2. Supplementary Methods

Preparation of Tat-rMsrA

The cDNA fragment encoding rMsrA was obtained from pcDNA3.1-rMsrA (kindly provided by Dr. Bertrand Friguet, Universite' Paris 7-Denis Diderot, France) using PCR. A 5' sense primer containing a BamHI site and a Tat gene code (5'-AAAGGATCCGGTTACGGTCGTAAGAAACGTAGACAGCGCAGACGTGGTATGCT CTCCGCCTCCAGAAGGAC-3'), and a 3' reverse complement primer with an XhoI site (5'-AAACTCGAGTTACTTTTTAATGGCCGTGGGACAGG-3') were used. Both the pET-32a (+) vector and amplified product were digested with BamHI and XhoI (MBI FERMENTAS, Burlington, ON, Canada), and the PCR fragment encompassing the complete rattus MsrA coding region was ligated into the restricted pET-32a (+) vector using phage T4 DNA ligase (MBI Fermentas, Burlington, ON, Canada). The nucleotide sequence of the cloned insert was confirmed by sequencing at least twice in each direction. BL21 cells were transformed with the recombinant plasmid and grown in LB medium containing 50 μg / ml ampicillin at 37 °C. When cells reached an absorbance at 600 nm of 0.7, IPTG (Amresco, Cochran solon, OH, USA) was added to a final concentration of 0.5 mM and the culture was continued for an additional 4 h. The cells were centrifuged at 5, 000 g for 10 min at 4 °C and the pellet was suspended in buffer A (20 mM Tris-HCl, 0.5 mM imidazole and 500 mM NaCl) and sonicated four times for 30 s. The lysate was centrifuged at 40, 000 g for 30 min at 4 °C. Supernatant was applied to a 4 ml Ni-Trap nickel-chelating column (qiagen GE Healthcare, Hilden, Germany) previously equilibrated with the same buffer A. Column was washed with buffer A containing 60 mM imidazole (Sigma-Aldrich, St. Louis, MO, USA) and the

recombinant peptide that contains full sequence of rMsrA was then eluted with buffer A containing 1 M imidazole. 5 ml of collection was treated with centrifugal ultrafiltration and the protein was concentrated. The N-terminal His-tag of recombinant peptide was removed using a Thrombin kit (New England Biolabs, Ipswich, MA, USA). Protein (500 μg) was digested for 24 h at 20 °C with 2 unit of thrombin. The purity of recombinant peptide was verified by SDS-PAGE stained with Coomassie Brilliant Blue. Recombinant peptide was stored at −80 °C until needed. Antibodies against rMsrA were used for immunological characteristics of recombinant peptide by western blotting analysis. Protein concentrations were determined by the Bradford method, using bovine serum albumin as a standard. Heat-inactivated MsrA was prepared by boiling recombinant peptide at 95 °C for 30 min and no MetO-reducing activity was detected.

Analysis of Tat-rMsrA activity

Tat-rMsrA activity was monitored by detecting both MetO-reducing activity and methyl sulfoxides-dependent oxidation of DTT. After preparation, the MsrA activity was controlled by a measurement of MetO-reducing activity based on the reduction of the substrate MetO to Met. Recombinant Tat-rMsrA (20 μg) were added into the reaction mixture containing 50 mM sodium phosphate (pH 7.4), 10 mM $MgCl_2$, 30 mM KCl, 20 mM DTT (Sigma-Aldrich, St. Louis, MO, USA) and 0.5 mM MetO (Sigma-Aldrich, St. Louis, MO, USA). The mixture was incubated for 30 min at 37 °C. The reaction was stopped by addition of diamide. We monitored the Met content using liquid chromatography coupled with electrospray ionization-ion trap mass spectrometry (LC-ESI-ITMS). MetO-reducing activity of

