Supplementary Information for

An inhibitor of leukotriene-A₄ hydrolase from bat salivary gland facilitates virus infection

Mingqian Fang, Xiaopeng Tang, Juan Zhang, Zhiyi Liao, Gan Wang, Ruomei Cheng, Zhiye Zhang, Hongwen Zhao, Jing Wang, Zhaoxia Tan, Peter Muiruri Kamau, Qiumin Lu, Qi Liu, Guohong Deng*, Ren Lai

Corresponding author: Ren Lai and Guohong Deng
Email: rlai@mail.kiz.ac.cn, gh_deng@hotmail.com.

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Materials and Methods

Bat collection and ethics statement

Ten mature *Myotis pilosus* bats (seven females and three males, 3–5 g) were captured from a cave in the Qijiao Mountain, Jinning District, Kunming, Yunnan Province, China. All experimental procedures involving animals complied with national legislation and were approved by the Institutional Animal Care and Use Committees at the Kunming Institute of Zoology, Chinese Academy of Sciences (approval ID: SMKX-20191015-33). All possible efforts were made to reduce the sample size and to minimize animal suffering. Human blood samples were collected according to the clinical protocols approved by the institutional Review Board of the Kunming Institute of Zoology (approval ID: SMKX-20191101-197). All human specimens were collected with the informed consent of patients prior to the study.

Submaxillary salivary gland protein analysis

After anesthesia with isoflurane, the bat submaxillary salivary glands were immediately removed and dissected. Three pairs of salivary glands were placed in 100 μl of RNA storage solution (BL560A, Biosharp, China) and stored at −80 °C. The other seven pairs were homogenized, with the resulting supernatant stored at −80 °C. The salivary-gland extract was loaded onto a Sephadex 200 Increase 10/300 (8.6 μm, 28990944, GE Healthcare, USA) gel filtration column pre-equilibrated with 0.01 M phosphate-buffered saline (PBS), pH 7.2. Proteins were eluted at a flow rate of 0.8 ml/min using the same buffer. Eluted fraction peaks were monitored at 280 and 215 nm, and the effluent of each peak was collected. Fractions with trypsin inhibiting activity were further purified using a Resource Q anion-exchange chromatography column (6-ml volume, 17117901, GE Healthcare, USA) on an AKTA Explorer fast protein liquid chromatography system (GE Healthcare, USA) with 0.01 M PBS, pH 7.2, and eluted at a flow rate of 1 ml/min with 1 gradient/min of NaCl. The absorbance of eluted peaks was monitored at 280 and 215 nm. Purified protein was subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) (12% gel concentrations) and Edman sequencing using a Protein Sequencer PPSQ-31A.

cDNA synthesis and identification of full-length cDNA sequence of MTX

Total RNA was extracted from the salivary glands of *M. pilosus* using Trizol (15596-018, Invitrogen, USA). Total RNA was used as a template and the first strand of cDNA was synthesized by long-distance (LD)-polymerase chain reaction (PCR) using a SMART™ cDNA Library Construction Kit (PT3000-1, Clontech, USA). The second strand was amplified using advantage polymerase using CDS III/3’ PCR primer 5’-ATTCTAGAGGCCAGGCAGGCGACATG-3’ and 5’ PCR primer 5’-AAGCAGTGTTATCAACGCAGAGT-
3’, provided by the SMART™ cDNA Library Construction Kit. Degenerate primers (5’- ATHGGNACNGCNTGAYGYSNA-3’, Y = C/T, H = A/T/C, N = A/C/G/T) were designed according to amino acid sequences determined by automated Edman degradation. Degenerate primers (mentioned above) and reverse complement primers combined with two vector primers were used for PCR under the following conditions: 5 min at 95 °C followed by 33 cycles of 30 s at 95 °C, 30 s at 56 °C, and 60 s at 72 °C. The PCR products were analyzed by agarose gel electrophoresis and recovered by a DNA Gel Extraction Kit (D2500-02, OMEGA, USA) and then sequenced on a DNA Analyzer (ABI3730xl) at the Tsingke Biological Technology Company (Beijing, China).

MTX and MTX mutant recombinant expression and purification

The DNA sequences encoding mature MTX and its mutant were chemically synthesized and cloned into the pET-32a (+) vector (Tsingke Biological, China). The cleavage site -DDDDK-, which is susceptible to enterokinase, was designed between the His-Tag and 5’ upstream of the MTX (or MTX mutant) coding sequence. The MTX (or MTX mutant)/pET-32a (+) construct was transformed into Escherichia coli strain BL-21 (DE3) for expression. The -DDDDK- site leads the His-Tag to be released from the recombinant MTX (or MTX mutant) protein. MTX (or MTX mutant) was purified using a Sephadex G-50 (10 × 250 cm; Superfine, Amersham Biosciences) gel filtration column combined with C8 reverse-phase high-performance liquid chromatography (RP-HPLC) (5 μm, 10 × 250 mm, XBridge, USA).

MTX polyclonal antibody preparation

Polyclonal antibodies were prepared as previously reported (1). In brief, purified MTX protein was mixed with an equal volume of Freund’s complete adjuvant (F5881, Sigma-Aldrich, USA) at a final concentration of 400 μg/ml. Subsequently, 200 μg of MTX was injected subcutaneously into the backs of New Zealand white rabbits (male, 3 months old, 2 kg). After 10 d, 100 μg of MTX in Freund’s incomplete adjuvant (F5506, Sigma-Aldrich, USA) was injected into the backs of the same rabbits. The third and fourth immunizations were executed with 100 μg of MTX in Freund’s incomplete adjuvant at 10-d intervals. Blood was collected at 10 d after the last injection. The blood was placed at room temperature for 2 h, and antisera were collected via centrifugation at 3000 rpm for 15 min at 25 °C. The immunoglobulin G (IgG) fraction in antisera was purified by saturated ammonium sulfate protein precipitation combined with diethylaminoethyl (DEAE)-cellulose chromatography (2). The antibody titer obtained by enzyme-linked immunosorbent assay (ELISA) (3) was 1:10 000.

