SYNAPSE 65:433–440 (2011)

Neuron-Glial Cell Communication in the Traumatic Stress-Induced Immunomodulation

HUI ZHAO,[1]* SHENG XIAO,[1] XIAOYAN KONG,[1] JUN WANG,[1] XIAODING CAO,[1] WU GENCHENG,[1] HORACE H. LOH,[2] AND PING-YEE LAW[2]

[1]*Department of Integrative Medicine and Neurobiology, Shanghai Medical College, Fudan University, Shanghai 200032, People's Republic of China*

[2]*Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota*

KEY WORDS ERK1/2; RSK-1; neuron; glial cell; neuroimmune modulation

ABSTRACT We have previously reported that neuron and glia could collaboratively govern the immunomodulation in traumatic rats. Herein, we characterized the sequential involvement of cortical neuron, microglia, and astrocytes in the traumatic stress-mediated neuroimmune modulation. At day 1 of trauma, transient extracellular signal related kinase 1/2 (ERK1/2) activation was initiated in neuron and microglia, which was accompanied by RSK-1 expression in the cytosol. At day 3 of trauma, persistent ERK1/2 activation occurred in astrocytes, which were destined for the nucleus leading to Elk-1 expression. Furthermore, the functional overlap of ERK1/2 and neuroligin 1 in astrocytes was strengthened at day 3 of trauma and responsible for the recovery from the immnosuppression. These effects could be disrupted by β-neurexin blockade. Altogether, we proposed the mechanism underlying the traumatic stress-induced immunosuppression, in which local activity ensured the initial establishment of neural circuitry in the frontal cortex. ERK1/2-signaling events are required for the temporal and spatial coordination between neuron and glial cells. **Synapse 65:433–440, 2011.** ©2010 Wiley-Liss, Inc.

INTRODUCTION

Within the central nervous system (CNS), microglia and astrocytes are the most important nonprofessional antigen-presenting cells, and they express low levels of major histocompatability complex (MHC) and costimulatory molecules and provide a key mechanism that endows the CNS to be immune privileged under healthy conditions (Freria et al., 2010; Kaneko et al., 2010; Liu et al., 2010; Streit et al., 1999). However, the roles that neurons have cannot be overlooked. Neurons could express MHC class I molecules after stimulation by interferon-γ; this expression can drive them to become targets of cytotoxic T cells in CNS infections and autoimmune diseases (Escande-Beillard et al., 2010). In our work, we found that both neuron and microglia could release IL-1β when subjected to traumatic stress by which immunosuppression was mediated (Zhao et al., 2002). Recently, it has shown that MHC class I molecules in neuron are also crucial for normal brain development and synaptic plasticity (Feng et al., 2010; Ribic et al., 2010; Walter et al., 2010). These findings underlie the potential capacity of neural circuitry to control cellular immune responses in pathological conditions.

Neurons communicate with glial cells in various ways (de Oliveira et al., 2009; Shono et al., 2009); the cross-talk between them is mediated by neuronal immune-regulatory molecules, cell-adhesion molecules, and other receptors. Neuroligins are synaptic cell-adhesion molecules, which might be involved in strengthening cell–cell contacts (Blundell et al., 2010; Jung et al., 2010). When neuroligin 1 is expressed in a non-neuronal cell, the forming of synapses between neuron and non-neuronal cell will be initiated, and neurexin 1 is consequently enriched in presynaptic nerve terminals contacting a neuroligin-expressing non-neuronal cell (Berninghausen et al., 2007; Chubykin et al., 2005). It was also reported that overexpression of neuroligins in transfected neurons would increase the number of synapses onto these neurons

Contract grant sponsor: National Key Basic Research Program of China; Contract grant number: 2007CB512502; Contract grant sponsor: Brain Research Institute Open Program

*Correspondence to: Hui Zhao, Department of Integrative Medicine and Neurobiology, Shanghai Medical College, 138# Yixueyuan Road, Shanghai 200032, People's Republic of China. E-mail: mayhzhao@yahoo.com

Received 9 April 2010; Accepted 16 August 2010

DOI 10.1002/syn.20861

Published online 17 September 2010 in Wiley Online Library (wileyonlinelibrary.com).

(Boucard et al., 2005; Chih et al., 2004), and synapse size was also increased. Therefore, postsynaptic neuroligins are proposed to generally activate presynaptic neurexins by dimerizing them and seem to induce local formation of presynaptic specializations (Dean et al., 2003; Varoqueaux et al., 2006). When concerning the traumatic stress-induced immunomodulation, it is unclear if neurexin/neuroligin has been linked and is capable to enhance local neural cells circuitry under this condition.

ERK1/2 activation is a well-established response in many cells to stimulation by mitogens and results in neuronal proliferation and differentiation in vitro (Pearson et al., 2001). Increased ERK activation is participated in age-dependent immunomodulation in traumatic rats (Xiao et al., 2009). ERK1/2 is also associated with cell survival in some in vivo systems and could be activated with spatial–temporal pattern. For example, ischemia-induced multiphasic ERK1/2 activation, which is including both an early onset, rapid increase during the first 30–60 min of reperfusion, and a later onset, protracted increase between 12 and 24 h after reperfusion (Hicks et al., 2000). It was assumed that the earliest phase of activation (1 h) occurred on neuron and microglia, whereas activation beyond day 1 was primarily restricted to astrocytes (Cha et al., 2001; Choi et al., 2006). Besides that, ERK1/2 activation is mediated by two distinct and independent-signaling events, namely, a Src/Yes/Fyn-dependent and heterotrimeric G-protein/PKCζ-dependent mechanism. In response to angiotensin II, the ability of ERK1/2 to remain within the cytoplasm or translocate into the nucleus is controlled by the above two mechanisms, respectively (Godeny and Sayeski, 2006; Shah et al., 2004). Consequently, we sought to define the signaling mechanisms underlying ERK activation in neural cells when challenged with traumatic stress. It is conceivable that neuron-glial cells communication involved by cell-surface molecules are likely operationally implicated in traumatic stress, which required for ERK1/2 activation, and finally build a complex network and achieve a distinct cellular outcome to sense and respond to brain insults.