recombinant peptide was at 420.2 ± 22.7 pmol of the product Met mg protein^{-1} min^{-1}. After heat-inactivation, no MetO-reducing activity was detected. Before every experiment, the activity of recombinant Tat-rMsrA was rapidly monitored by colorimetric determination of methyl sulfoxides-mediated oxidation of DTT. MsrA-catalyzed oxidation of DTT was performed in the reaction system (pH 8.0, 200 μl) including $MgCl_2$ 10 mM, KCl 30 mM, Tris-HCl 25 mM, DMSO 2.5 M, DTT 2.5 mM and MsrA 20 μg. Different concentrations of recombinant rMsrA peptide (active or non-active) were added into the reaction buffer and incubated for indicated time. Due to the potential reductive activity of DTT, this reaction was performed in the air-tight and complete darkness. After reaction, 100 μl of the reaction mixture and 100 μl DTNB (4 mM) was added to each well and incubated at 37 °C for 10 min. Absorbance at 412 nm (OD_{412}) was measured with a micro-plate reader (ELx800; BioTek, Winooski, VT, USA). In each group, OD_{412} in the wells containing a same system incubated for 0 min were monitored and defined as initial values. Compared to initial values, the decreased OD_{412} (△A) in the wells after reaction were monitored and calculated. △A in the wells containing a reaction system with heat-inactivated MsrA peptide were defined as △Acon, which reflected a non-enzymatic oxidation of DTT. △A in the wells containing a reaction system with active rMsrA peptide incubated for indicated time were defined as $\triangle A_{Total}$, which reflected a total oxidation of DTT. After deduction of non-enzymatic oxidation of DTT, △A induced by active MsrA were calculated and defined as: $\triangle A_{MsrA} = \triangle A_{Total} - \triangle Acon$. Only when the $\triangle A_{MsrA}$ is above 2.0, the recombinant rMsrA peptide was used in the experiment.

RT-PCR

Total RNA was extracted from microglia using TRIZOL (Invitrogen, Carlsbad, CA, USA), according to the protocol provided by the manufacturer. The concentration and purity assessment using a UV-visible spectrophotometer (P360; Implen, Munich, Germany) was performed at 260 and 280 nm. Single-strand cDNA was synthesized from 2 μg RNA with the RevertAidTM First Strand cDNA Synthesis system (Transgene, Beijing, China). Primers used for RT-PCR analysis of MsrA were as follows: MsrA (116 bp) 5'-TGCCAGGACGCACAGAGT-3' (forward), 5'-GAAGCAGCCCATTCCAAA-3' (reverse). The first-strand product (2 μl) was used as a template in each 20 μl PCR reactions, under the following conditions: initial denaturation at 94 °C for 5 min, PCR cycles: 94 °C for 30 s, 57.2 °C for 30 s, 72 °C for 1 min repeated for 40 cycles, final elongation at 72 °C for 5 min. The PCR products were resolved on 2% agarose gel and analyzed by GENIUS bio-imaging system (Kodak, Rochest, NY, USA). Size markers were obtained from MBI Fermentas (Burlington, ON, Canada) and used to verify the size of PCR products.

Western blotting

After treatments, slices which were carefully separated from the inserts and cells were lysed in ice-cold lysis buffer (50 mM Tris–HCl, 1 mM EDTA, 100 mM NaCl, 20 mM NaF, 3 mM Na_3VO_4, 1 mM phenylmethanesulfonyl fluoride, 1% Nonidet P-40 (v / v), 2.0l g / ml aprotinin, 2.0l g / ml leupeptin and 2.0l g / ml pepstatin A). Equal amounts of protein from above lysates were loaded in each lane and separated on 10-15% SDS / PAGE gels. Protein (20 μg) were separated electrophoretically and then transferred to nitrocellulose membranes

