Tumor Necrosis Factor-α Induces Neurotoxicity via Glutamate Release from Hemichannels of Activated Microglia in an Autocrine Manner*

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Glutamate released by activated microglia induces excitoneurotoxicity and may contribute to neuronal damage in neurodegenerative diseases, including Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, and multiple sclerosis. In addition, tumor necrosis factor-α (TNF-α) secreted from activated microglia may elicit neurodegeneration through caspase-dependent cascades and silencing cell survival signals. However, direct neurotoxicity of TNF-α is relatively weak, because TNF-α also increases production of neuroprotective factors. Accordingly, it is still controversial how TNF-α exerts neurotoxicity in neurodegenerative diseases. Here we have shown that TNF-α is the key cytokine that stimulates extensive microglial glutamate release in an autocrine manner by up-regulating glutaminase to cause excitoneurotoxicity. Further, we have demonstrated that the connexin 32 hemichannel of the gap junction is another main source of glutamate release from microglia besides glutamate transporters. Although pharmacological blockade of glutamate receptors is a promising therapeutic candidate for neurodegenerative diseases, the associated perturbation of physiological glutamate signals has severe adverse side effects. The unique mechanism of microglial glutamate release that we describe here is another potential therapeutic target. We rescued neuronal cell death in vitro by using a glutaminase inhibitor or hemichannel blockers to diminish microglial glutamate release without perturbing the physiological glutamate level. These drugs may give us a new therapeutic strategy against neurodegenerative diseases with minimum adverse side effects.

Central nervous system inflammation, including microglial activation, likely contributes to the neurotoxicity observed in neurodegenerative diseases, such as Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, and multiple sclerosis (1–5). Excitoneurotoxicity may play an important role in these neurodegenerative diseases (6, 7). Microglia have been shown to act not only as antigen-presenting cells but also effector cells that can injure other cells in the central nervous system directly in vitro and in vivo (8). Thus, inhibition of microglial activation is considered a therapeutic candidate for several neurodegenerative diseases. On the other hand, microglia also have neuroprotective effects mediated by neurotrophin release, glutamate uptake, and sequestering of neurotoxic substances (8–12). Therefore, any therapeutic approach should inhibit the deleterious effects of microglia without diminishing their protective role. We sought to identify the source of these toxic factors produced by activated microglia.

Recently, we demonstrated that activated microglia release large amounts of glutamate and that microglial neurotoxicity is mediated primarily by NMDA3 receptor signaling (13). In addition to glutamate, activated microglia release pro-inflammatory cytokines, such as interleukin (IL)-1β, IL-6, interferon (IFN)-γ, and tumor necrosis factor-α (TNF-α) (14–18), which also promote neuronal damage. TNF-α from activated microglia is a major neurotoxic cytokine that induces neurodegeneration through the silencing of cell survival signals and caspase-dependent cascades, including promotion of Fas ligand signals (1, 19–22). However, direct neurotoxicity by TNF-α is relatively weak, because it also activates neuroprotective factors, including MAPK and expression of nuclear factor-kB (NF-kB) (23, 24). Accordingly, it is still controversial how TNF-α exerts neurotoxicity in neurodegenerative diseases.

In this study, we have demonstrated that TNF-α is the key cytokine that stimulates extensive microglial glutamate release in an autocrine manner by up-regulating glutaminase to cause excitoneurotoxicity. Moreover, glutamate originating from microglia is released through the connexin (Cx)32 hemichannel of the gap junction more significantly than glutamate transporters such as excitatory amino acid transporter (EAAT) and

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3 The abbreviations used are: NMDA, N-methyl-D-aspartate; IL, interleukin; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor; TNFR, TNF receptor; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; NF-κB, nuclear factor-κB; IκB, inhibitor of NF-κB; Cx, connexin; EAAT, excitatory amino acid transporter; TUNEL, terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling; MTS, 3-(4,5-dimethyl-thiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; DON, 6-diazoo-5-oxy-L-norleucine; THA, β-threo-β-hydroxyaspartic acid; AAA, l-2-aminoacidopinic acid; GA, 18α-glycyrrhetinic acid; RT, reverse transcription; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase.
transport system Xc–. We rescued neuronal cell death in vitro by using a glutaminase inhibitor or hemichannel blockers to diminish microglial glutamate release without perturbing physiological glutamate level. Thus, these drugs may give us a new therapeutic strategy against neurodegenerative diseases with minimum adverse side effects.

