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Neuroprotective Immunity: T Cell-Derived Glutamate Endows Astrocytes with a Neuroprotective Phenotype

Sanjay K. Garg,1*† Ruma Banerjee,2*† and Jonathan Kipnis2*‡

A well-controlled T cell response to CNS injury may result in increased neuronal survival. However, the precise mechanism of T cell-induced neuroprotection is unknown. In this study, we report the unexpected finding that during culture of T cells, high levels of glutamate accumulate, which are efficiently cleared if T cells are cocultured with astrocytes. The T cell-derived glutamate elicits in turn, the release of neuroprotective thiols (cysteine, glutathione, and cysteinyl-glycine) and lactate from astrocytes. Media obtained from astrocytes conditioned in the presence of T cells reduce neuronal apoptosis induced by oxidative stress in primary neuronal cultures from 48 ± 14 to 9 ± 4% (p < 0.001). Inhibition of glutamate-dependent signaling during astrocyte-T cell cocultivation by a glutamate uptake inhibitor, t-aspartic acid β-hydroxamate, abolishes this neuroprotective effect. The ability of astrocytes to clear extracellular glutamate is impaired under conditions of oxidative stress. We demonstrate that T cells, via secreted cytokines, restore glutamate clearance capacity of astrocytes under oxidative conditions. Furthermore, under normoxic conditions, glutamate-buffering capacity of astrocytes is increased upon cocultivation with T cells. It is known that, following CNS injury, astrocytes can respond with beneficial or destructive effects on neurons. However, the context and signaling mechanisms for this dual astrocytic response are unknown. Our results implicate T cells as potential determinants of the context that elicits a protective role for astrocytes in the damaged CNS. The Journal of Immunology, 2008, 180: 3866–3873.

The outcome of a CNS insult depends not only on the magnitude of the primary injury but also on the extent of secondary degeneration (1). The complexity of cell types activated in response to CNS injury poses challenges to dissecting their individual contributions to the process. Following mechanical injury, the blood-brain barrier is breached and this allows infiltration of immune cells into the CNS (2, 3). Historically, immune inflammation has been considered to be detrimental to neural tissue. However, recent studies have revealed a critical role for T cells in neuroprotection (4–6). This notion was based on the observation that in rodents, passive transfer of encephalitogenic (disease-inducing) T cells reactive to myelin basic protein increases postinjury neuronal survival relative to those of controls (6, 7). It was shown, moreover, that the beneficial effect of these T cells is not merely the result of experimental manipulation, but is a physiological response to a CNS insult, because animals devoid of endogenous T cells showed a worse outcome of CNS injury than their wild-type counterparts (8, 9). The mechanism underlying T cell-induced neuroprotection after CNS injury has not been completely elucidated. As neurons do not express MHC class II molecules, they are unable to engage in direct interactions with T cells. The major potential partners for the neuroimmune dialogue in the CNS are microglia. Indeed, several lines of evidence suggest that T cells interact with microglia to switch their phenotype from neurotoxic to neuroprotective (10). Although microglia heavily populate the site of injury and express MHC class II molecules abundantly, their ability to affect neuronal functioning is limited compared with that of astrocytes. Astrocytes also express MHC class II molecules and while their ability for Ag presentation is debatable, they can interact with T cells (11).