MATERIALS AND METHODS

Animals

SD adult male rats (Animal Center of Chinese Academy of Sciences, 200–250 g) were used in this study. The animals were housed in groups (five per cage) in a controlled environment on a 12-h light–dark cycle and allowed to acclimate for a minimum of 5 days before conducting experiments. Water and food were available at all times. All protocols were approved by the Committee on Research Animal Care of Fudan University, and the principles and procedures are outlined in the NIH Guide for the Care and Use of Laboratory Animals.

The surgical trauma stress was performed as previously described (Zhao et al., 2002). Briefly, rats were anesthetized with pentobarbital sodium (35 mg/kg, i.p.) and then were incised longitudinally to a length of 6 cm along the dorsal median line and 5 cm along the abdominal median line. Five minutes after surgery, the wounds were sutured, and the animals were kept warm under standard housing conditions. No postoperative infection occurred. The operation was performed 48 h after implanting a cannula, and tissue samples were taken 1, 3, and 7 days after operation.

Intracellular injection of drugs

Implantation of the cannula was performed stereotaxically under anesthesia, and the stainless steel guide cannula (0.5 mm in diameter) with an inserted cannula (0.25 mm in diameter) was implanted into the right lateral ventricle (posterior 0.5, lateral 1.5, and horizontal 4.5) and fixed on the skull with dental cement. Minocycline (50 μg, Santa Cruz Biotechnology, Santa Cruz, CA), glial toxin fluorocitrate (5 nM, Sigma, St. Louis, MO), and neurexin-1β antibody (10 μg, Santa Cruz Biotechnology, Santa Cruz, CA) were dissolved and injected over 10 s via the cannula at a volume of 10 μl. Rats from the control group were injected with sterilized PBS. At the end of each experiment, the position of the cannula was assessed by histological examination. Data were collected only from experiments in which correct insertion of the cannula was verified. Animals were killed 24 h after injection.

Lymphocyte proliferation and NK cell activity

For lymphocyte proliferation, spleens were pressed through stainless steel mesh, and red blood cells were lysed by treatment with NH_4Cl solution. Then, cell suspension of 1×10^7 cells/ml in a final volume of 200 μl of complete tissue-culture medium (RPMI 1640 supplemented with 10% heat activated fetal calf serum, 2 mM L-glutamine) was seeded in triplicate in a U-bottomed 96-well plate in the presence and absence of concanavalin A (Con A, 1 mg/L, Sigma, St. Louis, MO). Plates were incubated at 37°C in 5% CO_2. After 48 h, cultures were labeled with 0.5 μCi of [^{3}H] thymidine (Amersham Biosciences, Piscataway, NY), and cells were harvested using a cell harvester 24 h later. Samples were counted in a liquid scintillation counter. Proliferation results are presented as the mean cpm ± SD of triplicate cultures.

For natural killer (NK) cell cytotoxicity, first, suspensions of YAC-1 lymphoma cells with a concentration of 2×10^5/ml at a final volume of 100 μl were targeted with 0.5 μCi of [^{3}H] thymidine and incubated at 37°C, 5% CO_2 for 6 h. Then, spleens were homogenized, and the resultant cell suspensions were pooled in the pres-

ence/absence of Con A and seeded in triplicate with the effector: target ratios of 50:1 for 16 h. Cytotoxic activity results were determined as follows:

Percent response = [(counts in tested well-counts in spontaneous response well)/(counts in maximum response well-counts in spontaneous response well)] $\times$ 100.

Cell cultures and treatments

For primary neuron culture, rat fetuses were removed from pregnant rats on embryonic day 18. Frontal cortex was dissected and collected in Hanks' balanced salt solution on ice. Cells were dissociated and plated at a density of 10^5 cells per well into 24-well tissue-culture plates pretreated with 0.1% polyethylenemine. Cells were maintained in serum-free Neurobasal medium containing B27 supplement (Gibco, Rockville, MD) (Buzas et al., 1998). After 3–4 days in culture, cells of neuronal morphology sent out long processes. By 10 days, MAP2 immunopositive cells were sorted by flow cytometry and treated with IL-1β (R&D systems, Minneapolis, MN; 20 ng/ml, 24 h) and IL-1ra (R&D systems, Minneapolis, MN; 10 ng/ml, 24 h) for the indicative times.

For primary astrocyte cultures, the dissociated cells were plated in untreated 24-well tissue-culture plates. The culture medium was Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM glutamine, and 50 U penicillin/50 μg/ml streptomycin, and the adherent cells were purified after 24-h plating. By 2 weeks, GFAP immunopositive cells were sorted by flow cytometry and treated with IL-1β and/or IL-1ra for the indicative times.

For microglia culture, mixed brain-cell cultures were prepared in tissue-culture flasks. After 10–20 days of culture, microglia were harvested by shaking the culture flasks for 1.5 h at 80 rpm. Harvested cells were seeded in 100-mm petri dishes and incubated for 10 min at 37°C. After removing nonadherent cells using a single wash with PBS, the attached microglia were maintained in the growth medium. The next day, the microglial cells were removed using PBS containing 1 mM EDTA. CD11b/c immunopositive cells were sorted by flow cytometry and treated with IL-1β and/or IL-1ra for the indicative times.

Immunofluorescent double-labeling

Rats were anesthetized with sodium pentobarbital and perfused transcardially with fixative (4% paraformaldehyde). Coronal brain sections (20 μm) were obtained using a cryostat. Sections were subjected to anti-pERK1/2 (1:1000, Cell Signaling Technology, Danvers, MA) at 37°C for 18 h and then transferred into Alexa Fluor® 488-conjugated anti-rabbit antiserum (1:1000, Invitrogen, Carlsbad, CA) for 1 h. Thereafter, sections were subsequently incubated with anti-neuroligin-1 (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA) and Alexa Fluor 594-conjugated anti-mouse antibodies. Data derived from each group were analyzed by Leika Q500IW image analysis system.