EXPERIMENTAL PROCEDURES

Cell Culture—All reagents except those specifically mentioned were obtained from Sigma-Aldrich. The protocols for animal experiments were approved by the Animal Experiment Committee of Nagoya University. Microglia in 24-well multidishes at a density of 5 × 10^4 cells/well were prepared from newborn C57BL/6 mice with the “shaking off” method as described previously (25). The purity of the cultures was >99% as determined by Fc receptor-specific immunostaining. Cultures were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (JRH Biosciences, Lenexa, KS), 5 µg/ml bovine insulin, and 0.2% glucose. Neuron cultures were prepared from C57BL/6 mice at embryonic day 17 as described previously (13). Briefly, cortices were dissected and freed of meninges. Cortical fragments were dissociated into single cells using dissociation solution, and they were resuspended in Neuron Medium (serum-free conditioned medium from 48-h rat astrocyte confluent cultures based on Dulbecco’s modified Eagle’s medium/F-12 with N2 supplement (Sumitomo Bakelite, Akita, Japan). Primary neuronal cells were plated on 12-mm polyethyleneimine-coated coverslips (Asahi Techno Glass Corporation, Chiba, Japan) in 24-well multidishes at a density of 5 × 10^4 cells/well. The purity of the cultures was >95% as determined by NeuN-specific immunostaining. To assess the direct neurotoxicity of pro-inflammatory cytokines, neurons at 10–13 days in vitro were incubated with Neuron Medium (Sumitomo Bakelite) containing 1 µg/ml lipopolysaccharide (LPS), 100 ng/ml IL-1β, 100 ng/ml IL-6, 100 ng/ml IL-10, 100 ng/ml IFN-γ, or 100 ng/ml TNF-α (R&D Systems, Minneapolis, MN). Cultures were evaluated 24 h after stimulation. To assess microglial neurotoxicity, microglia were also cultured in the same media conditions described above. After a 24-h incubation, microglia-conditioned medium was applied to each well containing 5 × 10^4 neurons at 10–13 days in vitro. Neurons were evaluated 24 h after medium exchange. To assess neuroprotective effect, activated microglia treated with LPS or TNF-α were incubated with each drug as follows: 100 µg/ml anti-mouse TNF-α-neutralizing antibody (TECHNE Corporation, Minneapolis, MN); 20 µg/ml anti-mouse TNFR1-neutralizing antibody (R&D Systems); 20 µg/ml anti-mouse TNFR2 neutralizing antibody (R&D Systems); p38 MAPK inhibitor, 10 µM SB203580 (Calbiochem, San Diego, CA); 10 µM JNK inhibitor (Calbiochem); MEK inhibitor, 10 µM PD98059 (Calbiochem); 100 µM ilkB kinase inhibitor (Calbiochem); glutaminase inhibitor, 1 mM DON; EAAT inhibitor, 100 µM THA; transport system Xc– inhibitor, 2.5 mM AAA; gap junction inhibitor, 100 µM carbenoxolone disodium (CBX; gap junction inhibitor), 50 µM GA; Cx32 mimetic peptide, 0.25 mg/l 32 gap 27 (SRPTEKTVFT, extracellular loop 2, position 182–191 of Cx32, Thermo Electron GmbH); Cx32 mimetic peptide, 0.25 mg/l 32 gap 27 (SRPTEKTVFT, extracellular loop 2, position 182–191 of Cx32, Thermo Electron GmbH); Cx43 mimetic peptide, 0.25 mg/l 32 gap 27 (SRPTEKTVFT, extracellular loop 2, position 182–191 of Cx43, Thermo Electron GmbH). The purity of all connexin mimetic peptides was >98%. After a 24-h incubation, microglia-conditioned medium was applied to neurons at 10–13 days in vitro. White bars, neurons treated with reagents directly; black bars, neurons incubated with reagent-treated microglia-conditioned medium. *, p < 0.05 versus control; **, p < 0.01 versus control. †, p < 0.01 versus control; ‡, frequency of bead-bearing neurons. **, p < 0.01 versus control. White bars, neurons incubated with LPS- or TNF-α-treated microglia-conditioned medium. The values are the means ± S.D. C–H, phase contrast images. C, control microglia. D, LPS-treated microglia. E, TNF-α-treated microglia. LPS- or TNF-α-treated microglia were activated and changed dramatically to the large amoeboid shape (D and E). F, control neurons; G, neurons incubated with LPS-treated microglia-conditioned medium for 24 h. H, neurons incubated with TNF-α-treated microglia-conditioned medium for 24 h. Control neurons bore few neuritic beads (F). In contrast, LPS- or TNF-α-treated microglia-conditioned medium induced numerous neuritic beading accompanied by neurite narrowing (G and H). Scale bar, 10 µm.
each well containing $5 \times 10^4$ neurons at 10–13 days in vitro. Evaluations of neuronal cultures were performed 24 h after medium exchange.