Astrocytes have the capacity to be both beneficial and destructive at sites of CNS injury (12–14) but the context that elicits these opposing astrogial phenotypes is not understood. Reactive astrogliosis occurs in response to virtually all forms of acute and chronic conditions in the CNS, although their precise function and contribution to either degeneration or protection are not completely understood (12, 15). Under normal physiological conditions, astrocytes regulate extracellular osmolarity, ionic composition, and pH, clear neurotransmitters from synaptic clefts, and provide growth factors and nutrients for neurons (16–19). Moreover, astrocytes also play a role in regulation of neuronal functions by affecting the strength and number of synapses (20, 21). In addition, astrocytes play an important role in antioxidant defense. They contain high concentrations of glutathione, a major intracellular antioxidant, and provide neurons with cysteine, a precursor for glutathione synthesis (22). Under pathological conditions, e.g., ischemia, the enhanced production of reactive oxygen species compromises the intrinsic antioxidant capacity of astrocytes and neurons and leads to oxidative stress and cell death. Several neurodegenerative disorders are also characterized by major perturbations in the glutathione system (23–26). Reactive astrocytes represent a good source of glutathione and the mitochondrial glutathione pool appears to be of major importance (27, 28). The pathological release of glutamate from damaged neurons is one of the major contributors of secondary degeneration following CNS injury. Astrocytes have a large capacity for clearing the excitatory

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neurotransmitter, glutamate, and play a critical role in maintaining very low concentrations (<2 μM) of this amino acid in the extracellular space (29, 30). However, oxidative stress compromises the glutamate-clearing capacity of astrocytes leading to accumulation of glutamate, which, at elevated concentrations, is neurotoxic (31, 32).

In this study, we tested the hypothesis that interactions between T cells and astrocytes serve to modulate the astroglial response to CNS damage. We demonstrate that T cells, by secreting glutamate and enhancing the glutamate clearance capacity of astrocytes, induce the latter to release the neuroprotectants, lactate and cysteine, which buffer neurons against excitotoxicity.

Materials and Methods

Isolation and preparation of murine primary cells

Astrocytes. Primary murine cortical astrocyte cultures were prepared as described previously (33). In brief, cortices were harvested from 1-day-old mice under deep isoflurane anesthesia. The cortices were dissociated in papain/DNase, plated in a T-75-cm² tissue-culture flask in DMEM/F12 medium containing 10% FBS and 2 mM l-glutamine, penicillin-streptomycin (10,000 U/ml; Invitrogen Life Technologies), and maintained in a 37°C incubator with an atmosphere of 5% CO₂. Every third day, half of the medium was replaced with fresh medium and cells were split once a week. At the end of the third passage, cells were seeded in a 24-well plate at a density of 5 × 10⁵/well in 1 ml of medium and incubated for a week (every third day half of the medium was changed) before being used for experiments.

Neurons. Dissociated neurons were prepared from embryonic day 16 (E16) to E18 mice and cultured in Neurobasal medium, B27 supplement, 2 mM l-glutamine, and penicillin-streptomycin (10,000 U/ml; Invitrogen Life Technologies) on poly-t-lysine (Sigma-Aldrich) coated glass coverslips (12 mm; Belco Glass) in 24-well plates as described previously (34). Cells were cultured at a density of 250,000 cells/well for 11 days in a humidified incubator with 5% CO₂ at 37°C before TUNEL staining.

T lymphocytes. Lymph nodes (axillary, inguinal, superficial cervical, mandibular, and mesenteric) and spleens were harvested and mashed. T lymphocytes (at a 1:1 ratio) or astrocytes plus 500 μM glutamate (t-BuOOH; Sigma-Aldrich), which is an organic peroxide, for 9–12 h. To examine whether T cells are able to endow astrocytes with a neuroprotective phenotype under conditions of oxidative stress, we preincubated astrocyte cultures with 200 μM t-BuOOH for 3 h and then stimulated them with either T cells or with 500 μM glutamate for the following 7 h. Conditioned medium from these cell cultures were transferred onto neuronal monolayers in the presence of 200 μM tert-butyl hydroperoxide (t-BuOOH; Sigma-Aldrich). Apoptosis of the neurons was determined using TUNEL staining (In Situ Cell Death Detection kit; Roche Diagnostics) as per the manufacturer’s protocol. OD was measured in a 96-well plate reader at 570 nm. Lactate concentrations in the samples were determined using a calibration curve generated for standards of known concentration. The background value for medium (blank) was subtracted for the final measurements.