Immunoprecipitation and Western blotting

Frontal cortex was sonicated with about seven volumes of protein extraction buffer-containing 20 mM HEPES (pH 7.5), 10 mM potassium chloride, 1.5 mM magnesium chloride, 1 mM ethylenediaminetetraacetic acid, 1 mM EGTA, and 1× complete protease inhibitor (Roche Applied Science). The sonicated sample was centrifuged at 10,000*g* for 15 min at 4°C, and the supernatant was incubated with anti-MAP2, GFAP, and CD11b/c (1:200) on a rotating platform overnight, followed by incubation with 20-μl protein G agarose beads (Pierce Biotechnology) for 2 h at 4°C. Beads were washed three times in lysis buffer, and proteins were extracted and resolved in SDS–polyacrylamide gel and transferred to a polyvinylidene difluoride membrane (PVDF, Amersham). The membrane was probed with antipERK1/2 (1:1000), antineuroligin-1 (1:1000), and subsequent alkaline phosphatase-conjugated secondary antibody (1:5000).

Cells (1.0×10^6 cells) were collected and centrifuged followed by resuspension in lysis buffer-1 (10 mM HEPES, 10 mM KCl, 0.1 mM EDTA, 1 mM DTT, and pH 7.9. protease inhibitor cocktail). NP40 was added followed by centrifugation, and the supernatant was collected as the cytoplasmic fraction. The pellet was then resuspended in lysis buffer-2 (10 mM HEPES, 400 mM NaCl, 1 mM EDTA, and 1 mM DTT, and pH 7.9. protease inhibitor cocktail). Suspension was vortexed and centrifuged, and supernatant was collected as the nuclear fraction. Lowry assays were used to determine the protein concentration, and 5 μg of protein was resolved in SDS–polyacrylamide gel and transferred to a PVDF membrane (Amersham Biosciences, Piscataway, NJ). The membrane was probed with primary antibodies including ERK1/2 (Novus Biological, Littleton, CO, 1:1000), ribosomal S6 kinase-1 (RSK-1), and ETS like transcription factor-1 (Elk-1; Santa Cruz, Santa Cruz, CA, 1:2000). Alkaline phosphatase-conjugated secondary antibody (1:5000) was subsequently used. The bands were detected by ECF substrate (Amersham Biosciences, Piscataway, NJ) and quantified using ImageQquant software.

Statistics

Data were represented as mean ± SEM and analyzed with Prism 5 software. For all data sets, normality and homocedasticity assumptions were reached, validating the application of the one-way ANOVA, followed by *t*-test with Bonfferroni correction for multiple comparisons. Differences were considered significant for $P < 0.05$.

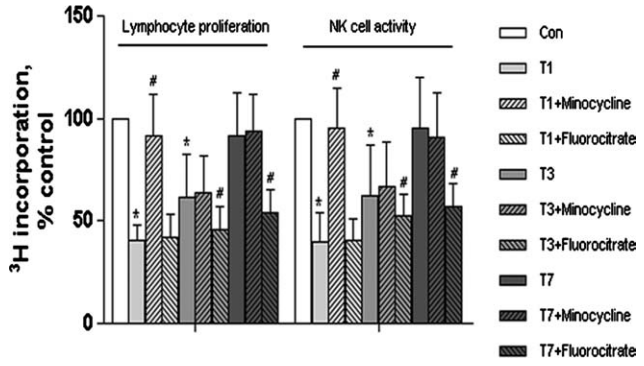

Fig. 1. Sequential involvement of microglia and astrocytes in the neuroimmune-modulation induced by traumatic stress. Rats were divided into 10 subgroups: Control, Trauma 1 day (T1), Trauma 1 day + icv injection of minocyline (50 μg, 24 h), Trauma 1 day + icv injection of glial toxin fluocitrate (5 nM, 24 h), Trauma 3 day (T3), Trauma 3 day + icv injection of minocyline, Trauma 3 day + icv injection of glial toxin fluocitrate, Trauma 7 day (T7), Trauma 7 day + icv injection of minocyline, and Trauma 7 day + icv injection of glial toxin fluocitrate ($n = 6$). Spleen cells were isolated, lymphocyte proliferation (**A**) and NK cell activity (**B**) were determined by [^{3}H] thymidine incorporation. Results are presented as mean ± SD. $^*P < 0.05$ vs. Control, $^\#P < 0.05$ vs. Respective trauma group.

RESULTS

Sequential involvement of microglia and astrocytes in the neuroimmune modulation induced by traumatic stress

Our previous results addressed that neuron and microglia in frontal cortex were implicated in the traumatic stress-mediated neuroimmune modulation (Xiao et al., 2009; Zhao et al., 2002). In this study, we consistently demonstrated that lymphocyte proliferation and NK cell activity were attenuated when challenged with traumatic stress. As illustrated in Figure 1, lymphocyte proliferation (A) and NK cell activity (B) were 40.5% ± 7.45% and 39.8% ± 14.0% control at day 1 of trauma. They gradually increased to 61.3% ± 21.4% and 62.2% ± 25.1% control at day 3, 91.3% ± 21.4% and 95.2% ± 25.1% control at day 7 of trauma, respectively.

Minocycline [50 μg, (Zhang et al., 2006)], a microglial activity inhibitor, and glial toxin fluorocitrate [5 nM, Watkins et al., 1997)], an astrocytes activity inhibitor, were used to examine the involvement of glial cells in the traumatic stress-induced neuroimmune modulation. Figure 1 demonstrated that minocycline could increase lymphocyte proliferation and NK cell activity at 1 day, but had no effect at 3 and 7 days of trauma. In contrast, glial toxin fluorocitrate could not alleviate immunosuppression occurred on day 1 of trauma, but could inhibit immune response recovery at 3 and 7 days of trauma.

Temporal and spatial ERK1/2 activation in neural cells induced by traumatic stress

Src-ERK1/2 signaling in the frontal cortex played an important role in the traumatic stress-induced immunomodulation (Xiao et al., 2009). By immunoprecipitation, we demonstrated that ERK1/2 activation in neuron and microglia was transiently increased at day 1 of trauma. However, the activation in astrocytes was persistent, which could be detected at 1, 3, and 7 days of trauma (Fig. 2A).