Assessment of Neuritic Beading—To assess neuritic beading, neurons were observed with phase-contrast microscopy 24 h after stimulation as described previously (13). More than 200 neurons in duplicate wells were assessed blindly in three independent trials. The ratio of bead-bearing neurons was calculated as a percentage of total cells.

Assessment of Cell Death—Cell death was assessed by the dye exclusion method with propidium iodide (Molecular Probes) as described previously (13, 26). More than 200 neurons in duplicate wells were assessed blindly in three independent trials with a conventional fluorescent microscope. The ratio of dead cells was calculated as a percentage of propidium iodide-positive cells among total cells. To detect apoptosis, we used the TUNEL assay with the in situ cell death detection kit (Roche Diagnostics, Basel, Switzerland) as described previously (13, 26). TUNEL assay was carried out 24 h after stimulation according to the manufacturer’s protocol. As a positive control, neurons were incubated with 10 nM staurosporin for 24 h. More than 200 neurons in duplicate wells were assessed blindly in three independent trials under a conventional fluorescent microscope. The ratio of apoptotic cells was calculated as a percentage of TUNEL-positive cells among total cells.

Assessment of Mitochondrial Impairment—To assess mitochondrial viability, we used the MTS assay with the CellTiter 96 Aqueous One-solution assay (Promega, Madison, WI) according to the manufacturer’s protocol as described previously (13, 26). Absorbance at 490 nm was measured in a multiple plate reader. Assays were carried out in six independent trials.

Assessment of Intracellular ATP Levels—To measure intracellular ATP levels, we used a luminometric assay with the ApoSENSOR cell viability assay kit (BioVision, Mountain View, CA) as described previously (13). Assays were carried out in six independent trials. ATP concentration was calculated as a percentage of control.

Assessment of Glutamate Release—To measure extracellular glutamate concentrations, we used the Glutamate Assay Kit colorimetric assay (Yamasa Corporation, Tokyo, Japan) as described previously (13). Assays were carried out in six independent trials.

Reverse Transcription (RT)-PCR Analysis of Microglial Glutaminase—To examine mRNA expression of microglial glutaminase, we used RT-PCR analysis, $1 \times 10^6$ microglia plated on 24-well multidishes were treated with 1 $\mu$g/ml LPS or 100 ng/ml TNF-$\alpha$ for 12 h. Total RNA was extracted from microglia using the RNeasy Mini Kit according to the manufacturer’s protocol (Qiagen). cDNAs encoding mouse glutaminase and GAPDH were generated by RT-PCR using Super Script II (Invitrogen) and Ampli TaqDNA polymerase (Applied Biosystems) with the following primers: glutaminase (sense), 5'-GTCAGATCTTGTGTCTCTGGTTC-3'; glutaminase (antisense), 5'-GTCGAAAGAGCAGTGCTTCATCATCAG-3'; GAPDH (sense), 5'-ACTCA- CGGCAAATTCACGAG-3'; and GAPDH (antisense), 5'- CCTGTGCTCCTAGCCCGTA-3'.