Lactate assay

Extracellular lactate concentration in the culture supernatants was measured using the Lactate Assay kit (BioVision) as per the manufacturer’s protocol. OD was measured in a 96-well plate reader at 570 nm. Lactate concentrations in the samples were determined using a calibration curve generated for standards of known concentration. The background value for medium (blank) was subtracted for the final measurements.

Statistics

The significance of differences in data between control and experimental groups was determined by two-way ANOVA followed by posttest Bonferroni adjustment. A value of p < 0.05 was considered to be statistically significant.

Results

Accumulation of glutamate in T cell cultures

While conducting limited metabolite profile analysis of extracellular medium, we found unexpectedly that T cell cultures exhibit substantial accumulation of glutamate (463 ± 136 μM after 24 h) (Fig. 1). Glutamate accumulation was analyzed by HPLC and enzymatic methods and its identity was confirmed by mass spectrometric analysis of the chromatographically purified peak (data not shown). When cocultivated with astrocytes, extracellular glutamate accumulation was greatly diminished and in fact could not be detected at 24 h.
Cultivation of T cells with astrocytes leads to extracellular thiol accumulation that is linked to T cell-derived glutamate

Under normoxic conditions, astrocytes support neuronal function by clearing extracellular glutamate efficiently and providing cysteine needed for biosynthesis of glutathione (30, 37). Indeed, a time-dependent accumulation of extracellular cysteine was observed, which was enhanced under cocultivation conditions (7.3 ± 3.3 μM for control vs 21 ± 1.9 μM at the 6-h time point for T cell-activated astrocytes, p < 0.001; Fig. 2a). Because astrocyte-conditioned medium at the 6-h time point was used for subsequent neuroprotection experiments, the quantitative data in the text refer to this time point, unless stated otherwise. To determine whether glutamate itself can induce cysteine accumulation from astrocytes, the latter were cultivated in the presence of 500 μM glutamate. A similar increase in extracellular cysteine was observed (7.3 ± 3.3 μM for control vs 16.2 ± 2.5 μM for glutamate-activated astrocytes, p < 0.001; Fig. 2a).

In principle, the observed increase in cysteine could result from stimulation of extracellular cystine uptake and/or activation of the transsulfuration flux, which provides a mechanism for increasing intracellular cysteine synthesis from methionine. Although a trend in greater cystine depletion was seen in the presence of T cells or exogenous glutamate, the difference was not statistically significant except at 24 h, when the enhanced consumption of extracellular cystine by activated astrocytes was observed (66 ± 10, 45 ± 12, and 51 ± 6 μM for control, T cell-activated astrocytes and glutamate-activated astrocytes, respectively, p < 0.05, at 24 h; Fig. 2b).

A change was seen in the expression of enzymes involved in the transsulfuration pathway, cystathionine β-synthase and γ-cystathionase, (data not shown), although this does not rule out the possibility of increased flux via activation of one or both enzymes. Extracellular cysteine, which is relatively abundant, is not efficiently imported by neurons (38). Astrogial glutathione export and its subsequent cleavage by γ-glutamyl transpeptidase to Cys-Gly by membrane-bound γ-glutamyl transpeptidase to be taken up by neurons where it is used to synthesize glutathione.
cell-activated astrocytes and glutamate-activated astrocytes, respectively; $p < 0.05$, Fig. 2c) and its cleavage product, Cys-Gly (3.2 ± 0.7, 9.4 ± 1.0, and 12.5 ± 1.9 µM for control, T cell-activated astrocytes, and glutamate-activated astrocytes, respectively, $p < 0.01$; Fig. 2d) was observed when astrocytes were cocultured with either T cells or glutamate.

