pulmonary Inflammation
Next, we evaluated the effect of Shp2 inhibition on NTHi-induced lung inflammation. PHPS1, a Shp2-specific pharmacological inhibitor, was administrated to mice 30 minutes before...
NTHi stimulation. Compared with controls, the level of inflammation was significantly attenuated in PHPS1-pretreated mice 12 hours after infection (Figure 2A). We found that Shp2 inhibition resulted in reduced levels of several proinflammatory cytokines, including IL-6, TNF-α, KC, and MIP-2, in lung tissue (Figure 2B). The decreased levels of IL-6 and TNF-α were found in the BAL fluid at indicated times (Figure 2C).

**Inactivation of Shp2 Skewed the Macrophage Phenotype During NTHi Infection**

During the course of NTHi-induced lung infection, the early and timely recruitment of neutrophils and macrophages to the lung plays an important role in eradication of the bacteria. It is known that macrophages are polarized toward an M1 phenotype in the early stage of bacterial infection and serve as a prompt to eliminate pathogens [15]. Our data showed that PHPS1, which inhibits Shp2 activity, significantly suppressed the expression of the M1-associated genes encoding IL-6, TNF-α, IL-12p40, and iNOS (Figure 3A–3D), whereas there was a marked elevation in expression of M2 marker genes, including those encoding Arg1 and Ym-1 (Figure 3E and 3F). These results indicate that Shp2 regulates macrophage phenotypic shift during NTHi infection.

**RelA/NF-κB Is Required for an M1 Macrophage Shift in Response to NTHi Infection**

To elucidate the molecular mechanisms responsible for Shp2-mediated regulation of M1-related genes, we analyzed mitogen activated protein kinases (MAPKs) and NF-κB. As expected, NTHi induced increased phosphorylation of ERK1/2, p38, and p65-NF-κB in primary BMDMs (Figure 4A). However, Shp2 gene deletion had no major effects on p-ERK1/2 and p-p38 in NTHi-stimulated cells, whereas it caused a remarkable decrease in phosphorylation of p65. Decreased NF-κB activation was found in Shp2+/− macrophages by the luciferase reporter assay, compared with that in controls (Figure 4B). To further determine whether the decrease in M1-associated gene transcription was correlated with the attenuated NF-κB activity in Shp2−/− cells, we constructed an IL-6 promoter reporter plasmid containing the NF-κB site [13, 33]. The results showed that IL-6–promoted activity was significantly lower in Shp2−/− macrophages (Figure 4C), which consistently indicated that NF-κB–dependent regulation of M1-associated genes was mediated by Shp2 signaling. Previous studies have demonstrated that RelA/p65 is involved in mediating M1-associated gene expression [13, 34–38]. Collectively, our results demonstrated that RelA/p65 mainly accounts for Shp2-mediated skewing of M1 genes.

**Endogenous Deletion of Shp2 Exhibited Decreased Inflammation via Regulation of Macrophage Phenotype in Pulmonary NTHi Infection**

Shp2−/− mice with deletion of Shp2 gene in monocytes/macrophages were created. Compared with the WT group, Shp2−/− mice showed a significant decline in the influx of inflammatory cells into alveolar spaces upon NTHi stimulation (Figure 5A).

Meanwhile, the total BAL cell counts and the percentage of neutrophils were also lower in Shp2−/− mice (Figure 5B). We further investigated the role of Shp2 in the regulation of macrophage phenotype. As expected, the results were consistent with those of PHPS1-treated mice. Inhibited expression of the prototypical M1 genes, including those encoding IL-6, IL-12, and iNOS, was observed in the NTHi-infected Shp2−/− BMDMs, compared with controls (Figure 5C), whereas expression of the M2 marker genes, such as that encoding Ym-1, was...
increased in infected-Shp2−/− cells (Figure 5D). Therefore, Shp2 appears to play a critical role in driving M1-associated gene expression upon bacterial invasion.

Shp2 Deletion Impaired Host Immunity Against NTHi
A remarkably increased bacterial burden was found in BAL fluid from Shp2−/− mice, compared with controls (Figure 6A). Bactericidal activity of macrophages is considered a critical feature of M1-polarized macrophages. The MKI, as described in Methods, was used to further evaluate the bactericidal activity in BMDMs. The BMDMs from Shp2−/− mice were less efficient in clearing NTHi. After 1 hour of treatment with 50 μg/mL gentamicin, cell culture showed no extracellular bacteria, which was consistent with the result from a previous study [30]. At this time point, the number of intracellular bacteria was not significantly different between WT and Shp2−/− cells (mean, 7.66 × 10^4 CFUs vs 5.78 × 10^4 CFUs; P = .25). We found that the antibacterial effect, measured by the MKI, in WT cells was markedly larger than that in Shp2−/− cells after 1 hour and 3 hours of additional exposure to gentamicin (1 μg/mL). After 5 hours of additional gentamicin treatment, both WT and Shp2−/− macrophages had almost eradicated all intracellular NTHi, and there was no difference in MKI between