by using a transfer cell system (Bio-Rad, California, USA). After blocking with 5% bovine serum albumin (BSA) / TBS / 0.1% Tween-20 (1 h at room temperature), membranes were incubated with the following primary antibodies: anti-ED1 at 1:200 dilution (Millipore, Billerica, MA, USA), anti-iNOS and anti-COX-2 at 1 : 200 dilution (Santa Cruz Biotechnology, Santa Cruz, USA), anti-MsrA and anti-Iba1 at 1 : 500 dilution (Abcam, Cambridge, UK), anti-ERK and anti-phosphorylated-ERK at 1 : 1000 dilution (Santa Cruz Biotechnology, Santa Cruz, USA), anti-β-actin at 1 : 2000 dilution (Santa Cruz Biotechnology, Santa Cruz, USA), anti-p38, anti-phosphorylated-p38, anti-JNK, anti-phosphorylated-JNK, anti-IκBα, anti-phosphorylated-IκBα and anti-phosphorylated p65 at 1 : 1000 dilution (Cell signaling Technology, Beverly, USA). Appropriate peroxidase-conjugated secondary antibodies were used to detect the proteins. Immunoblots were developed on microchemi (DNR, Jerusalem, Israel) by using the enhanced chemiluminescence technique (ECL, Pierce, Rockford, USA). All assays were performed at least three times. ImageJ software was used to determine the densitometry of immunoblots relative to the loading control.

Immunolabeling

To prepare brain slices, male SD rats (200-250 g) were deeply anesthetized with sodium pentobarbital (60 mg / kg) and perfused with warm (37 °C) saline and 4% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4). Brains were then removed and postfixed in 4% paraformaldehyde for at least 24 h. Following transfer to 30% sucrose solution overnight at 4 °C, the brains were chipped into coronal sections (25 μm thickness) with a freezing

microtome (CM1900; Leica Microsystems, Wetzlar, Germany). For cultured microglia, cells were fixed with 4% paraformaldehyde in 0.01 M phosphate-buffered saline (PBS, pH 7.4) for 30 min and then rinsed three times with PBS for 10 min each.

Then, the slices or cells were then permeabilized with PBS containing 0.3% Triton X-100 for 30 min and blocked by 3% BSA-PBS for 30 min at room temperature. The slices or cells were probed with appropriate antibodies against p65, MsrA and / or Iba1 at 1 : 100 dilution (Santa Cruz Biotechnology, Santa Cruz, USA) in PBS containing 0.3% TritonX-100 and 1% BSA overnight at 4 °C. Slices were rinsed in PBS three times for 10 min each, and then incubated with donkey anti-rabbit fluorescein isothiocyanate (FITC)-conjugated second antibody and / or donkey anti-goat CY3-labeled second antibody at 1 : 200 dilution (Pierce, Rockford, USA) in PBS containing 0.3% TritonX-100 and 1% BSA for 1 h at room temperature. After washed three times in PBS, slices were mounted on glass slides with 30% glycerin and imaged using a fluorescence microscope (System Microscopy IX70; Olympus, Tokyo, Japan) or a confocal laser scanning microscope (FV500; Olympus, Tokyo, Japan).

Measurements of intracellular ROS

The levels of intracellular ROS were determined by the 2′, 7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) assay (47). H_2DCFDA diffuses through the cell membrane and is deacetylated by intracellular esterase to non-fluorescent 2′, 7′-dichlorodihydrofluorescein (DCFH). DCFH reacts with ROS to form 2′, 7′-dichlorofluorescein (DCF), the fluorescent product. In brief, primary microglial cells were seeded in a 96-well plate and loaded with H_2DCFDA (20 μM, Sigma-Aldrich, St. Louis, USA) for 1 h, followed by pretreatment with

Tat-rMsrA or non-active Tat-rMsrA (0.5 μM) for 1 h, and then treatment with LPS (100 ng / ml) (Sigma-Aldrich, St. Louis, USA). After incubation at 37 °C for 30 min, the fluorescence images were immediately taken using a fluorescence microscope (System Microscopy IX70; Olympus, Tokyo, Japan). DCF intensities of cells were counted using Image-Pro Plus (IPP) software.