IL-1β was envisioned as the crucial mediator in the traumatic stress-induced immunosuppression (Zhao et al., 2002). Therefore, we determined to clarify the discordant induction of ERK1/2 activation by IL-1β in neural cells. As illustrated in Figures 2B–2D, upon IL-1β exposure, pERK1/2 expression in neuron and microglia was increased about threefold within 1 h and returned to normal level at the following time points. In astrocytes, pERK1/2 expression was persistent, which sustained threefold increase from 30 min to 5 h.

Then, we focused on the effect exerted by IL-1β treatment for 1 h. Using Western blot analysis, we found that the alteration of ERK1/2 signaling was mainly concentrated in the cytosol in neuron and microglia. In neuron, the relative density of pERK1/2 expression was increased to 421.3% ± 43.8% control by IL-1β and restored by IL-1ra (98.3% ± 19.5% control). RSK-1 expression in the cytosol was in parallel altered by IL-1β and IL-1ra, and the relative densities were 397.5% ± 41.9%, and 101.2% ± 23.7% control, respectively. In microglia, IL-1β could also increase pERK1/2 and RSK-1 expression in the cytosol, the relative densities were 365.2% ± 41.6% and 347.2% ± 55.1% control, respectively, and the effect could be reversed by IL-1ra (101.1% ± 21.1% and 94.6% ± 21.0% control).

In astrocytes, however, IL-1β-induced ERK1/2 signaling was translocated into nucleus, and pERK1/2 expression in nucleus was increased to 326.2% ± 31.0% control by IL-1β and returned to control level by IL-1ra exposure (97.4% ± 11.7% control). In the meantime, Elk-1 expression in the nucleus was also potentiated by IL-1β (357.8% ± 57.2% control) and restored by IL-1ra (99.5% ± 19.8% control).

Functional correlation of ERK1/2 activation and neuroligin-1 in the traumatic rats

By immunoprecipitation, we demonstrated that pERK1/2 expression in the neuroligin-1 complex was gradually increased by traumatic stress. Time-course examination displayed that they began to colocalize with each other in frontal cortex at day 3 and sustained to 7 days of trauma (Fig. 3A). Overlap of neuroligin-1 and GFAP immunoreactive cells was analyzed by the immunofluorescent double-labeling technique. Under laser confocal scanning photography, neuroligin-1 immunoreactive cells were shown by green immunofluorescence, and GFAP immunoreactive cells were shown by red immunofluuorescence

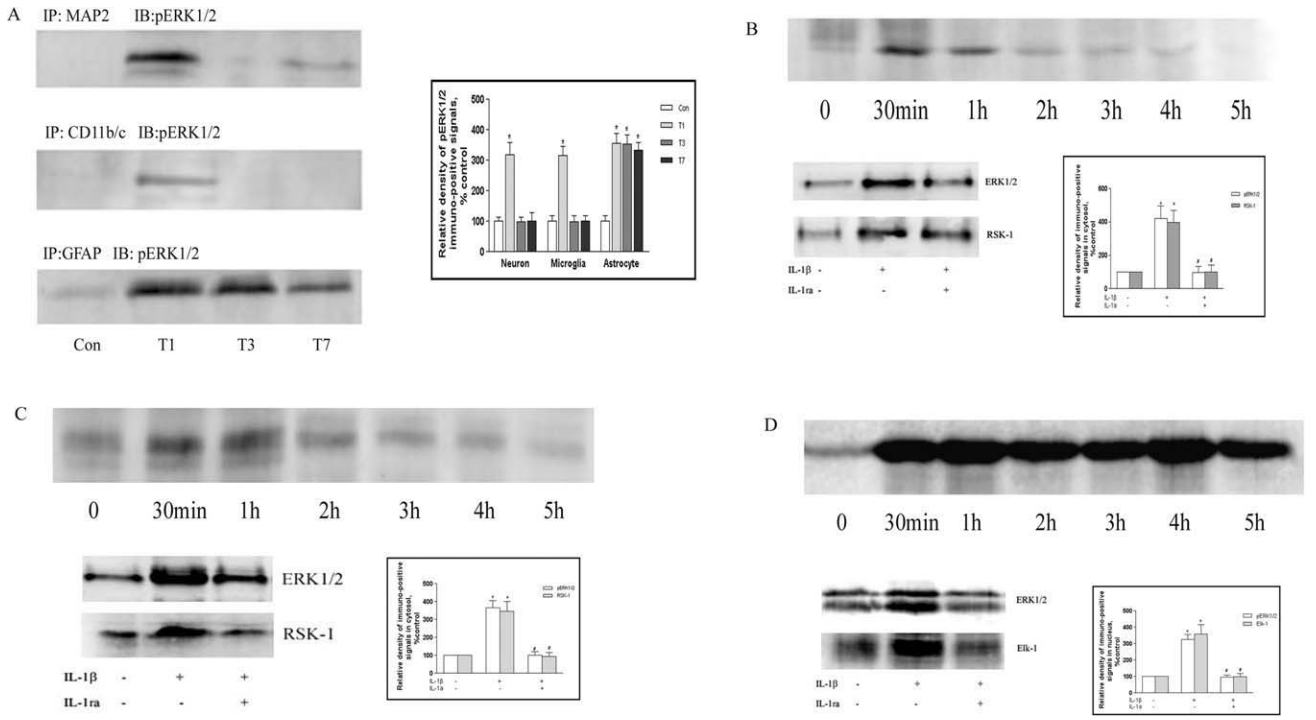

Fig. 2. Temporal and spatial ERK1/2 activation induced by traumatic stress. Rats were divided into four subgroups: Control, Trauma 1 day, Trauma 3 day, and Trauma 7 day ($n = 6$). **A**: Frontal cortex was separated and immunoprecipitation with anti-MAP2, GFAP, CD11b/c, and ERK1/2 activation was analyzed by Western Blot (A). After IL-1β and/or IL-1ra treatment, neuron (**B**), microglia (**C**), and astrocytes (**D**) were collected, and cytoplasmic and nuclear fractions were extracted and resolved in SDS–PAGE gel. The membrane was probed with antibodies including pERK1/2, RSK-1, and Elk-1, and bands were detected by ECF substrate and quantified using ImageQquant software. Data were calculated as percentage of control, and each value represents mean ± SD of three independent experiments.

and colocalized cells by yellow immunofluorescence. At 3 and 7 days of trauma, double-staining cells were significantly increased in frontal cortex (Fig. 3B). This time-dependent alteration was matched with that of pERK1/2 expression.