Tissue distribution analysis of MTX

The M. pilosus tissues, including submaxillary salivary gland, heart, liver, muscle, skin, and blood, were homogenized in RIPA buffer (R0278, Sigma-Aldrich, USA) mixed with proteinase inhibitor cocktail (EDTA-Free, 100× in DMSO, HY-K0010, MedChemExpress, USA) using a glass homogenizer on ice to extract
proteins. The homogenates were centrifuged at 12,000 rpm for 30 min at 4 °C, and the protein concentration of the supernatants was analyzed using a BCA Protein Assay Kit (T9300A, Takara, Japan). A total of 50 μg of protein was subjected to 12% SDS-PAGE for immunoblot analysis. A homemade polyclonal antibody against MTX was used to determine the bat tissue distribution of MTX by immunohistochemical and ELISA analyses.

**Stability analysis in mouse plasma**

After injection of 2 mg of MTX into the tail veins of mice, 10 μl of blood was drawn from the tail every 2 h. Plasma was collected by centrifugation at 3000 rpm for 15 min at 4 °C, and 1 μl of plasma was then used for ELISA. MTX in plasma was adsorbed onto a 96-well flat-bottom immunoplate at 4 °C overnight. After washing, the plate was blocked with 1% bovine serum albumin (BSA) in 0.01 M PBS with 0.2% Tween-20 (PBST) for 1 h at 37 °C and then incubated with anti-MTX polyclonal antibody (1:3,000) with 1% BSA for 2 h at 37 °C. After washing, a secondary antibody (1:2,000, horseradish peroxidase (HRP)-labeled, 7074s, CST, USA) was used. The color was developed using a TMB Single-Component Substrate Solution (RP1200-500, Solarbio, China), and the reaction was stopped with ELISA Stop Solution (C1058-500, Solarbio, China). Optical density at 450 nm was measured using a plate reader (Epoch Etock, BioTek, USA).

**Effects of MTX on enzymatic activities of serine proteases**

The effects of MTX on serine proteases, including trypsin, elastase, and plasmin, were assayed using corresponding chromogenic substrates. The testing enzyme (enzyme concentration of reaction system: 2 nM trypsin (T6424, Sigma, USA), 20 nM elastase (E8140, Sigma, USA), or 10 nM plasmin (P1867, Sigma, USA)) was incubated with different concentrations of MTX (0–210 nM) in 60 μl of 50 mM Tris-HCl buffer (pH 7.4) for 5 min, after which specific concentrations of the chromogenic substrates were added. The chromogenic substrate (10 or 20 μM) for trypsin and plasmin was Gly-Arg-p-nitroanilide dihydrochloride (G8148, Sigma, USA) and the chromogenic substrate (65 or 130 μM) for elastase was N-methoxysuccinyl-Ala-Ala-Pro-Val-p-nitroanilide (M4765, Sigma, USA). Absorbance at 405 nm was monitored immediately and the kinetic curve was recorded using an enzyme-labeled instrument (Epoch BioTek, USA) for 30 min. A Dixon plot curve was used to calculate the Ki of MTX to inhibit the proteases.

**Co-immunoprecipitation**

Healthy plasma (1 μl) was first mixed with 1 μg of MTX in 10 μl of PBS for 10 min, and the anti-MTX antibody (5 μg) or control IgG (5 μg, NI01, Sigma, USA) was mixed and incubated in 30 μl of Tris-HCl buffer (25 mM, pH 7.4) for 16 h at 4 °C. Protein A agarose (20 μl, P2006, Beyotime, China) was then added to couple with the anti-MTX antibody and incubated for 3 h at 4 °C. After centrifugation at 2500 rpm for 10 min at 4 °C, loading buffer (10 μl, 4 × CW0027A, CWBIO, China) was added, followed by
boiling for 10 min to obtain the coupled proteins. All proteins were subjected to 12% SDS-PAGE separation. The target gel lane was subjected to in-gel digestion and liquid chromatography-tandem mass spectrometry (LC-MS, Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer, ThermoFisher Scientific, USA). The database retrieval algorithm used was Percolator. The database used for searching was the Proteome Reference Database of Humans in UniProt.

Surface plasmon resonance (SPR) analysis

SPR was performed according to the manufacturer’s instructions. In brief, a CM5 sensor chip (BR100012, GE, USA) was first activated with 0.4 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and 10 mM N-hydroxysuccinimide at a flow rate of 5 μl/min for 20 min. Leukotriene (LT) A4 hydrolase (LTA₄H, 10007817, Cayman, USA) was diluted (20 μg/ml) with 200 μl of sodium acetate (10 mM, pH 5) and then flowed across the activated surface to couple with the chip to a 5,000-target response value (RU). The remaining activated sites on the CM5 sensor chip were blocked by 75 μl of ethanolamine (1 M, pH 8.5). Real-time detection was recorded using a Biacore 3000 at a flow rate of 20 μl/min. Serially diluted MTX (100, 200, 400, and 800 nM in 20 mM Tris-buffer, pH 7.4) was applied to analyze the interactions with LTA₄H coupled on the surface of the chip. The dissociation constant (equilibrium dissociation constant) for binding was determined using the BIA evaluation program.

Native PAGE

Basic native PAGE was used to further analyze the interactions between MTX and LTA₄H. In brief, human LTA₄H (2 μg) and different dosages of MTX (1–4 μg) were first incubated in 30 μl of Tris-HCl buffer (50 mM, pH 7.4) at 37 °C for 10 min, and then applied to 8% pre-coagulated gel (PG00810-N, Solarbio, China) to analyze complex formation between MTX and LTA₄H in a running buffer (0.05 M Tris, 0.38 M glycine, pH 8.9) at 200 V constant voltage for 1 h.

Protein-protein docking

To model the LTA₄H-MTX complex, we used the known crystal structure of LTA₄H (Protein Data Bank ID: 5N3W) and the homology model of the MTX (Protein Data Bank ID: 1TBR) protein for protein docking. After running Molecular Dynamics (MD) simulation for a short time (10 ps) to obtain the optimized structure model, MTX was docked to LTA₄H by ZDOCK (4). Protein-protein docking was guided by the SPR experimental data (Fig. 1C), whereby the residues that disrupted binding were forcefully included in the interface, and residues that did not affect binding were not forcefully included. About 2,000 structure complexes were generated and ranked according to the ZRANK scoring function (5). The best ZDOCK pose between the two conformations was used as the representative of LTA₄H-MTX interaction and verified by mutation and in vivo experiments (Fig. S10B, C, and Fig. 5E-G). The design of mutation experiments was guided by the docking pose (amino acid sequence of the MTX mutant was obtained by
replacing the MTX peptide 73-ACTMEYMPHCGSDGVEYGNK-92 that binds to the hydrophobic region of LTA₄ with 73-ACTMLYMFFCGSNGVNYANM-92). Candidate interference peptides for the LTA₄-MTX complex were designed according to the docking pose.