Flow Cytometry—The expression level of the microglial gap junction was detected with flow cytometry. $5 \times 10^4$ microglia plated on 24-well multidishes were treated with 1 $\mu$g/ml LPS or 100 ng/ml TNF-$\alpha$ for 24 h. Cells were labeled with mouse monoclonal anti-mouse Cx32 or Cx43 antibody (1:200, Chemicon, Temecula, CA) for 20 min at 4 °C followed by labeling with Alexa 488-conjugated secondary antibody (1:300, Molecular Probes) for 20 min at 4 °C. Fluorescence signals were measured with a flow cytometer (Cytomics FC500, Beckman Coulter, Fullerton, CA).

Statistical Analysis—All results were analyzed by one-way analysis of variance with a Tukey-Kramer post hoc test using Statview software version 5 (SAS Institute Inc., Cary, NC).
RESULTS

First, we examined neuronal damage caused by microglial cytokines by applying the pro-inflammatory cytokines IL-1β, IL-6, IFN-γ, and TNF-α and anti-inflammatory cytokine IL-10 to neurons or microglia. As LPS treatment induces release of these cytokines from microglia, we applied LPS to these cells. Recently, we demonstrated that neuritic beading, focal bead-like swellings in dendrites and axons, is an early pathological feature of neuronal cell dysfunction that precedes neuronal death (13). We quantified neuronal damage as the ratio of neuritic bead-bearing cells to those with normal morphology, as well as the ratio of dead cells to living ones. Direct application of LPS, pro-inflammatory cytokines, and anti-inflammatory cytokines to neurons induced little neuritic beading (Fig. 1A, white bars) or neuronal death (Fig. 1B, white bars). TNF-α only weakly induced neuritic beading and neuronal death. Interferon-γ also induced some neuritic beading but resulted in little neuronal death. In contrast, conditioned medium from microglia treated with LPS or TNF-α induced numerous beads in

FIGURE 3. TNF-α promotes microglial glutamate release through the TNFR1 pathway in an autocrine manner. A, extracellular glutamate concentration. B, frequency of bead-bearing neurons. C, frequency of dead neurons. LPS- or TNF-α-treated microglia were incubated with or without reagents for 24 h. Thereafter, neurons were incubated with each microglia-conditioned medium for 24 h. α-TNF, anti-TNF-α-neutralizing antibody; α-R1, anti-TNF1-neutralizing antibody; α-R2, anti-TNF2-neutralizing antibody; TNF1, 1 ng/ml TNF-α; TNF10, 10 ng/ml TNF-α; TNF100, 100 ng/ml TNF-α. *, p < 0.05 versus control. **, p < 0.01 versus control. †, p < 0.05 versus neurons incubated with LPS or TNF-α-treated microglia-conditioned medium. The values are the means ± S.D.

FIGURE 4. TNF-α induces microglial glutamate release from Cx32 hemichannels of gap junctions through activating glutaminase. A, extracellular glutamate concentration. B, frequency of bead-bearing neurons. C, frequency of dead neurons. Neurons were incubated with TNF-α-treated microglia-conditioned medium with or without reagents for 24 h. α-p38, p38 MAPK inhibitor SB203580; α-MEK, MEK inhibitor PD98059; α-JNK, JNK inhibitor; α-IKK, IkB kinase inhibitor; DON, glutaminase inhibitor 6-diazo-5-oxo-L-norleucine; THA, EAAT inhibitor DL-threo-β-hydroxyaspartic acid; AAA, transporter system Xc-inhibitor l-2-aminoacidic acid; CBX, gap junction inhibitor carbenoxolone disodium; GA, gap junction inhibitor 1α-glycyrrhetinic acid; GAP24, Cx32 mimetic peptide GAP24; GAP27, Cx32 mimetic peptide GAP27; GAP27, Cx43 mimetic peptide GAP27. *, p < 0.05 versus control; †, p < 0.05 versus neurons incubated with TNF-α-treated microglia-conditioned medium; **, p < 0.05 versus neurons incubated with TNF-α- and AAA-treated microglia-conditioned medium. The values are the means ± S.D.
most neurites and marked neuronal death (Fig. 1, A and B, black bars; Fig. 1, G and H). Consistent with these findings, LPS- or TNF-α-treated microglia had changed morphologically to the large amoeboid shape and appeared activated (Fig. 1, D and E). As described previously (13, 27, 28), very little apoptotic neuronal cell death was detected by TUNEL assay (data not shown). This neuronal damage was almost completely inhibited by the NMDA receptor antagonist MK801 (Fig. 1, A and B). These observations are consistent with a critical role for glutamate in the neuronal damage by LPS- and TNF-α-activated microglia. Moreover, neuronal death in LPS- and TNF-α-activated microglia is likely to be excitotoxicity through NMDA receptor signaling.