Glutamate and cystine trafficking by astrocytes are linked via their transport systems (Fig. 3). Glutamate is imported via $X_{\text{AG}}^-$, a $\text{Na}^+\text{K}^+$-dependent transporter. Enhanced uptake of T cell-derived glutamate by astrocytes is predicted to activate the $\text{Na}^+\text{K}^+$-ATPase and the antiporter, $x_{\text{AG}}^{-}$, which uses the glutamate gradient to import cysteine (39, 40). Inhibition of the $X_{\text{AG}}^-$ transporter when astrocytes and T cells are cocultured impairs both cysteine accumulation (8.1 ± 2.1, 15.3 ± 1.9, and 10.9 ± 1.6 µM for control, T cell-activated astrocytes and AβH plus T cell-activated astrocytes, respectively; Fig. 4a) and glutamate clearance (Fig. 4b).

**FIGURE 4.** The inhibitory effect of AβH on cysteine release (a) and glutamate clearance (b). Astrocytes were incubated with T cells in the presence or absence of 400 µM AβH for the indicated times and the concentration of extracellular cysteine and glutamate were measured. Data are the mean ± SD and are representative of two independent experiments performed in duplicates on different batches of cells. Statistical analysis using repeated two-way ANOVA revealed significant inhibition in cysteine release and glutamate clearance by astrocytes as a function of time and treatment with AβH. Asterisks on the graph indicate the results of paired comparisons (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant).

**FIGURE 5.** Analysis of extracellular lactate during interaction of astrocytes with T cells. Culture medium from murine astrocytes incubated alone (□), or with T cells (▪), or glutamate 500 µM (□) were collected at the indicated time points and the concentration of lactate (a) was analyzed. b, Extracellular lactate accumulation after astrocytes were incubated with T cells in the presence or absence of 400 µM AβH for 6 h. Data are the mean ± SD and are representative of six (a) or two (b) independent experiments performed in duplicates on different batches of cells. Statistical analysis using repeated two-way ANOVA revealed significant changes in lactate production by astrocytes as a function of time and treatment with either T cells or glutamate or AβH; a ($F = 67.54$; DF = 2; DFd = 18; $p = 0.0001$). Bonferroni posttests were used to compare individual points. One-way ANOVA followed by Bonferroni posttests were performed for analysis of the data in b. Asterisks on the graph indicate the results of paired comparisons (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ns, not significant).

T cell-derived glutamate activates anaerobic metabolism in astrocytes and release of lactate

Activation of the $\text{Na}^+/\text{K}^+$ ATPase depletes astrocytic ATP reserves triggering a shift in energy metabolism toward anaerobic glycolysis (39, 40). Under these conditions, pyruvate is converted to lactate, which serves to quickly regenerate NAD$^+$. Lactate is subsequently released by astrocytes to be taken up by neurons where it is reconverted to pyruvate and serves as a fuel molecule (Fig. 3). To evaluate whether these metabolic changes are induced in astrocytes in response to T cell-derived glutamate, extracellular lactate levels were compared when astrocytes were cultivated in the absence or presence of T cells or 500 µM glutamate (Fig. 5a).

Extracellular lactate concentrations increased significantly when either T cells or 500 µM glutamate were added to astrocytes (1.5 ± 0.1, 4.0 ± 0.9, and 3.9 ± 0.1 mM in control, T cell-activated astrocytes and glutamate-activated astrocytes, respectively, $p < 0.01$; Fig. 5a) and were diminished in the presence of a glutamate uptake inhibitor (3.9 ± 0.4 mM vs 2.5 ± 0.3 mM in the absence or presence of AβH, respectively, $p < 0.01$; Fig. 5b).