**Effects of MTX on epoxide hydrolase and aminopeptidase activity of LTA₄**

The epoxide hydrolase activity of LTA₄ was assayed using RP-HPLC combined with ELISA. Firstly, the LTA₄ methyl ester (20010-500, Cayman, USA) was hydrolyzed to LTA₄ using alkaline hydrolysis buffer (ice methanol: 50% NaOH = 9:1) at 4 °C for 3 h. Different concentrations (0.4–10 μM) of MTX or MTX mutant were incubated with LTA₄ (100 nM, 10007817, Cayman, USA) in 40 μl of Tris-HCl buffer (100 mM, pH 7.4) for 5 min at 37 °C. The LTA₄ solution (10 μl) described above was added to a final concentration of 50 μM, and the reaction mixture was incubated for 20 min at 37 °C. Lastly, the reaction was stopped by the addition of 1 volume of methanol. The samples were analyzed by RP-HPLC using a C₁₈ column (5 μm, 4.6 × 250 mm, XBridge, USA). The elution buffer was a mixture of methanol/water/acetic acid (70/30/0.0025; v/v/v) and absorbance was monitored at 270 nm with a flow rate of 1 ml/min. To identify the peak of LTB₄, the effluent of each peak was collected and used for mass spectrometric qualitative analysis. The peak of LTA₄ was determined by the retention time of the LTA₄ control. The LTA₄ hydrolysis rate was calculated based on the peak area integration value (LTA₄ hydrolysis ratio = (1 – LTA₄ peak area of treatment group / LTA₄ peak area of medium (Med) group) %).

The content of LTB₄ in the reaction solution was analyzed by ELISA (CEA562Ge, USCN, China).

The aminopeptidase activity of LTA₄ was assayed using a chromogenic substrate. In brief, LTA₄ (100 nM) was incubated with 1 μM Bestatin (HY-B0134, MedChemExpress, USA) or different concentrations (0.4–10 μM) of MTX in 60 μl Tris-HCl buffer (100 mM, pH 7.4) for 5 min, and 500 μM of aminopeptidase chromogenic substrate (L-lysine p-nitroanilide dihydrobromide, L7002-250MG, Sigma, USA) was then added. Absorbance at 405 nm was monitored immediately, and the kinetic curve was recorded using an enzyme-labeled instrument (Epoch BioTek, USA) for 15 min.

**Effect of MTX on PLA₂, COX₁/₂, and P450 enzyme activity**

For cytochrome p450 (CYP450) enzyme activity analysis, 100 mg of the mouse liver tissue was fully homogenized in 1 ml PBS, centrifuged at 8000 rpm, and the supernatant was taken for further analysis. After incubating 20 μl liver homogenate with 10 μl different concentrations of MTX (0.4–10 μM) in PBS at 37°C for 5 min, the activity was measured using the CYP450 enzyme activity assay kit (GMS18017.1, Biosharp, China) according to the instructions. For phospholipase A2 (PLA2) and total cyclooxygenase (COX) enzymes activity analysis, incubating 20 μl human plasma with 80 μl different concentrations of MTX (0.4–10 μM) in PBS at 37°C for 5 min, and the PLA2 (PLA2-2-G, Comin, China) and COX activity assay kit (760151, Cayman, USA) were used for activity determination according to the instructions, respectively.
Assays of LTA4H concentration in plasma of mice stimulated by H1N1

The influenza A virus (IAV: H1N1 PR8) was provided by the National Institute for Viral Disease Control and Prevention (China). C57BL/6J male mice (Charles River Laboratories, USA) were infected nasally by H1N1 (10 μl, 10^3 tissue culture infection dose 50% (TCID$_{50}$)) (n = 6). Plasma from different groups of mice after 0, 1, 3, and 5 d of stimulation was collected to measure the concentrations of LTA4H and LTB4 by ELISA.

Cell lines

The human umbilical vein endothelial cells (HUVEC), non-small cell lung cancer (NSCLC) line A549, human lung fibroblast cell line Medical Research Council-5 (MRC-5), and Madin-Darby Canine Kidney (MDCK) cells were purchased from the Conservation Genetics CAS Kunming Cell Bank. Cells were grown in a 37 °C humidified 5% CO$_2$ atmosphere. We used M200 medium (M-200-500, Cascade Biologics, USA) containing 10% fetal bovine serum (FBS, 35-081-CV, Corning, USA), 2% low serum growth supplement (LSGS, S-003-10, Cascade Biologics, USA), and 1% penicillin-streptomycin solution (P1400, Solarbio, USA) for HUVEC cultivation, RPMI1640 medium (10-040-CVR, Corning, USA) containing 10% FBS and 1% penicillin-streptomycin solution for A549 cell cultivation, MRC-5 cell-specific complete medium (CM-0161, Procell, CHN) for MRC-5 cell cultivation, and Dulbecco’s Modified Eagle Medium (DMEM) (10-013-CVR, Corning, USA) containing 10% FBS and 1% penicillin-streptomycin solution for MDCK cell cultivation.

Isolation of polymorphonuclear neutrophils (PMNs) and plasmacytoid dendritic cells (pDCs)

Healthy human peripheral blood treated with 1.5% EDTA-Na$_2$ anticoagulant agent was collected from the Kunming Blood Center, Yunnan, China. The PMNs were isolated using Polymorphprep (AS1114683, Axis-Shield, Norway) as per the manufacturer’s protocols. In brief, 5 ml of blood was carefully layered over 5 ml of Polymorphprep, with the sample then centrifuged at 800 rpm for 30 min in a swing-out rotor at 22 °C. After centrifugation, two leukocyte bands were visible (PMNs in the upper layer, mononuclear cells in the lower layer) and were harvested using a pipette. The fractions of the two bands were diluted with one volume 0.01 M PBS. The cell suspension was transferred to a 3-ml tube and centrifuged three times at 800 rpm for 30 min at 22 °C. The isolated PMNs were used for subsequent cell migration experiments. A pDC Isolation Kit II (130-097-415, Miltenyi, USA) was used to further isolate pDCs from mononuclear cells in accordance with the manufacturer’s instructions. PMNs and pDCs were cultured in RPMI1640 complete medium at 37 °C and 5% CO$_2$.