Next we examined extracellular glutamate concentrations in our microglial cultures. Only conditioned media from microglia treated with LPS or TNF-α contained higher levels of glutamate (Fig. 2A), which is consistent with a previous report (35). Previously, we demonstrated that NMDA receptor signaling induces a rapid drop in intracellular ATP levels by inhibiting mitochondrial respiratory chain complex IV activity (13). Conditioned media from LPS- or TNF-α-treated microglia also induced a rapid drop in intracellular ATP levels and mitochondrial dysfunction in neurons (Fig. 2, B and C). These findings are consistent with extracellular glutamate elevation.

TNF-α is a ligand for TNFR1 and TNFR2. TNF-α promotes further microglial TNF-α production in an autocrine manner through TNFR1 signaling (29). We assessed whether this TNF-α autocrine loop participates in microglial neurotoxicity and which TNF receptor mediates microglial glutamate release. First, LPS- or TNF-α-treated microglia were incubated with or without neutralizing antibodies for TNF-α or TNFR for 24 h. Subsequently, each microglial conditioned medium was applied to the neurons. Assessments were performed 24 h after medium exchange. TNF-α induced microglial glutamate release and subsequent neuronal damage in a dose-dependent manner (Fig. 3). Thereafter, neutralizing TNF-α with function-blocking antibodies reduced LPS- and TNF-α-induced microglial glutamate release and subsequent neuronal damage. Blockade of TNFR1 with function-blocking antibodies also suppressed LPS- and TNF-α-induced microglial glutamate release and subsequent neuronal damage, whereas blockade of TNFR2 had no effect (Fig. 3). Thus, TNF-α, acting through TNFR1 in an autocrine manner, is the key cytokine that promotes glutamate release from activated microglia in response to LPS to cause excitotoxicity.

TNF-α activates NF-κB through at least three MAPK pathways, including the p38 MAPK pathway, MEK1/2 pathway, and JNK pathway. However, none of the MAPK inhibitors we examined affected TNF-α-induced glutamate release and neurotoxicity (Fig. 4). Further, the inhibition of NF-κB activation with an IκB kinase inhibitor did not have significant effects on TNF-α-induced glutamate release and neurotoxicity. Thus, NF-κB does not appear to mediate glutamate release; other unknown pathways may be involved.

In general, cells utilize two pathways to synthesize glutamate (30–33). In one route of synthesis, glutamate dehydrogenase converts α-ketogluatric acid to glutamate. Cellular homeostasis of glutamate levels is maintained primarily by this glutamate dehydrogenase pathway. In the other, glutaminase produces glutamate from glutamine. Cellular uptake of glutamine is through glutamine transporters. We assessed the contribution of the glutaminase pathway to TNF-α-induced microglial glutamate release by using a glutamine-free culture medium. Glutamine starvation abolished the effect of TNF-α treatment (Fig. 4A). It also inhibited the TNF-α-induced neurotoxicity of microglial conditioned media almost completely (Fig. 4, B and C). In a separate experiment, the glutaminase inhibitor DON suppressed TNF-α-induced glutamate release and subsequent neurotoxicity to levels similar to glutamine starvation. RT-PCR analysis revealed that both LPS and TNF-α up-regulated the mRNA expression of glutaminase in microglia (Fig. 5A). We conclude that TNF-α-induced glutamate synthesis in microglia is primarily through the glutaminase pathway.