Effect of neurexin-1β and neuroligin-1 on the traumatic stress induced immunodepression

Neuroimmune modulation of neurexin-1β was finally addressed in this study. It was found that lymphocyte proliferation and NK cell activity were not affected at day 1 of trauma when intracerebraventricular (icv) injection of neurexin-1β antibody. But this treatment could interrupt the immune response improvement at days 3 and 7 of trauma. In parallel, ERK1/2 activation in the neuroligin-1 complex was decreased around threefold at days 3 and 7 of trauma by icv injection of neurexin-1β antibody.

DISCUSSION

We have demonstrated in the previous report that severe stress induced by surgical trauma resulted in the disturbance of central responses followed by decreased splenocyte proliferation and NK cell activity (Zhao et al., 2001) and reduced production of a number of cytokines (Zhao et al., 2002). The intriguing hypothesis is that IL-1β from neuron and activated microglia could closely examine the endangered challenge, and neuron–microglia signaling network might be required for the neuroimmune modulation.

In this study, we found that brain-driven immunoregulatory mechanisms seem to be largely dependent on neural-cell activity. When microglia activity was blocked by minocycline, traumatic stress-mediated immunodepression could not be initiated. In contrast, the immune-response improvement that could not be achieved upon astrocytes activity was inhibited by glial toxin fluorocitrate. The data suggested that, at the early stage of traumatic stress, microglia were activated and triggered the immunosuppression, whereas astrocytes were activated at the later stage, which was mainly responsible for the recovery from the brain insults.

ERKs are known as mitogen-activated protein kinases, mediating intracellular signaling in all eukaryotic cells. ERK1 and ERK2 are the two most-studied members of a family of serine/threonine protein kinases, which are most abundant in brain and are activated when brain faces pathology (Jones and Bergeron, 2004; Rothman et al., 2005). In the traumatic rats, we observed that Fyn-ERK1/2 signaling was involved in the age-dependent immunomodulation (Xiao et al., 2009). IL-1β, the major mediator in the

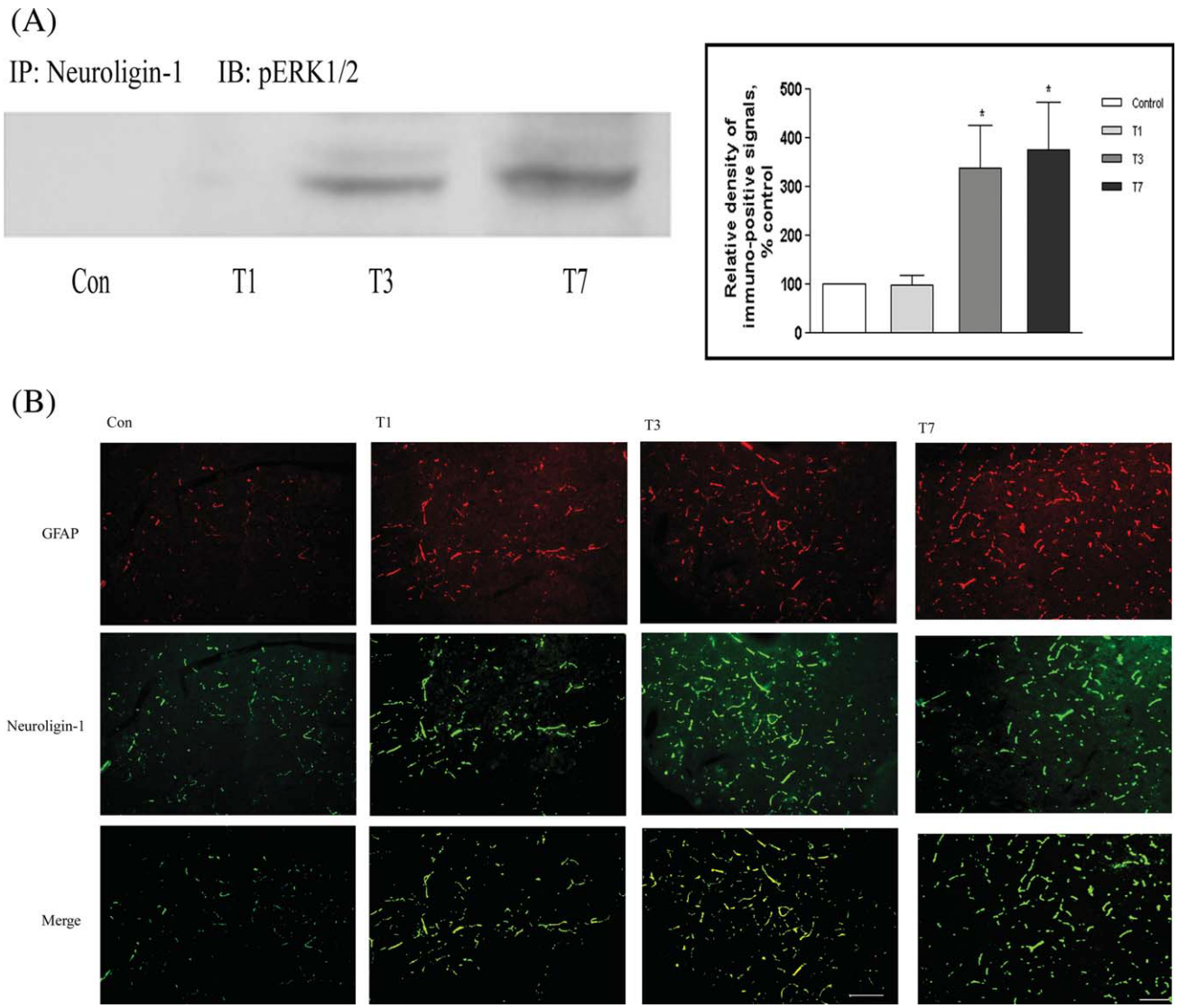

Fig. 3. Functional correlation of ERK1/2 activation and neuroligin-1 in the traumatic rats. **A**: Rats were divided into four subgroups: Control, Trauma 1 day, Trauma 3 day, and Trauma 7 day (n = 6). Frontal cortex was separated and immunoprecipitation with antineuroligin-1 and ERK1/2 activation was analyzed by Western Blot. **B**: Rats were divided into four subgroups: Control, Trauma 1 day, Trauma 3 day, and Trauma 7 day (n = 6). Immunofluorescent double-labeling shows the colocalization of neuroligin-1 (green) and that of GFAP (red) in frontal cortex. Scale bars, 50 μm.