Virus amplification and titration

DMEM (2 ml) containing 100 TCID$_{50}$ virus was added to a 100 x 20 mm cell culture dish containing 80% MDCK cells without culture medium, then gently shaken and placed in an incubator (37 °C, 5% CO$_2$) for
90 min to allow the virus to be adsorbed completely on the cell surface. We then added 8 ml of DMEM containing 2 μg/ml trypsin (TPCK-Treated, 4370285, SIGMA, USA), 10 % FBS, and 1% penicillin-streptomycin solution. After 3 d of culture at 37 °C and 5% CO2, the supernatant was harvested and titrated by TCID50 assay, and virus titer was calculated according to the Reed-Muench formula (6).

**Effects of MTX on proinflammatory factors expression induced by H1N1 infection**

The A549 cells, MRC-5 cells, pDCs, and HUVECs were seeded in a 12-well plate, respectively. After the cells adhered to the wall and grew to 70% confluence, they were washed with 0.01 M PBS and the medium was replaced. Different concentrations of MTX (0–2 μM) or LTB4 receptor (BLT1, LTB4 receptor 1; BLT2, LTB4 receptor 2) mixed antibodies (anti-BLT1 antibody, 0.5 μg/ml, 120111, Cayman, USA; anti-BLT2 antibody, 0.5 μg/ml, A15479, ABclonal, China) were added to H1N1-infected (0.1 MOI, Fig. S5) or non-H1N1-infected wells for 12 or 24 h at 37 °C with 5% CO2. Cells were lysed in RIPA buffer (R0278-50ML, SIGMA, USA) with protease inhibitor cocktail (100×, MedChemExpress, USA) for immunoblot analysis of H1N1 PR8 nucleoprotein (NP), LTA4H, and antiviral innate immune response receptor RIG-I (DDX58). Cells were lysed in TRIzol reagent for RNA isolation to detect NP, LTA4H, TNF-α, IL-1β, IL-6, IL-8, and IFN-β expression by quantitative real-time PCR (qRT-PCR). The cell supernatant was used to determine the concentrations of LTA4H, LTB4, tumor necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), interferon-β (IFN-β), prostaglandin E2 (PGE2), and cysteinyl leukotrienes (CysLTs) by ELISA. To detect the binding protein of MTX and LTA4H in the cell supernatant, we added MTX antibody and protein A agarose to enrich the MTX-LTA4H binding protein for immunoblot analysis. The LTA4H antibody was used to identify the pull-down LTA4H protein that binds to MTX.

**Effects of MTX on activation of LTB4 receptor signaling**

The A549 cells, MRC-5 cells, pDCs, and HUVECs were seeded in 12-well plates. After the cells adhered to the wall and grew to 90% confluence, they were washed with PBS and replaced with the new medium. The cells received the same treatment as described above ("Effects of MTX on proinflammatory factors expression induced by H1N1 infection") and were cultured for another 1 h. Cells were washed with PBS and incubated with a crosslinking reagent (0.5 M disuccinimidyl suberate, 21555, Thermo, USA) for 15 min at room temperature. After terminating the reaction with 0.5 M Tris, cells were washed with ice-cold PBS and lysed in 100 μL of RIPA buffer with protease and phosphatase inhibitors (100×, HY-K0022, MedChemExpress, USA). BLT1 dimerization was detected by immunoblot analysis. Cells were lysed in RIPA buffer with protease and phosphatase inhibitors for immunoblot analysis of phosphorylated protein kinase B (p-AKT), phosphorylated extracellular-signal-regulated kinase (p-ERK), phosphorylated signal transducer and activator of transcription 1 (p-Stat1), p-Stat3, phosphorylated Interferon regulatory factor 3 (p-IRF3), and p-IRF7. We used the NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (78833,
Thermo, USA) to extract nuclear proteins from cells according to the manufacturer’s instructions to detect activation of the nuclear factor-κB (NF-κB) subunit (p65) signaling pathway.

**Effects of exogenous addition of LTB₄ on inflammatory signaling pathway and viral infection**

A549 cells were seeded in a 12-well plate. After the cells adhered to the wall and grew to 80% confluence, they were washed with PBS and replaced with a new medium. Different concentrations of LTB₄ (100, 1 nM), U75302 (70705, Cayman, USA, BLT1 antagonist, 100 nM), and MTX (2 μM) were added to H1N1-infected or non-H1N1-infected wells at 37 °C with 5% CO₂. After 1 h of H1N1 infection, cells were lysed in RIPA buffer with protease and phosphatase inhibitors for immunoblot analysis of signaling pathway proteins p65, AKT, and ERK, and their phosphorylated proteins. After 24 h of virus infection, cells were lysed in RIPA buffer for immunoblot analysis of LTA₄H and NP protein, and cells were lysed in TRIzol reagent for RNA isolation to detect NP gene expression. The cell supernatant was harvested and titrated by TCID₅₀ assay.

**Effects of MTX on neutrophil chemotaxis**

The HUVECs and A549 cells (1 × 10⁵) were seeded in 6-well plates equipped with an 8-mm cell slide. After 4 h of culture (37 °C and 5% CO₂) to allow the cells to adhere to the wall, different concentrations of MTX (0–2 μM) or mixed BLT1-BLT2 antibodies with or without H1N1 were added to the medium. A transwell chamber (0.8 μm, Thermo Fisher Scientific, USA) was inserted into the plate, and PMNs were then seeded in the transwell chamber. After 24 h of co-cultivation, the cell slide in the plate was removed and placed in 4% paraformaldehyde for 30 min to fix the cells and detect PMN chemotaxis by immunofluorescence. Cells in the lower layer were lysed in TRIzol reagent for RNA isolation to detect NP expression by qRT-PCR. The co-culture medium was collected to measure the contents of LTA₄H, LTB₄, and chemokine IL-8 by ELISA. Cells in the lower layer were collected and the number of neutrophils was analyzed by flow cytometry.