Next, we focused on glutamate release. Glutamate is considered to be secreted through exocytosis or glutamate transporters such as EAATs and transporter system Xc− (34–38). Recent reports demonstrate that astrocytes release glutamate and ATP through hemichannel of gap junctions (39, 40). Additionally, TNF-α reportedly enhances the expression of hemichannels on microglia (41). Thus, we examined the effects of gap junction and glutamate transporter inhibitors in our culture system. The gap junction inhibitors carbenoxolone disodium and GA markedly reduced TNF-α-induced microglial glutamate release and subsequent neurotoxicity more significantly than the transporter system Xc− inhibitor AAA, whereas the EAAT inhibitor THA did not (Fig. 4). These data support a model in which the hemichannel of the gap junction is another main source of glutamate release from microglia besides transporter system Xc−. Thereafter, we examined the effect of the hemichannel-specific blocker using connexin mimetic peptides in our system as described previously (42). Blockade of Cx32 with 32gap27 or 32gap27 strikingly diminished TNF-α-induced microglial glutamate release and subsequent neurotoxicity, whereas blockade of Cx43 with 32gap27 did not (Fig. 4). Interestingly, flow cyto-

**FIGURE 5.** TNF-α up-regulates both glutaminase mRNA expression and cell surface expression of Cx32 in microglia. A, RT-PCR data demonstrate that both LPS and TNF-α up-regulate the mRNA expression of microglial glutaminase. B, flow cytometric data show that both LPS and TNF-α enhance the expression of Cx32 on the microglial cell surface.
metric analysis of either LPS- or TNF-α-treated microglia exhibited up-regulated cell surface expression of Cx32 (Fig. 5B), whereas neither LPS nor TNF-α affect microglial surface expression of Cx43 (data not shown). Hence, Cx32 hemichannels of gap junctions are the novel principal site of TNF-α-induced glutamate release from microglia.

**DISCUSSION**

In this study, we provide the first evidence that TNF-α is the key cytokine that promotes excitotoxicity by inducing microglial glutamate release in an autocrine manner (Fig. 6). Recent studies have reported that TNF-α enhances excitotoxicity through synergistic stimulation of TNFR and NMDA receptor (43) and by inhibition of astroglial glutamate uptake through the glutamate transporter (44). We observed that TNF-α directly induces glutamate release from microglia and subsequent excitotoxic neuronal death. We demonstrated that glutamine starvation and a glutaminase inhibitor strikingly reduce glutamate release to the control levels. Cellular homeostasis of glutamate levels may be maintained primarily by the glutamate dehydrogenase pathway (30–33). Our data reveal that TNF-α stimulates microglia to produce large amounts of redundant glutamate through up-regulation of glutaminase but not glutamate dehydrogenase. Thus, inhibition of glutaminase did not suppress the glutamate production of control cultures. In other words, the glutaminase inhibitor only affected induced glutamate synthesis but did not perturb essential glutamate production. Therefore, glutaminase inhibitors may prevent neurotoxicity by activated microglia specifically without serious adverse effects.

Further, we demonstrated that the Cx32 hemichannel of gap junction is another principal source of TNF-α-induced glutamate release from microglia besides transporter system Xc−. Moreover, TNF-α enhances the surface expression of Cx32 hemichannels. Activated microglia can undergo dramatic morphological change and dynamic movement. Accordingly, gap junctions of activated microglia may be more openly exposed to the extracellular space than those of other central nervous system cells. Thus, inhibitors of gap junctions may be more effective in suppressing glutamate release from microglia, and this specificity may translate into fewer adverse side effects during treatment.

Treatment with TNF-α-neutralizing antibodies is an effective therapy for autoimmune diseases (45). This therapy, however, has serious adverse side effects, including an increased risk for infections such as tuberculosis. Thus, TNF-α-neutralizing therapy is not readily applicable to neurodegenerative diseases, even though TNF-α may play an important role in the progression of neurodegenerative diseases. Blockade of NMDA receptors is another promising therapy for neurodegenerative diseases (46). However, adverse effects increase in a dose-dependent manner. The present study provides two new possibilities for therapeutic targets, the enzyme glutaminase and gap junctions. Inhibitors directed against these targets may be specific therapeutic candidates for activated microglia-mediated neurodegenerative diseases (Fig. 6). We are planning more thorough studies to investigate this issue.

In conclusion, we have demonstrated that TNF-α induces microglial glutamate release from Cx32 hemichannels of gap junctions through the up-regulating glutaminase to promote excitotoxicity. Either the glutaminase inhibitor or gap junction inhibitors may be a novel effective strategy for the treatment of neurodegenerative diseases with minimum adverse side effects.

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**FIGURE 6. A schematic model of TNF-α-induced glutamate release and excitotoxicity.**

TNF-α-induced Excitotoxicity via Microglia
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