**T cells improve glutamate clearance capacity of astrocytes under normoxic and toxic conditions**

T cells were previously shown to improve microglial glutamate uptake (10). We examined whether T cells can similarly enhance astrocytic capacity for glutamate removal. To test this, astrocytes were cultured with 500 µM glutamate in the presence and absence of T cells. Under normoxic conditions, activated T cells modestly enhance (by ~20%) the basal glutamate clearance rate by astrocytes (71 µM × h$^{-1}$; Fig. 6a). However, under oxidative stress conditions induced by 800 µM t-BuOOH, T cells confer a markedly protective effect on astrocytic glutamate uptake. Thus, in contrast to almost complete abrogation of glutamate clearance by oxidatively stressed astrocytes, the presence of T cells restores glutamate clearance to a rate of ~14 µM × h$^{-1}$ (Fig. 6b). This protection is simulated by IFN-$\gamma$ and IL-2 alone but not by IL-4 (Fig. 6c), suggesting the involvement of a Th1 type of response. Potential differences in the magnitude of glutamate release by different subpopulations of T cells (Th1 vs Th2) remains to be established.
T cell endow astrocytes with a neuroprotective phenotype

Based on our model (Fig. 3), we predicted that medium conditioned by cocultivation of astrocytes with T cells should be neuroprotective. To test this, mouse cortical primary neurons were treated with 200 \( \mu \text{M} \) t-BuOOH and protection from apoptosis was assessed by addition of conditioned cell culture medium. Astrocyte-conditioned medium did not significantly affect the extent of neuronal apoptosis compared with controls that received fresh medium (57 ± 18 vs 48 ± 14%, \( p > 0.05 \); Fig. 7a). However, when oxidatively stressed neurons were cultured in medium conditioned by cocultivation of astrocytes and T cells, a >5-fold decrease in apoptotic (TUNEL positive) cells was observed in comparison to astrocyte-conditioned medium alone (48 ± 14 vs 9 ± 4%, \( p < 0.001 \); Fig. 7a). In addition, when 500 \( \mu \text{M} \) glutamate replaced T cells during astrocyte culture, the resulting astrocyte-conditioned medium also afforded a similar degree of neuronal protection (Fig. 7a). The glutamate uptake inhibitor, AβH, added during coculture of astrocytes and T cells completely abolished the neuroprotective

FIGURE 6. Comparison of the glutamate-clearing ability of untreated (a), and t-BuOOH-treated (b) astrocytes in the presence or absence of T cells or cytokines. Astrocytes were incubated with 500 \( \mu \text{M} \) glutamate ± T cells (a), or 500 \( \mu \text{M} \) glutamate + 800 \( \mu \text{M} \) t-BuOOH ± T cells (b), or 500 \( \mu \text{M} \) glutamate + 800 \( \mu \text{M} \) t-BuOOH ± IL-2 (25 ng/ml) or IL-4 (25 ng/ml) or IFN-\( \gamma \) (25 ng/ml) (c). At the indicated time points, culture medium was collected and the concentration of extracellular glutamate was measured. Results show the mean ± SD of samples from duplicate cultures. ANOVA revealed significant changes in glutamate clearance by astrocytes as a function of time and incubation with T cells or IL-2 and IFN-\( \gamma \) alone but not IL-4; \( a (F = 393.93; DFn = 1; DFd = 16; p = 0.0001), b (F = 9798.84; DFn = 1; DFd = 40; p = 0.0001), c (F = 9798.84; DFn = 4; DFd = 40; \( p = 0.0001 \)). Bonferroni posttests were used to compare individual points. Asterisks on the graph indicate the results of paired comparisons (*, \( p < 0.05 \); **, \( p < 0.01 \); *** , \( p < 0.001 \); ns, not significant).

FIGURE 7. The neuroprotective effect of T cell- or glutamate-primed astrocytes. a, Primary mouse cortical neurons were treated with 200 \( \mu \text{M} \) t-BuOOH for 9–12 h in the presence of either fresh medium, or conditioned medium from astrocytes or conditioned medium from T cell- or 500 \( \mu \text{M} \) glutamate-stimulated astrocytes. Bar graphs represent the mean ± SEM of apoptotic neurons measured by TUNEL labeling as a percentage of total cells (labeled by Hoechst). The data were analyzed by counting at least 4000 cells for each treatment and three independent experiments were performed. b, Statistical analysis using one-way ANOVA followed by Bonferroni posttests was performed and individual \( p \) value pairs are presented in the table (*, \( p < 0.05 \); **, \( p < 0.01 \); *** , \( p < 0.001 \); ns, not significant). c, Representative micrographs of apoptotic neurons are presented.