**Effects of MTX on H1N1 infection in mice**

Wild-type (WT) C57BL/6J and LTA₄H⁻/⁻ male mice were purchased from Charles River Laboratories. Five-week-old mice were anesthetized with sodium pentobarbital (80 mg/kg) and then infected with H1N1 (10 μl, 10³ TCID₅₀) through the nasal cavity with or without intranasal administration of MTX (50 μg dissolved in 10 μl of 0.9% salt water per mouse) or BLT1/2 antibody mixture (5 μg of BLT1 antibody mixed with 5 μg of BLT2 antibody dissolved in 10 μl of 0.9% salt water per mouse). Five-week-old male C57 BL/6J mice were inoculated intranasally with 10³ TCID₅₀ of H1N1 PR8 alone (IAV) or in combination with MTX (50 μg per mouse), and all mice were given intranasal COX inhibitor piroxicam (100 ng per mouse) to eliminate the interference of COX product prostaglandins (PGs) on the experiment. Immediately following H1N1 infection to five-week-old male C57 BL/6J mice, LTB₄ (0.1 mg/kg, 20110, Cayman, USA), MTX antibody
(0.25 mg/kg), or peptide IM14 (KLVVDLTDIDPDVA; for MTX-LTA4H interaction interference) (2 mg/kg) in
100 μl of PBS was intravenously administered to evaluate possible antiviral effects. Five-week-old male
C57 BL/6J mice (wild-type) or LTA4H−/− mice inoculated intranasally with 10^3 TCID50 of H1N1 PR8 (IAV)
alone or in combination with MTX (50 μg per mouse, IAV/MTX), LTB4 (50 ng per mouse), or MTX-LTB4
mixture. Over the following 14 d, both body weight and survival status of the mice were recorded daily.
Cells were obtained from isolated lungs on the first day after infection using a Lung Dissociation Kit (130-
095-927, Miltenyi, USA) to detect immune cell chemotaxis through flow cytometry. Mouse tissues were
collected after virus infection for 1, 3, and 9 d, respectively. These tissues were used for checking virus
titer, cell apoptosis, tissue lesions, cytokines, and NP expression by lipidomic analysis (LC.Bio.Tech,
China), immunofluorescence, immunoblotting, ELISA, TdT-mediated dUTP Nick-End Labeling (TUNEL)
staining, hematoxylin and eosin (H&E) staining, TCID50 assay, and/or qRT-PCR.

H&E staining

We conducted H&E staining using a commercially available kit (G1120-100, Solarbio, China) as per the
manufacturer’s protocols. Briefly, after deparaffinization and rehydration, the 5-μm sections were stained
with hematoxylin solution for 3 min followed by dipping in acid ethanol (1% HCl in 70% ethanol) five times
and rinsing in distilled water. The sections were then stained with eosin solution for 1 min followed by
dehydration with graded alcohol and clearing in xylene. The sections were sealed with neutral balsam
(G8590-100, Solarbio, China), and examined using an EVOS XL Core Imaging System (Life
Technologies, USA).

Immunofluorescence and TUNEL staining

Immunofluorescence staining was carried out as described previously (7). In brief, cells or 5-μm tissue
sections were permeabilized with 0.2 % Triton X-100 (T0694, Amresco, USA) for 30 min, washed with
PBS, and blocked in 2% BSA for 1 h. The cells or slides were incubated with primary antibodies (anti-
H1N1 nucleoprotein antibody, bs-4976R, Bioss, China; or anti-LTA4H antibody, GTX54332, Genetex,
USA; or anti-myeloperoxidase antibody, AF3667, R&D Systems, USA; or anti-histone H3 antibody,
ab195277, Abcam, USA, 1:500) overnight at 4 °C. After washing, they were incubated with fluorescent
secondary antibodies (1:400; Alexa Fluor® 488 donkey anti-rabbit IgG, ab150073, Abcam, USA; or Alexa
Fluor® 647 donkey anti-rabbit IgG, ab150075, Abcam, USA; or Cy5® goat anti-mouse IgG, ab6563,
Abcam, USA) at room temperature for 1 h in the dark, then washed thoroughly and sealed with ProLong
Gold Antifade Reagent with DAPI (8961S, CST, USA). For TUNEL staining, tissue sections were
processed for immunostaining of apoptotic nuclei using an Apoptosis Detection Kit (40307ES20,
YEASEN, China) as per the manufacturer’s protocols. Immunostaining was detected using an Olympus
FluoView 1000 Confocal Microscope (Japan).

Immunoblot analysis
Samples were separated by 12% SDS-PAGE, followed by electrophoretic transfer to polyvinylidene fluoride (PVDF) membranes. The membranes were then blocked with 10% BSA in Tris-buffered saline with Tween-20 (TBST, 50 mM Tris, 150 mM NaCl, 0.1% Tween-20) at room temperature for 1 h and incubated with primary antibodies at 4 °C for 16 h. After washing with TBST, the membranes were incubated with secondary antibody at room temperature for 1 h and developed with an Enhanced Chemiluminescence Kit (PA112, Tiangen, China) using ImageQuant LAS 4000 mini (GE Healthcare, USA). ImageJ software was used for image analysis. The following primary antibodies were used: anti-H1N1 nucleoprotein (bs-4976R, Bioss, China), anti-LTA-H (GTX54332, Genetex, USA), anti-BLT1 (ADI-905-793-100, Enzo Life Sciences, USA), anti-AKT (9272S, CST, USA), anti-p-AKT (4060S, CST, USA) anti-ERK (9101 L, CST, USA), anti-p-ERK (9102 L, CST, USA), anti-histone H3 (ab195277, Abcam, USA), anti-DDX58 (DF6107, Affinity, USA), anti-p-IRF7 (AF8486, Affinity, USA), anti-p-IRF3 (29047S, CST, USA), anti-p-Stat1(9167S, CST, USA), anti-p-Stat3 (9145S, CST, USA), and anti-GAPDH (T0004, Affinity, USA). HRP-linked anti-rabbit (7074s, CST, USA) or anti-mouse (7076s, CST, USA) antibody was used as the secondary antibody.