traumatic stress-induced immnuodepression (Zhao et al., 2002), was associated with Fyn signaling in neural cells (data not shown). Herein, we demonstrated that ERK1/2 could be temporal-spatially activated in neuron and glial cells. In neuron and microglia, IL-1β-induced ERK1/2 activation was less than 1 h and accompanied by cytosol RSK-1 phosphorylation. Although, in astrocytes, IL-1β-induced ERK1/2 activation was persisted more than 5 h, which subsequently translocates into the nucleus and leads to Elk-1 activation. Based on reports, ERK1/2 activation could be modulated by Src- and PKCδ-signaling mechanisms, which resulted in ERK1/2 sustaining in cytosol *or* nuclear translocation, respectively (Godeny et al., 2006; Shah et al., 2004). As such, we assumed that distinct ERK1/2-signal pathways were triggered by traumatic stress and transient ERK1/2 activation in neuron, and microglia were contributed to early events of immunosuppression, and sustained activation in astrocyte reflected the recovery of the immune responses.

Thereafter, we identified the functional correlation of ERK1/2 and neuroligin-1 at day 3 of trauma. In the meantime, the enhanced neuroligin-1 expression was concentrated in astrocytes. As well known, neuroligins and neurexins bind to each other in a tight complex with nanomolar affinity (Araç et al., 2007; Chen et al., 2008; Fabrichny et al., 2007). Neurexin-1β existed and functioned predominantly at the presynaptic terminal (Barrow et al., 2009), acting by binding to neuroligin-1,

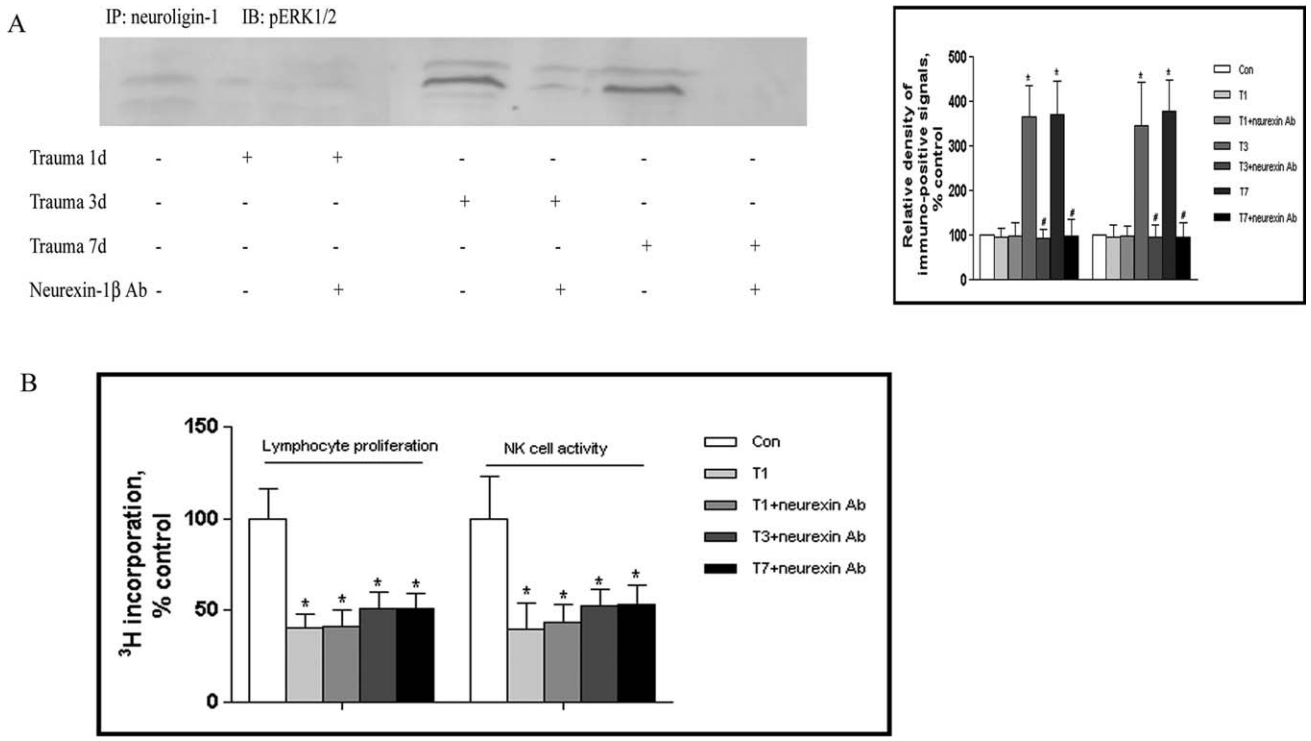

Fig. 4. The effect of neurexin-1β and neuroligin-1 on the traumatic stress induced immunodepression. Rats were divided into seven subgroups: Control, Trauma 1 day, Trauma 1 day + icv injection of neurexin-1β Ab (10 μg, 24 h), Trauma 3 day, Trauma 3 day + icv injection of neurexin-1β Ab, Trauma 7 day, and Trauma 7 day + icv injection of neurexin-1β Ab, (n = 6). **A**: Frontal cortex was separated, and immunoprecipitation with anti-neuroligin-1 and ERK1/2 activation was analyzed by Western Blot. **B**: Spleen cells were isolated, and lymphocyte proliferation and NK cell activity were determined by [^{3}H] thymidine incorporation. Results are presented as mean ± SD. $^*P < 0.05$ vs. Control, $^{\#}P < 0.05$ vs. respective trauma group.

which is exclusively present in excitatory synapses (Boucard et al., 2005; Chih et al., 2006; Comoletti et al., 2006) and is required for synaptic transmission. It is also reported that neuroligin-1 performed central function in the initial induction of synapse formation and functional organization, namely, synapse validation (Chubykin et al., 2007). Then, the present observation pointed that neurons may form synapse onto non-neuronal cells and exchange information with glial cells, by which neural cells develop a conceptual framework for the neuroimmune modulation phenotype.