### Figure 5.
Endogenous deletion of Src homology 2 domain-containing phosphatase 2 exhibited decreased inflammation via regulation of macrophage phenotype during nontypeable Haemophilus influenzae (NTHi) infection. Shp2−/− and wild-type (WT) mice were euthanized 12 hours after infection with NTHi. A, Hematoxylin and eosin staining of lung tissue (100 × original magnification). B, The number of total cells and cell differentials in bronchoalveolar lavage (BAL) fluid were counted. Bone marrow-derived macrophages (BMDMs) derived from Shp2−/− mice were treated with NTHi for the indicated times. C, Relative messenger RNA (mRNA) levels of M1-related genes and protein levels of inflammatory cytokines (interleukin 6 [IL-6] and tumor necrosis factor α [TNF-α]) in BMDMs were measured. D, Relative mRNA levels of M2-related genes in BMDMs were examined. There were 5–8 mice per group. * P < .05. Abbreviations: Arg1, arginase 1; IL-12, interleukin 12; iNOS, inducible nitric oxide synthase; PBS, phosphate-buffered saline; YM-1, chitinase 3–like protein 3.
the 2 groups (Figure 6B). Consistently, a remarkable reduction in NO production was observed in BAL fluid from NTHi-infected Shp2−/− mice and cell supernatant from Shp2−/− BMDMs (Figure 6C and 6D). Given the antimicrobial property of NO, the attenuated production of NO may contribute, in part, to the delayed bacterial clearance of NTHi in Shp2−/− mice.

**DISCUSSION**

NTHi is the most common cause for bacterial exacerbations during chronic COPD and community-acquired pneumonia. Macrophages are central immune cells participating in the first line of host defense against NTHi. Macrophages exhibit phenotypic heterogeneity and functional plasticity [39]. The molecular mechanisms underlying macrophage polarization, particularly upon infection, are largely unknown. Here, we demonstrate that, upon NTHi infection, host macrophages are skewed toward an antimicrobial M1 phenotype via activation of Shp2-dependent p65–NF-κB signaling. Therefore, Shp2 holds a critical role in regulating pulmonary inflammation and bacterial clearance in the acute infection model of NTHi.

As an intracellular classical PTP, Shp2 functions to remove phosphate groups from tyrosine-phosphorylated molecules, thereby regulating cell proliferation, cell differentiation, and organ development [40, 41]. Shp2 has been shown to be involved in a wide variety of diseases, like atherosclerosis, gastric carcinoma, and glioma [24–26, 42–44]. Shp2 positively regulated platelet-derived growth factor BB–induced migration of aortic smooth muscle cells and neointima formation [24]. Shp2 was reported to induce a growth factor–like response in gastric epithelial cells by forming a *Helicobacter pylori* CagA–Shp-2 complex [25]. Recent data showed that Shp2 positively prompted tumor growth factor β1 (TGF-β1)–induced epithelial-mesenchymal transition, which was repressed by Hook1, a novel Shp2-interacting protein. The Shp2-Hook1 complex may be involved in tumor metastases [45]. Of note, Shp2 is known to be universally and constitutively expressed in the lungs [46–48]. Shp2 was also shown to participate in the pathogenesis of acute lung injury. It was found to coordinate the tyrosine phosphorylation profile of vascular endothelial cadherin, β-catenin, and p190 RhoGAP and the activity of RhoA, thereby keeping the integrity of the adherin junction complex [47]. Tao et al reported that Shp2 inactivation augmented IL-4–mediated M2 polarization. Further studies revealed that Shp2 deletion in macrophages promoted the association of Janus kinase 1 (JAK1) with IL-4Ra and enhanced IL-4–mediated JAK1/signal transducer and activator of transcription 6 activation, thereby resulting in M2 skewing of macrophages [23]. They also identified a novel role of Shp2 in maintaining pulmonary surfactant homeostasis. Shp2 was demonstrated to play a central role in mediating fibroblast growth factor/Grb-2–associated binders (GAB)/ERK signaling. Shp2 deregulation induced spontaneous pulmonary fibrosis [27]. Shp2 is involved in the regulation of eosinophil differentiation, and Shp2 blockade in myeloid cells alleviated allergic airway inflammation [49]. By modulation of TGF-β1 activity, Shp2 in airway epithelial cells led to airway remodeling and lung dysfunction in a mouse model of asthma [22]. Cigarette smoke can induce Shp2 expression in airway epithelial cells. Selective inhibition or conditional knockout of Shp2 in lung epithelia attenuated pulmonary inflammation in...
cigarette smoke–exposed mice. A further in vitro study revealed that cigarette smoke extract mediated interleukin 8 release via Shp2-regulated epidermal growth factor receptor/GAB/MAPK signaling [21]. Therefore, Shp2 could be a potential target for therapeutic intervention for airway inflammatory diseases.

NF-κB is recognized as a central player in controlling macrophage polarization and inflammatory response. NF-κB p50 promotes the recruitment of polymerase II to M2-associated genes, while p65 facilitates expression of the M1-associated genes with inflammatory response and antibacterial activity [13, 35–38, 50]. Sophisticated modulation of NF-κB activity represents a critical mechanism to keep the balance between host antibacterial inflammatory response and tissue injury. We previously revealed that Akt1 negatively regulated RelA/p65 in M1-associated gene transcription via miR127-SOCS1 signaling [13]. In the current work, we found that p65 phosphorylation levels were markedly downregulated in Shp2−/− cells following NTHi stimulation, which subsequently led to decreased expression of proinflammatory M1-associated genes like IL-6 and TNF-α and of antimicrobial genes such as iNOS. Luciferase assay results further indicated selective effects of RelA/p65 on M1-associated gene expression. Compared to controls, a disadvantage in bacterial clearance was also found in Shp2−/− cells and mice. Therefore, activation of Shp2-regulated macrophage function favored the host response against pathogens, which was evidenced by a robust inflammatory response and significantly reduced bacterial burden.

Taken together, our study establishes a central role for Shp2 in macrophage polarization and bacterial clearance. Manipulation and optimization of Shp2-mediated signaling offer a promising therapeutic strategy for infectious diseases.

Notes

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