ELISA

LTA-H, LTBs, and cytokines in the samples were measured using ELISA kits according to the manufacturers’ instructions, including a mouse IL-6 ELISA kit (DKW12-2060-096, Dakewe Biotech, China), mouse IL-1β ELISA kit (DKW12-2012-096, Dakewe Biotech, China), mouse TNF-α ELISA kit (DKW12-2720-096, Dakewe Biotech, China), mouse CXCL1 ELISA kit (EMC104.96, NeoBioscience, China), mouse LTA-H ELISA kit (KTE71157, Abbkine, China), human IL-6 ELISA kit (DKW12-1-60-096, Dakewe Biotech, China), human TNF-α ELISA kit (DKW12-1720-096, Dakewe Biotech, China), human IL-8 ELISA kit (DKW12-1012-096, Dakewe Biotech, China), human IFN-β ELISA kit (SEA222Hu-96T, USCN, China), human LTA-H ELISA kit (SED236Hu, USCN, China), LTβ ELISA kit (CEA562Ge, USCN, China), PGE2 ELISA kit (CEA538Ge, USCN, China), CysLTs ELISA kit (JM-05092H, Besoon, China).

qRT-PCR

Total RNA was extracted with TRizol reagent (Invitrogen, USA) following the manufacturer's instructions. To quantize virus RNAs, cDNA was reverse transcribed using SuperScript III Reverse Transcriptase (18080044, Invitrogen, USA) with the following primers: 5'-GACGATGCAACGGCTGGTCTG-3' for NP vRNA and 5'-AGTAGAAACAAGGGTATTTTCTTTTCTTTTCTTTTCTTTT-3' for NP cRNA. Single-stranded cDNA was then subjected to qRT-PCR using EvaGreen 2X qPCR MasterMix (MasterMix-S/R/LR/iC, ABM, Canada) with the following specific primers: 5'-AGCATTGGTCCAACACTCTCCTT-3' and 5'-GACGATGCAACGGCTGGTCTG-3' for NP. For the quantization of cell RNA, cDNA was reverse transcribed using 5X All-In-One RT MasterMix with an AccuRT Genomic DNA Removal Kit (G492, ABM,
qRT-PCR was performed on a CFX-96 Touch Real-Time Detection System (Bio-Rad). Other primer sequences are listed in Table S1.

Flow cytometry

Fluorescent-dye-conjugated antibodies were purchased from BD Biosciences (USA) (anti-CD16, 560829; anti-CD28, 740466; and anti-CD8, 563068), Cell Signaling Technology (USA) (anti-CD45, 41104S; anti-CD11b, 16538S; anti-CD11c, 37664S; and anti-CD3, 38527S), Biorbyt (UK) (anti-MPO, orb3082), eBioscience (USA) (anti-MHC-CII, 11-5321-82), and BioLegend (USA) (anti-CD125, 153404). For flow cytometry of neutrophils (anti-CD45, anti-CD11c, anti-CD11b, and anti-MPO), eosinophils (anti-CD45, anti-CD125, and anti-CD16), CD8+ T cells (anti-CD45, anti-CD8, and anti-CD28), and NK cells (anti-CD45, anti-CD16, and anti-CD3), isolated cells were surface stained using a subset of antibodies. Dead cells were excluded by propidium iodide (PI) staining. Staining was carried out as described previously (7) and cells were analyzed on a BD LSRFortessa Cell Analyzer (BD Biosciences, Franklin Lakes, NJ, USA).

Statistical analysis

Data are given as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) and Fisher’s protected $t$-tests were used for statistical analyses. $P$-values of $\leq 0.05$ were considered significant. Analyses were performed using SPSS v22.0 and GraphPad Prism v6.0.
Figure S1. MTX purification and characterization from submandibular salivary gland of *Myotis pilosus*. A, Image of *M. pilosus*. B, Salivary gland extract was separated using Sephadex 200 Increase 10/300 GL filtration. C, Fraction IV (arrow) from 'B' showing trypsin inhibitory activity was selected for further purification by a Resource Q anion-exchange column. D, A purified inhibitor of trypsin (peak V, arrow) from 'C' was collected for mass spectrometry analysis (up: non-reduced; down: reduced by DTT (3 mM) and 12% SDS-PAGE(down right). E, cDNA sequence encoding MTX precursor. The signal peptide
is shown in italics. Peptide sequence determined by Edman degradation is in red, and the sequence of recombinant protein purified from *E. coli* is underlined. F, Dixon plot analysis of inhibitory constant (*K*<sub>i</sub>) of MTX on trypsin, elastase, and plasmin, respectively. F-H, Data are representative of three independent experiments.