Finally, we directly demonstrated that neurexin-1β was implicated in the recovery from the traumatic stress-induced immunosuppression, but not in the initiation. In the meantime, the functional overlap of ERK1/2 activation and neuroligin-1 was disrupted by central modulation of neurexin-1β. As reported, glial cells are typically described as the resident macrophage occupying the parenchyma of the CNS (Gehrmann and Banati, 1995; Giulian and Tapscott, 1988), which has long been considered brain intrinsic antigen-presenting cells. Neurons usually have been regarded as victims of activated glial cells. Damaged neurons initiate an early activation of glial cells and direct the neuron-glia communication. By this way, neurons inform glial cells about their status and control glial cells function (Aldskogins et al., 1998; Basu et al., 2002; Hung et al., 2009; Ladeby et al., 2005). Our present observation supported the idea that potential interaction could be established between neuron and glial cells. Their communication temporally and spatially concentrates or recruits related signal proteins and evolutionarily facilitates neuroimmune modulation.

Indeed, the function of neurexin-1β binding in neuroligin-1 regarding the traumatic stress-induced neuroimmune modulation has not yet been examined directly. This issue is important for understanding how synaptic cell adhesion shapes synaptic transmission in brain. We now addressed this issue by functional modulation of neurexin-1β, as a consequence, the association of neuroligin 1 and ERK1/2 activation in astrocytes was interrupted. Therefore, we presumed that neuron-glial signaling is optimally activated and clustered within its respective cargos and facilitating ERK1/2 activation, which might resolve the dichotomy of neural cell activities as well as the traumatic stress-induced immune consequences.

REFERENCES

Aldskogius H, Kozlova EN. 1998. Central neuron-glial and glial-glial interactions following axon injury. Prog Neurobiol 55:1–26.

Araç D, Boucard AA, Ozkan E, Strop P, Newell E, Südhof TC, Brunger AT. 2007. Structures of neuroligin-1 and the neuroligin-1/neurexin-1β complex reveal specific protein-protein and protein-Ca^{2+} interactions. Neuron 56:992–1003.

Barrow SL, Constable JR, Clark E, El-Sabeawy F, McAllister AK, Washbourne P. 2009. Neuroligin1: A cell adhesion molecule that recruits PSD-95 and NMDA receptors by distinct mechanisms during synaptogenesis. Neural Dev 18:4–17.

Basu A, Krady JK, Enterline JR, Levison SW. 2002. Transforming growth factor beta1 prevents IL-1β-induced microglial activation, whereas TNFα- and IL-6-stimulated activation are not antagonized. Glia 40:109–120.

Berninghausen O, Rahman MA, Silva JP, Davletov B, Hopkins C, Ushkaryov YA. 2007. Neurexin Iβ and neuroligin are localized on opposite membranes in mature central synapses. J Neurochem 103:1855–1863.

Blundell J, Blaiss CA, Etherton MR, Espinosa F, Tabuchi K, Walz C, Bolliger MF, Südhof TC, Powell CM. 2010. Neuroligin-1 deletion results in impaired spatial memory and increased repetitive behavior. J Neurosci 30:2115–2129.

Boucard AA, Chubykin AA, Comoletti D, Taylor P, Südhof TC. 2005. A splice code for trans-synaptic cell adhesion mediated by binding of neuroligin 1 to α- and β-neurexins. Neuron 48:229–236.

Buzás P, Eysel UT, Kisvárday ZF. 1998. Functional topography of single cortical cells: an intracellular approach combined with optical imaging. Brain Res Brain Res Protoc 3:199–208.

Cha H, Lee EK, Shapiro P. 2001. Identification of a C-terminal region that regulates mitogen-activated protein kinase kinase-1 cytoplasmic localization and ERK activation. J Biol Chem 276: 48494–48501.

Chen MH, Chuang YJ, Tseng FG. 2008. Self-masked high-aspect-ratio polymer nanopillars. Nanotechnology 19:50–53.

Chih B, Afridi SK, Clark L, Scheiffele P. 2004. Disorder-associated mutations lead to functional inactivation of neuroligins. Hum Mol Genet 13:1471–1477.

Chih B, Gollan L, Scheiffele P. 2006. Alternative splicing controls selective trans-synaptic interactions of the neuroligin-neurexin complex. Neuron 51:171–178.

Choi JS, Kim HY, Cha JH, Lee MY. 2006. Ischemic preconditioning-induced activation of ERK1/2 in the rat hippocampus. Neurosci Lett 409:187–191.

Chubykin AA, Liu X, Comoletti D, Tsigelny I, Taylor P, Südhof TC. 2005. Dissection of synapse induction by neuroligins: effect of a neuroligin mutation associated with autism. J Biol Chem 280: 22365–22374.

Chubykin AA, Atasoy D, Etherton MR, Brose N, Kavalali ET, Gibson JR, Südhof TC. 2007. Activity-dependent validation of excitatory versus inhibitory synapses by neuroligin-1 versus neuroligin-2. Neuron 54:919–931.

Comoletti D, Flynn RE, Boucard AA, Demeler B, Schirf V, Shi J, Jennings LL, Newlin HR, Südhof TC, Taylor P. 2006. Gene selection, alternative splicing, and post-translational processing regulate neuroligin selectivity for β-neurexins. Biochemistry 45:12816–12827.

de Oliveira GP, Maximino JR, Lin CJ, Chadi G. 2009. A method to immunolabel rodent spinal cord neurons and glia for molecular study in specific laser microdissected cells involved in neurodegenerative disorders. J Mol Histol 40:217–225.

Dean C, Scholl FG, Choih J, DeMaria S, Berger J, Isacoff E, Scheiffele P. 2003. Neurexin mediates the assembly of presynaptic terminals. Nat Neurosci 6:708–716.