FIGURE 8. The neuroprotective effect of T cells or glutamate-primed astrocytes on neuronal cell line. a, Transformed neuronal cell cultures, MES 21.2, were intoxicated with 200 \( \mu \text{M} \) t-BuOOH for 9 h in the presence of either fresh medium, or conditioned medium from astrocytes or conditioned medium from T cell- or 500 \( \mu \text{M} \) glutamate-stimulated astrocytes. Bar graphs represent mean ± SEM of apoptotic neurons measured by TUNEL labeling as a percentage of total cells (labeled by Hoechst). This analysis was performed by counting at least 2000 cells for each treatment and two independent experiments were performed. b, Statistical analysis using one-way ANOVA followed by Bonferroni posttests was performed and individual \( p \) values pairs are presented in the table (*, \( p < 0.05 \); **, \( p < 0.01 \); *** , \( p < 0.001 \); ns, not significant).
FIGURE 9. Phenotypic modulation of oxidatively stressed astrocytes by T cells or glutamate priming. Astrocytes were pretreated with either DMSO (vehicle) or with 200 μM t-BuOOH for 3 h and then incubated with either T cells (1:1), or glutamate (500 μM) or with no additions for another 7 h. Primary mouse cortical neurons (a) and MES 21.2 cell lines (b) were treated with 200 μM t-BuOOH for 9–12 h in the presence of either fresh medium (control), or astrocyte-conditioned medium (ACM) or conditioned medium from t-BuOOH-treated astrocytes or conditioned medium from T cell- or 500 μM glutamate-stimulated and t-BuOOH-treated astrocytes (+T cells or +Glu, respectively). Bar graphs represent the mean ± SEM of apoptotic neurons measured by TUNEL labeling as a percentage of total cells (labeled by Hoechst). The data were analyzed by counting at least 5000 cells in (a) and 2500 cells in (b) for each treatment and two independent experiments each in duplicate were performed. Statistical analysis using the Student t test (two-tailed pair) was performed (*, p < 0.05; **, p < 0.01; ***, p < 0.001; ns, not significant).

Discussion

The spread of damage after CNS injury is mediated by numerous factors, among which are NO, deprivation of growth factors, impaired blood supply and a general metabolic deficit. Another common characteristic of injured CNS is the increased presence of infiltrating immune cells that have been suggested to contribute to the pathology and the spread of damage (2). However, some studies have shown that in the event of an acute injury or chronic neurodegenerative conditions, T cells are recruited by and accumulate in the CNS (41, 42), where they rescue neurons from degeneration. Moreover, a well-controlled boost of autoimmune activity following injury increases the number of surviving neurons (43). Alternative mechanisms that may underlie T cell-mediated neuroprotection have been proposed, such as production of neutrotrophic factors by T cells (44) and induction of glutamate clearance by microglia (10). However, none of these mechanisms fully address the robust neuroprotective effect induced by T cells.

In this study, we report the unexpected finding that T cells are a source of high extracellular glutamate that is taken up by astrocytes and elicits the secretion of neuroprotective molecules. In addition to induction of neuroprotective molecules, T cells are also capable of protecting the glutamate clearance capacity of astrocytes impaired by oxidative stress. To our knowledge, this is the first demonstration that high levels of glutamate generated by T cells signal metabolic changes in astrocytes that lead to a neuroprotective phenotype. These results suggest that interaction between T cells and astrocytes plays a role in the molecular mechanism underlying T cell-mediated neuroprotection in CNS injury.