**Figure S2. Purification of recombinant MTX and MTX mutant.** A, 12% SDS-PAGE analysis of MTX and MTX mutant (mu-MTX) fusion proteins from *E. coli*. Lane 1, un-induced bacterial lysate of *E. coli* transformed by MTX expression vector; Lane 2, induced bacterial lysate containing soluble MTX fusion protein (0.5 mM IPTG); Lane 3, un-induced bacterial lysate of *E. coli* transformed by mu-MTX expression vector; Lane 4, induced bacterial lysate containing soluble mu-MTX fusion protein (0.5 mM IPTG); Lane 5, MTX fusion protein after Ni-NTA agarose affinity purification; Lane 6, mu-MTX fusion protein after Ni-NTA agarose affinity purification. B, 12% SDS-PAGE detection of MTX (Lane 7) and MTX mutant (Lane 8) fusion protein after enterokinase (5 U, 25 °C for 18 h) cleavage. Red arrows point to MTX or MTX mutant protein. C, Purification of mature MTX (left) and MTX mutant (right) by RP-HPLC. Purity analysis of recombinant MTX and MTX mutants by 12 % SDS-PAGE (D) (Lane 9, MTX; Lane 10, MTX mutant) and RP-HPLC (E) (left, MTX; right, MTX mutant), and the purity was 99.1 % and 98.5 %, respectively. F, Purified recombinant MTX (left) (treated with 3 mM DTT) (upper right corner: non-reduced MTX) and MTX mutant (right) (treated with 3 mM DTT) were analyzed by MALDI-TOF-MS.
Figure S3. LC-MS analysis of proteins pulled down by immunoprecipitation. A, 12% SDS-PAGE analysis of MTX-target proteins pulled from human plasma using immunoprecipitation. B, LC-MS analysis identified one binding protein as LTA4H, with resulting sequence fragments underlined. C, Secondary mass spectrometry analysis showing red underlined peptides in ‘B’.
Figure S4. MTX has no effects on the aminopeptidase activity of LTA₄H, PLA₂, COX₁/₂, and P450 enzymes. A, Enzyme kinetics analysis of the effect of MTX on the aminopeptidase activity of LTA₄H. Bestatin is the positive control. B, Fluorescence spectrophotometry to detect the effect of MTX on the activity of mouse liver cytochrome p450 enzyme (CYP450). C, Spectrophotometric detection of the effect of MTX on human plasma phospholipase A₂ (PLA₂) enzyme activity. D, Spectrophotometric detection of the effect of MTX on the total COX enzyme activity in human plasma.
Figure S5. MTX augments H1N1 infection in HUVECs by inhibiting LTB4 receptor signaling and the LTA4-LTA4H-LTB4 inflammatory axis. A, HUVECs were stimulated in the presence (+) or absence (-) of different concentrations of MTX or BLT1/2 antibody mixture (BLT1 antibody, 0.5 μg/ml; BLT2 antibody, 0.5 μg/ml) and infected with H1N1 (IAV) for 24 h. Immunoblot analysis of NP (H1N1 nucleoprotein) and LTA4H in HUVECs (A, left), and quantification of signaling intensity of NP and LTA4H using GAPDH as the loading control (A, right). B, Effects of MTX on trimerization and dimerization of LTB4 receptor 1 (BLT1) in HUVECs induced by H1N1 (IAV) infection for 1 h in the presence or absence of anti-BLT1/2 antibody or different concentrations of MTX, determined by immunoblot analysis. Cell lysates were cross-linked for 15 min with disuccinimidyl suberate. Quantification of signaling intensity of trimer and dimer using GAPDH as the loading control. C, Effects of MTX on downstream signals of LTB4 receptor activation promoted by H1N1 infection for 1 h, as determined by immunoblot analysis. Quantification of signaling intensity of p-ERK and p-AKT using GAPDH as the loading control; quantification of p65 signaling intensity using histone as the loading control. D-I, LTA4H (D), LTB4 (E), TNF-α (F), IL-8 (G), IL-6 (H) and IL-8 (I) production from ‘A’. Each symbol (A, right; B, down; C, right; and D-I) indicates an individual technical replicate in one experiment, and small horizontal lines (A, right; B, down; C, right; and D-I) indicate mean ± SEM. Data are representative (A, left; B, up; and C, left) of and are from (A, right; B,
down; C, right; and D-I) three independent experiments. *$p < 0.05$, **$p < 0.01$ by one-way ANOVA with Fisher’s protected $t$-tests.
Figure S6. The effects of MTX on LTB₄, LTA₄H, TNF-α, IL-6, IL-1β, IL-8, and NP expression in A549, HUVEC, MRC-5, and pDC cells after virus infection analyzed by qRT-PCR or ELISA. A-D, pDCs (A, C) or MRC-5 cells (B, D) were infected with H1N1 for 12 or 24 h in the presence or absence of MTX or BLT1/2 antibody mixture (BLT1 antibody, 0.5 μg/ml; BLT2 antibody, 0.5 μg/ml), NP gene expression was determined by qRT-PCR (relative to 12 h IAV group), and the concentration of LTB₄, LTA₄H, TNF-α, IL-6, IL-1β, and IL-8 was detected by ELISA (24 h). A549 cells (E), HUVECs (F), MRC-5 cells (G), and pDCs (H) were stimulated in presence (+) or absence (-) of different concentrations of MTX or BLT1/2 antibody mixture (BLT1 antibody, 0.5 μg/ml; BLT2 antibody, 0.5 μg/ml) and infected with H1N1 (IAV) for 24 h. Expression levels of IL-6, TNF-α, IL-1β, IL-8, and LTA₄H were determined by qRT-PCR (relative to control group). Each symbol (A-H) indicates an individual technical replicate in one experiment, and small horizontal lines (A-H) indicate mean ± SEM. Data are from (A-H) three independent experiments. *p < 0.05, **p < 0.01 by one-way ANOVA with Fisher’s protected t-tests.

Figure S7. MTX augments H1N1 infection in A549 cells by inhibiting LTB₄ receptor signaling and LTA₄-LTA₄H-LTB₄ inflammatory axis. A, Blot from ‘Figure 2C’ ii-v. B, Blot from ‘Figure 2D’. C, A549 cells treated with U75302 (BLT1 antagonist, 100 nM), MTX (2 μM), or LTB₄ in the presence or absence of H1N1. After 24 h of virus infection in each treatment group, contents of LTA₄H and NP protein in the cells were detected by immunoblot analysis. Quantification of signaling intensity of NP and LTA₄H using GAPDH as the loading control. Each symbol (C, middle and right) indicates an individual technical replicate in one experiment, and small horizontal lines indicate mean ± SEM. Data are representative (A;
B; and C left) of three independent experiments. *p < 0.05, **p < 0.01 by one-way ANOVA with Fisher’s protected t-tests.

**Figure S8. Effects of MTX on IFN signaling pathway. A-D (up), ELISA analysis of IFN-β in cell supernatants of A549 cells (A, up), HUVECs (B, up), MRC-5 cells (C, up), and pDCs (D, up) stimulated for 24 h with different concentrations of MTX in the presence (+) or absence (−) of BLT1/2 antibody (BLT1 antibody, 0.5 μg/ml; BLT2 antibody, 0.5 μg/ml) or H1N1 (IAV). A-D (down), qRT-PCR analysis of IFN-β expression in these cells. E&F, Immunoblot analysis of phosphorylated (p-) Stat1, Stat3, IRF7, IRF3, and GAPDH in A549 cells stimulated for 1 h by H1N1 (E) and quantification of phosphorylated IRF3 (F). G&H, Immunoblot analysis of DDX58 in A549 cells treated for 24 h (G) and quantification of DDX58 (H). GAPDH was used as the loading control. Each symbol (A-D; F; and H) indicates an individual technical replicate in one experiment, and small horizontal lines (A-D; F; and H) indicate mean ± SEM. Data are representative (E and G) of and are from (A-D; F; and H) three independent experiments. *p < 0.05, **p < 0.01 by one-way ANOVA with Fisher’s protected t-tests.
Figure S9. MTX inhibits neutrophil chemotaxis to facilitate H1N1 infection. A, Effects of MTX on neutrophil chemotaxis in HUVEC-PMN cell co-culture system. Neutrophil activation is indicated by MPO expression in HUVECs. MPO and NP were analyzed by immunofluorescence 24 h after H1N1 (IAV) infection. Scale bars, 50 μm. Concentrations of LTA₄ (B), LTB₄ (C), and IL-8 (D) in HUVEC-PMN co-culture system supernatant were analyzed by ELISA, and NP gene expression (E) was detected by qRT-PCR. Each symbol (A, right; and B-E) indicates an individual technical replicate in one experiment, and small horizontal lines (A, right; and B-E) indicate mean ± SEM. Data are representative (A) of and are from (A, right; and B-E) three independent experiments. *p < 0.05, **p < 0.01 by one-way ANOVA with Fisher’s protected t-tests.
Figure S10. MTX inhibits the formation of neutrophil extracellular traps (NETs) to promote H1N1 infection. A, Effects of MTX (50 μg per mouse) on NET formation in lungs of five-week-old male wild-type (WT) or LTA<sup>H<sup>+</sup></sup> mice by immunofluorescence analysis after 1 d of H1N1 infection (intranasally inoculated with 10<sup>3</sup> TCID<sub>50</sub> of H1N1 PR8). BLT1/2 antibody, 5 μg of BLT1 antibody mixed with 5 μg of BLT2 antibody dissolved in 10 μl of 0.9% salt water per mouse; Scale bars, 50 μm. B, Relative
The quantification of NET area from ‘A’. NET structure was determined by the detection of citrullinated histone and MPO. C, Relative quantification of LTA₄H expression from ‘A’. D, Five-week-old male mice were inoculated intranasally with 10⁵ TCID₅₀ of H1N1 PR8 alone (IAV) or in combination with MTX (50 μg per mouse), one day later, the arachidonic acid metabolites in mouse lungs was quantitatively analyzed by lipidomics. Each symbol (B, C) indicates an individual technical replicate in one experiment. Small horizontal lines (B, C) indicate mean ± SEM. Data are representative (A) of three independent experiments. *p < 0.05, **p < 0.01 by one-way ANOVA with Fisher’s protected t-tests.

