



Tumor necrosis factor- α modifies the effects of Shiga toxin on glial cells



Hue Leu^{a,b}, Naotoshi Sugimoto^{a,c,*}, Masaki Shimizu^a, Tomoko Toma^a, Taizo Wada^a, Kunio Ohta^a, Akihiro Yachie^a

^a Department of Pediatrics, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

^b Dan Phuong General Hospital, Hanoi, Vietnam

^c Department of Physiology, Graduate School of Medical Science, Kanazawa University, Kanazawa, Japan

ARTICLE INFO

Article history:

Received 22 January 2016

Received in revised form 16 May 2016

Accepted 26 May 2016

Available online 4 June 2016

Keywords:

Nuclear factor- κ B

Shiga toxin

Encephalopathy

Outbreak

ABSTRACT

Shiga toxin (STX) is one of the main factors inducing hemorrhagic colitis and hemolytic-uremic syndrome (HUS) in infections with STX-producing *Escherichia coli* (STEC). Approximately 62% of patients with HUS showed symptoms of encephalopathy in the 2011 Japanese outbreak of STEC infections. At that time, we reported elevated serum concentrations of tumor necrosis factor (TNF)- α in patients with acute encephalopathy during the HUS phase. In the current study, we investigated whether TNF- α augments the effects of STX in glial cell lines and primary glial cells. We found that TNF- α alone or STX in combination with TNF- α activates nuclear factor- κ B (NF- κ B) signaling and inhibits growth of glial cells. The magnitude of the NF- κ B activation and the inhibition of cell growth by the STX and TNF- α combination was greater than that obtained with TNF- α alone or STX alone. Thus, this in vitro study reveals the role of TNF- α in glial cells during STEC infections.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Shiga toxin (STX), which is produced by some strains of *Escherichia coli*, causes hemorrhagic colitis and hemolytic-uremic syndrome (HUS) [1]. 20 to 50% of HUS cases involve central nervous system (CNS) complications that are the most common cause of death in STEC infections [1,2]. However, approximately 62% of patients with HUS showed symptoms of encephalopathy in the 2011 Japanese outbreak of infection with STX-producing *E. coli* (STEC) [3,4]. Encephalopathy is a syndrome involving cerebral dysfunction with organic and inorganic causes, including cytokine storm and toxic reaction [5,6].

We previously reported that the serum concentration of tumor necrosis factor (TNF)- α was higher in patients with acute encephalopathy than in patients without encephalopathy during the HUS phase in the 2011 Japanese outbreak of STEC infections [7]. This study suggested that TNF- α is a critical factor leading to encephalopathy in STEC infections.

The brain contains mainly neuronal and glial cells. Glial cells are the most abundant cells in the brain and play key roles in cerebral

homeostasis, plasticity, and inflammation. Glial cells are functional components of the blood-brain barrier (BBB) [8]. Therefore, glial cell hypofunction and death impair cerebral homeostasis [9] and are thought to cause CNS complications, including encephalopathy [8].

In this study, we investigated the effect of TNF- α with or without STX on glial cells in vitro. Initially, we studied the effects of TNF- α alone on death of and inflammatory responses in glial cells. The subsequent analysis demonstrated that the combination of STX and TNF- α led to greater cell death and inflammatory responses than those noted with TNF- α alone in glial cells. Possibly, the role of TNF- α in sensitizing glial cells to STX contributes to the development of CNS complications in STEC infections.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), TNF- α , and Cell Counting Kit 8 were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Shiga toxin-2 (STX) was obtained from Nacalai Tesque (Kyoto, Japan). Fetal bovine serum (FBS) was obtained from Invitrogen Corporation (Carlsbad, CA, USA). Anti-phospho-specific nuclear factor- κ B (NF- κ B) p65 (Ser536), anti-phospho-specific c-Jun. N-terminal kinase (JNK; Thr183/Tyr185), anti-phospho-specific p38 mitogen-activated protein kinase (MAPK; Thr180/Tyr182), anti- β -actin, and horseradish-peroxidase-conjugated anti-rabbit IgG antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA).