Escande-Beillard N, Washburn L, Zekzer D, Wu ZP, Eitan S, Ivkovic S, Lu Y, Dang H, Middleton B, Bilousova TV, Yoshimura Y, Evans CJ, Joyce S, Tian J, Kaufman DL. 2010. Neurons preferentially respond to self-MHC class I allele products regardless of peptide presented. J Immunol 184:816–823.

Fabrichny IP, Leone P, Sulzenbacher G, Comoletti D, Miller MT, Taylor P, Bourne Y, Marchot P. 2007. Structural analysis of the synaptic protein neuroligin and its β-neurexin complex: Determinants for folding and cell adhesion. Neuron 56:979–991.

Feng J, Zhou Y, Campbell SL, Le T, Li E, Sweatt JD, Silva AJ, Fan G. 2010. Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. Nat Neurosci 13:423–430.

Freria CM, Zanon RG, Santos LM, Oliveira AL. 2010. Major histocompatability complex class I expression and glial reaction influence spinal motoneuron synaptic plasticity during the course of experimental autoimmune encephalomyelitis. J Comp Neurol 518: 990–1007.

Gehrmann J, Banati RB. 1995. Microglial turnover in the injured CNS: Activated microglia undergo delayed DNA fragmentation following peripheral nerve injury. J Neuropathol Exp Neurol 54:680–688.

Giulian D, Tapscott MJ. 1988. Immunoregulation of cells within the central nervous system. Brain Behav Immun 2:352–358.

Godeny MD, Sayeski PP. 2006. ERK1/2 regulates ANG II-dependent cell proliferation via cytoplasmic activation of RSK2 and nuclear activation of elk1. Am J Physiol Cell Physiol 291:C1308–C1317.

Hicks SD, Parmele KT, DeFranco DB, Klann E, Callaway CW. 2000. Hypothermia differentially increases extracellular signal-regulated kinase and stress-activated protein kinase/c-Jun terminal kinase activation in the hippocampus during reperfusion after asphyxial cardiac arrest. Neuroscience 98:677–685.

Hung J, Chansard M, Ousman SS, Nguyen MD, Colicos MA. 2009. Activation of microglia by neuronal activity: Results from a new in vitro paradigm based on neuronal-silicon interfacing technology. Brain Behav Immun 24:31–40.

Jones NM, Bergeron M. 2004. Hypoxia-induced ischemic tolerance in neonatal rat brain involves enhanced ERK1/2 signaling. J Neurochem 89:157–167.

Jung SY, Kim J, Kwon OB, Jung JH, An K, Jeong AY, Lee CJ, Choi YB, Bailey CH, Kandel ER, Kim JH. 2010. Input-specific synaptic plasticity in the amygdala is regulated by neuroligin-1 via postsynaptic NMDA receptors. Proc Natl Acad Sci USA 107:4710–4715.

Kaneko T, Kaneko M, Chokechanachaisakul U, Kawamura J, Kaneko R, Sunakawa M, Okiji T, Suda H. 2010. Artificial dental pulp exposure injury up-regulates antigen-presenting cell-related molecules in rat central nervous system. J Endod 36:459–464.

Ladeby R, Wirenfeldt M, Garcia-Ovejero D, Fenger C, Dissing-Olesen L, Dalmau I, Finsen B. 2005. Microglial cell population dynamics in the injured adult central nervous system. Brain Res Brain Res Rev 48:196–206.

Liu Y, Bando Y, Vargas-Lowy D, Elyaman W, Khoury SJ, Huang T, Reif K, Chitnis T. 2010. CD200R1 agonist attenuates mechanisms of chronic disease in a murine model of multiple sclerosis. J Neurosci 30:2025–2038.

Pearson G, Robinson F, Gibson TB, Xu BE, Karandikar M, Berman K, Cobb MH. 2001. Mitogen-activated protein (MAP) kinase pathways: Regulation and physiological functions. Endocr Rev 22:153–183.

Ribic A, Zhang M, Schlumbohm C, Mätz-Rensing K, Uchanska-Ziegler B, Flügge G, Zhang W, Walter L, Fuchs E. 2010. Neuronal MHC class I molecules are involved in excitatory synaptic transmission at the hippocampal mossy fiber synapses of marmoset-monkeys. Cell Mol Neurobiol 30:827–839.

Rothman DM, Shults MD, Imperiali B. 2005. Chemical approaches for investigating phosphorylation in signal transduction networks. Trends Cell Biol 15:502–510.

Shah BH, Yesilkaya A, Olivares-Reyes JA, Chen HD, Hunyady L, Catt KJ. 2004. Differential pathways of angiotensin II-induced extracellularly regulated kinase 1/2 phosphorylation in specific cell types: Role of heparin-binding epidermal growth factor. Mol Endocrinol 18:2035–2048.

Shono Y, Kamouchi M, Kitazono T, Kuroda J, Nakamura K, Hagiwara N, Ooboshi H, Ibayashi S, Iida M. 2010. Change in intracellular pH causes the toxic Ca^{2+} entry via NCX1 in neuron- and glia-derived cells. Cell Mol Neurobiol 30:453–460.

Streit WJ, Walter SA, Pennell NA. 1999. Reactive microgliosis. Prog Neurobiol 57:563–581.

Varoqueaux F, Aramuni G, Rawson RL, Mohrmann R, Missler M, Gottmann K, Zhang W, Südhof TC, Brose N. 2006. Neuroligins determine synapse maturation and function. Neuron 51:741–754.

Watkins, Martin D, Ulrich P, Tracey KJ, Maier SF. 1997. Evidence for the involvement of spinal cord glia in subcutaneous formalin induced hyperalgesia in the rat. Pain 71:225–235.

Xiao S, Wang J, Jiang JW, Cao XD, Wu GC, Zhao H. 2009. Characterization of Fyn signaling on the age-dependent immuno-modulation on traumatic rats. Brain Res 1255:162–169.

Zhao H, Du LN, Jiang JW, Wu GC, Cao XD. 2001. Modulatory effect of orphanin FQ on the IL-1β transcripts in hippocampus. Sheng Li Xue Bao 53:209–214.

Zhao H, Huang HW, Wu GC, Cao XD. 2002. Effect of orphanin FQ on interleukin-1β mRNA transcripts in the rat CNS. Neuroscience 114:1019–1031.