Figure S11. Inhibiting viral infection by interfering with the inhibitory activity of MTX on LTA₄H and the addition of exogenous LTB₄. A-C, Five-week-old male C57 BL/6J mice inoculated intranasally with 10⁵ TCID₅₀ of H1N1 PR8 (IAV) alone or in combination with MTX (50 μg per mouse, IAV/MTX). Effects of intravenous injection of LTB₄ (0.1 mg/kg), MTX antibody (0.25 mg/kg), or designed peptide IM14 (2 mg/kg) on MTX-promoted virus infection were evaluated. D-F, Five-week-old male C57 BL/6J mice were treated with LTB₄ or PBS by intranasal instillation (IN) or intravenous injection (i.v.). One day later, the
concentration of PGE$_2$, IL-6, and TNF-α in mouse lung homogenate was analyzed by ELISA. G-I, Five-week-old male C57 BL/6J mice were inoculated intranasally with $10^3$ TCID$_{50}$ of H1N1 PR8 alone (IAV) or in combination with MTX (50 μg per mouse), and all mice were given intranasal COX inhibitor piroxicam (100 ng per mouse) to eliminate the interference of COX product PGs on the experiment. A, G, Immunofluorescence analysis of NP or H&E staining on day 9 after the infection. Scale bars, 50 μm. B, H, H1N1 titers in lungs of mice on days 1, 3, and 9 after the infection. C, I, Daily body weight (relative to initial body weight) of mice within 14 d. Each symbol (B; D-F; and H) indicates individual mouse in one experiment. Small horizontal lines (B; C [n=10]; D-F; H; and I [n=6]) indicate mean ± SEM. Data are representative (A, G) of and are from (B -F; H; and i) three independent experiments. **p < 0.01 by one-way ANOVA with Fisher’s protected t-tests.

Figure S12. Flow cytometric analysis of ‘Figure 4J-N’. Cells isolated from lung tissues were stained with anti-CD45, anti-CD11c, anti-CD11b, anti-MPO, anti-CD16, anti-CD125, anti-CD3, anti-CD8, and anti-CD28 antibodies. Activated neutrophils (A) were identified as CD45+CD11c+CD11b+MPO+. CD8+ T cells (B) were identified as CD45+CD8+ and activated CD8+ T cells (B) were identified as CD45+CD8+CD28+. CD16+CD3- NK cells (C) were identified as CD45+CD16+CD3-. MHC-CII+CD11c+ DCs (D) were identified as CD45+CD3+MHC-CII+CD11c+. Eosinophils (E) were identified as CD45+CD125+CD16.
Figure S13. Key sites for interaction between MTX and LTA₄H. A, 3D structure represents the interaction between MTX and LTA₄H by docking and molecular dynamics. MTX (red) inhibits the activity of LTA₄H by blocking substrate entry into active sites (green circle) of LTA₄H. B&C, Effects of MTX mutation (B) or designed peptide IM14 (C) on MTX-LTA₄H interactions using RP-HPLC to analyze LTA₄ hydrolysis and LTB₄ production (Box 1, LTA₄ alone (50 μM); Box 2, LTA₄ and LTA₄H (100 nM); Box 3, LTA₄, LTA₄H, and 10 μM MTX; Box 4, LTA₄, LTA₄H, and 10 μM mu-MTX in ‘B’ or 2 μM IM14 in ‘C’). LTA₄ hydrolysis ratio = (1 – LTA₄ peak area of treatment group / LTA₄ peak area of Med group)%. ELISA showed the concentration of LTB₄ in reaction solution (D, E). The black arrow indicates the LTA₄ peak. The red arrow indicates the LTB₄ peak. Each symbol (B and C, right; D; and E) indicates an individual
technical replicate in one experiment, and small horizontal lines (B and C, right; D; and E) indicate mean ± SEM. Data are representative of (B, left; C, left) or from (B and C, right; D; and E) three independent experiments. NSp > 0.05, *p < 0.05, **p < 0.01 by one-way ANOVA with Fisher’s protected t-tests.

Figure S14. Graphical representation of MTX facilitation of IAV infection in mammalian hosts by targeting LTA4-LTA4H-LTB4 inflammatory axis to inhibit immunity. Bats spread IAV to mammalian hosts through saliva. Submaxillary salivary gland protein MTX specifically interacts with LTA4H to inhibit its hydrolase activity and reduce LTB4 formation, thus weakening LTB4 receptor-mediated inflammatory immune responses, which, in turn, down-regulate LTA4H expression. Negative feedback regulation effect of MTX on LTA4H and high-efficiency inhibition of LTA4H hydrolase activity greatly reduces content of LTB4 in the victim’s body, thereby preventing activation of the LTB4 receptor, and inhibiting its downstream inflammation signaling pathway and antiviral signaling pathways. Decrease in content of inflammatory factors, chemokines, and antiviral factors in the body weakens its antiviral ability and ultimately promotes virus infection in the body. Green dashed line indicates that the process is blocked; red line indicates that production is promoted.

Table S1. Primer sequences for qRT-PCR.
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