Although our results appear to contradict an earlier study, which reported that autoreactive T cells down-regulate glutamate transporters on astrocytes (45), it should be noted that these results were obtained using preactivated Ag (myelin basic protein)-specific autoreactive T cells at a higher concentration (3:1, T cells:astrocyte ratio) than used in our study. Our preliminary data also indicate that a higher T cell:astrocyte ratio as well as higher levels of glutamate abrogate the neuroprotective phenotype of astrocytes and exacerbate neuronal death. Therefore, the results of this study further emphasize the importance of tight regulation of the magnitude of the T cell response to CNS injury.

We demonstrate that enhanced uptake of T cell-derived glutamate by astrocytes is correlated with an increase in the extracellular Cys-Gly concentration, and appears to be associated with a decrease in the extracellular cysteine pool (Fig. 3). Several studies have shown that uptake of glutamate by astrocytes is associated with a decrease in extracellular cystine and an increase in glutathione synthesis by astrocytes (46–50).
Our observation that glutamate production by T cells can signal a protective response, seems paradoxical because high extracellular glutamate is known to be neurotoxic (51). However, during transendothelial migration, T cells first encounter astrocytes as they penetrate the blood-brain barrier formed in part by astrocytic end-feet. We propose that a spatial segregation of T cell-astrocyte interaction may be important for this glutamate-based neuroprotective response. Microglia also express glutamate transporters (10, 52–55), whose levels are further increased in activated microglia following nerve injury (56). Microglia have been suggested to potentially function as the “middenmen” in T cell-mediated neuroprotection (10, 57), due to their abundance at the epicenter of injury sites. In contrast, modulation of the neuroprotective role of astrocytes by T cells is not well-studied. Our model for glutamate-triggered neuroprotection by astrocytes (Fig. 3) could also be relevant to the role of microglia at sites of injury and merits testing.

Under oxidative stress conditions as occur during nerve injury, glutamate uptake by astrocytes is impaired (58–60) and astrocytes can even exacerbate the injury by releasing more glutamate (61, 62). Thus, T cell-enhanced thiol release by astrocytes could serve dual functions: first, by making cysteine, the limiting reagent in glutathione biosynthesis, more readily available to neurons, it increases the antioxidant capacity of neurons (63) and second, by remodeling the extracellular redox environment to be more reducing, counters reactive oxygen species. Remarkably, glutamate alone is able to recapitulate the effect of T cells on thiol release by astrocytes. These results suggest that glutamate originating from T cells can potentially signal induction of a neuroprotective response in astrocytes.

Because augmentation of glutamate clearance capacity by oxidatively stressed astrocytes is mimicked by soluble cytokines, it suggests that T cells need to be reactivated at injury sites following Ag recognition. Hence, the potential ability of T cells to modulate a neuroprotective response via glutamate is consistent with previously published observations suggesting that recognition between the neuroprotective T cells and Ag is required before initiation of the neuroprotective immune response (64).

This study offers a plausible explanation for the apparently contradictory observations on the role of T cells in neuronal survival under acute and chronic neurodegenerative conditions (65, 66). The number of astrocytes at the site of injury, and their ability to express major histocompatibility molecules that can interact with infiltrating T cells, will determine the outcome of the T cell-mediated inflammatory response. A high number of T cells can be neurotoxic due to the high levels of secreted glutamate. If T cells cannot adequately support damaged astrocytes, then, glutamate secreted by T cells could lead to direct neuronal toxicity. Alternatively, if the number of T cells is low (due to the magnitude of the endogenous response or due to immunodeficiency), then the glutamate signal may be insufficient to induce astrocytic activation. When the T cell numbers are boosted above the spontaneous level (by passive or active vaccination) but do not exceed the threshold tolerated by astrocytes, a neuroprotective response is predicted.

These results suggest a mechanism for T cell-dependent neuroprotection that needs to be tested further in vivo and, if confirmed, could have the potential to lead to the development of novel therapeautic approaches for acute and chronic CNS conditions. Circumvention of the need for T cell- or glutamate-dependent activation of astrocytes would reduce the risk for potential adverse effects induced by T cell therapy.

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