Abbreviations: BBB, blood-brain barrier; CNS, central nervous system; JNK, c-Jun. N-terminal kinase; HUS, hemolytic-uremic syndrome; NF- κ B, nuclear factor- κ B; STX, Shiga toxin; STEC, STX-producing *Escherichia coli*; TNF- α , tumor necrosis factor- α .

* Corresponding author at: Department of Pediatrics, Graduate School of Medical Science, Kanazawa University, 13-1 Takara-machi, Kanazawa 920-8640, Japan.

E-mail addresses: nevergiveup.hue@gmail.com (H. Leu), ns@med.kanazawa-u.ac.jp (N. Sugimoto), shimizum@staff.kanazawa-u.ac.jp (M. Shimizu), tomoko-t@staff.kanazawa-u.ac.jp (T. Toma), taizo@staff.kanazawa-u.ac.jp (T. Wada), kohta@staff.kanazawa-u.ac.jp (K. Ohta), yachie@staff.kanazawa-u.ac.jp (A. Yachie).

2.2. Cell culture

B92 rat cells [10] and primary rat glial cells [11] were gifted by Dr. Ohno-Shosaku (Kanazawa University, Japan). The glial fibrillary acidic protein, a marker of astrocyte, was expressed in primary cells that Dr. Ohno-shosaku provided, indicating astrocytes [9]. The cells were maintained in DMEM containing 10% FBS at 37 °C in a 5% CO₂ incubator. Subcultures were required to maintain that both cells were in the logarithmic phase of growth during culture.

2.3. Cell proliferation assay

Cell proliferation was analyzed using Cell Counting Kit 8. B92 rat glial cells and primary glial cells were seeded in 96 well-plates at a density of 3×10^3 cells/well. After 24-h incubation, the cells were then treated with TNF- α and/or STX2 for 48 h. Next, the cells were incubated with 10 μ L WST-8 for 2 h. The absorbance of the colored formazan product produced by mitochondrial dehydrogenases in metabolically active cells was recorded at 450 nm as the background value. Cell proliferation was expressed as a percentage of absorbance obtained in treated wells relative to that in untreated (control) wells.

2.4. Western blotting

Western blotting was performed as described previously [12,13].

2.5. Statistical analysis

Data are presented as the mean \pm SEM from at least three independent experiments. Statistical analysis was conducted using a Kruskal Wallis non-parametric ANOVA followed by a Bonferroni Inequality. Results were considered statistically significant when *P*-values were less than 0.05 or 0.01.

3. Results

3.1. TNF- α induces downregulation of cell growth and upregulation of NF- κ B activity in B92 glial cells

First, we examined the effects of TNF- α on growth of cultured glial cells. TNF- α (50–500 ng/mL) considerably inhibited cell growth (Fig. 1A) and cleaved caspase-3 (Suppl. Fig. S1) in a concentration-dependent manner in B92 glial cells.

Next, we examined the effects of TNF- α on inflammation in cultured glial cells. TNF- α (5–500 ng/mL) significantly phosphorylated NF- κ B in a concentration-dependent manner in B92 glial cells (Fig. 1B), indicating TNF- α induced activation of NF- κ B signaling.

3.2. STX facilitates TNF- α -induced downregulation of cell growth and TNF- α -induced upregulation of NF- κ B activity in B92 glial cells

Our previous study showed that STX (0.03–3 ng/mL) did not inhibit growth of B92 glial cells [14]. Likewise, in the current study, STX (3 ng/mL) alone failed to alter growth of B92 glial cells; however, it significantly facilitated TNF- α -induced downregulation of growth of these cells (Fig. 2A).

Although STX alone failed to phosphorylate NF- κ B, it facilitated TNF- α -induced NF- κ B phosphorylation in B92 glial cells (Fig. 2B).