Superoxide assay

The amounts of extracellular superoxide was determined by measuring the superoxide dismutase (SOD)-inhibitable reduction of the tetrazolium salt WST-1. Briefly, primary microglia-enriched cultures in the 96-well plate were washed twice with phosphate-buffered saline (PBS). Cells were then incubated at 37 °C for 1 h with vehicle control or Tat-rMsrA / non-active Tat-rMsrA (0.5 μM) in PBS (50 μl / well). Thereafter, 50 μl of PBS with and without SOD (50 U / ml, Sigma-Aldrich, St. Louis, USA) was added to each well along with 50 μl of WST-1 (1 mM, Sigma-Aldrich, St. Louis, USA) in PBS and 50 μl of vehicle or LPS (100 ng / ml). Thirty minutes later, absorbance at 450 nm was read with a micro-plate reader (Sunrise A-5082; Tecan, Salzburg, Australia). The different absorbance observed in the presence and absence of SOD was considered to be the production of produced superoxide.

Luminol-enhanced chemiluminescence assay

Hydroxyl radical was generated by the addition of ferrous iron to the buffer solution. Freshly prepared $FeSO_4$ (0.5 mM) was injected to $NaHCO_3$ / Na_2CO_3 buffer (50 mM $NaHCO_3$ and 50 mM Na_2CO_3, pH 10.2) plus luminol (0.1 mM) mixture. Luminol

chemiluminescence was measured at 25 °C using a chemiluminometer (Lumat LB9507; EG&G BERTHOLD, Bad Wildbad, Germany). The effects of various concentrations of methionine (0.1, 1 and 10 mM) in the presence or absence of Tat-rMsrA / non-active Tat-rMsrA (1 μM) were examined prior to addition of the stimulant, $FeSO_4$. Duplicate assays were performed in all experiments.

Methionine sulfoxide assay

Microglial cells were lysed in ice-cold lysis buffer (50 mM Tris–HCl, 1 mM EDTA, 100 mM NaCl, 20 mM NaF, 3 mM Na_3VO_4, 1 mM phenylmethanesulfonyl fluoride, 1% Nonidet P-40 (v / v), 2.0l g / ml aprotinin, 2.0l g / ml leupeptin and 2.0l g / ml pepstatin A). The lysates were recovered by centrifugation at 12, 000 g for 15 min. After denaturation, equal amounts of lysates were treated with 0.5 mM H_2O_2 in the presence of Tat-rMsrA or non-active Tat-rMsrA (1 μM) for 40 min at 37 °C. Then the lysates were boiled in sample buffer without reducing agent at 95 °C for 5 min, and detection of methionine sulfoxide was performed with the methionine sulfoxide immunoblotting kit (Cayman, Ann Arbor, MI, USA) according to the manufacturer's protocol.

Rat model of acute neuroinflammation

LPS was injected into the dentate gyrus of hippocampus to evoke neuroinflammation in rats. All experiments were carried out on SD rats (200-250 g). Two stainless-steel guide cannulas (22 gauge) were bilaterally implanted using a stereotactic holder under aseptic conditions. Each double-guide cannula with inserted dummy cannula and dust cap was fixed

to the skull with dental cement. Bilateral cannula were delivered into the dentate gyrus of the hippocampal formation at the stereotaxic coordinates, relatives to the bregma, as follows: 2.2 mm lateral, 3.7 mm posterior and 3.8 mm ventral from top of the skull. The animals were allowed to recover for 7 d before the experiments started. On the day of the experiment, sterilized physiological saline (2 μl) or Tat-rMsrA / non-active Tat-rMsrA (2 μl, 3 mg / ml) was infused at a constant rate of 1 μl / min through a stainless steel cannula of outside tip diameter 0.28 mm, connected to a 5 μl microsyringe. The cannula was maintained in place for 5 min to limit reflux along the injection track. At day 2 post injection, rats were injected with 2 μl sterilized physiological saline or LPS (5 mg / ml, dissolved in 2 μl of sterilized physiological saline) into the dentate gyrus of hippocampus for 24 h. Then, brains were extracted and processed for immunohistochemistry and immunoblotting.