3.3. STX facilitates TNF- α -induced activation of JNK and p38 MAPK in B92 glial cells

STX alone phosphorylated JNK but not p38 MAPK in B92 glial cells, indicating that it activates JNK but not p38 MAPK (Fig. 3). In contrast, TNF- α alone phosphorylated both JNK and p38 MAPK in B92 glial cells, indicating that it activates both JNK and p38 MAPK (Fig. 3). The

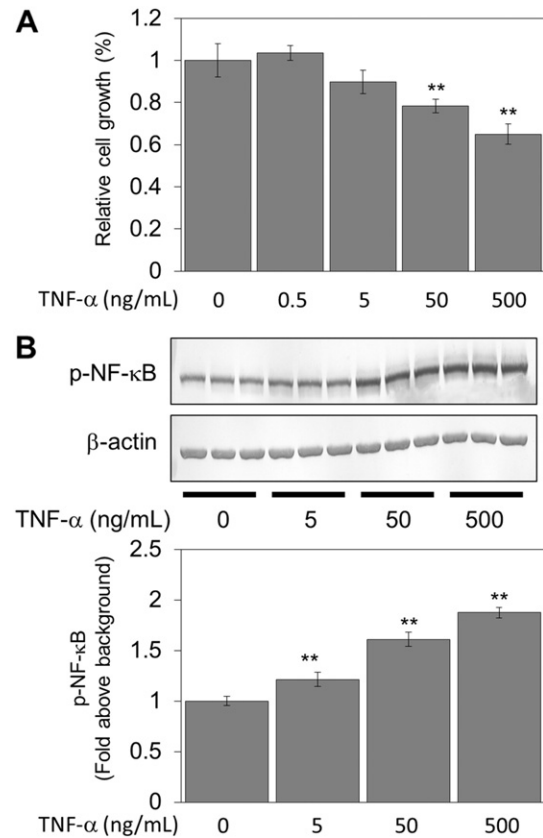


Fig. 1. TNF- α induces decrease in cell proliferation and increase in phosphorylation of NF- κ B in B92 glial cells. (A) The effects of TNF- α on cell growth at 48 h after treatment. (B) The effects of TNF- α on phosphorylation of NF- κ B at 30 min after treatment. Data is representative of at least three independent experiments. Each column represents the mean \pm SEM. ***P* < 0.01 versus vehicle control (0 ng/mL).

combination of STX and TNF- α strongly phosphorylated both JNK and p38 MAPK in glial cells. STX significantly facilitated TNF- α -mediated phosphorylation of JNK (Fig. 3).

3.4. STX facilitates TNF- α -induced upregulation of NF- κ B activity in primary glial cells

TNF- α , but not STX, phosphorylated NF- κ B in primary B92 glial cells (Fig. 4). Although STX alone failed to activate NF- κ B, it significantly facilitated TNF- α -induced NF- κ B phosphorylation, indicating the activation of NF- κ B (Fig. 4).

3.5. STX facilitates TNF- α -induced activation of JNK and p38 MAPK in primary glial cells

TNF- α , but not STX, phosphorylated both JNK and p38 MAPK in primary glial cells (Fig. 5). STX alone failed to activate JNK or p38 MAPK, similar to its effect on NF- κ B activity. However, STX significantly facilitated TNF- α -induced JNK and p38 MAPK phosphorylation in primary cells (Fig. 5).

4. Discussion

In the current study, we demonstrated that TNF- α inhibits cell growth and activates NF- κ B, JNK, and p38 MAPK in a concentration-dependent manner in glial cells. STX combined with TNF- α decreased cell growth and activated NF- κ B, JNK, and p38 MAPK to a greater extent than STX alone or TNF- α alone. These results suggest that TNF- α modifies disease progression in STEC infections.

Download English Version:

<https://daneshyari.com/en/article/5831750>

Download Persian Version:

<https://daneshyari.com/article/5831750>

[Daneshyari.com](https://daneshyari.com)