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Indoleamine 2,3-dioxygenase 1 is upregulated in activated microglia in mice cerebellum during acute viral encephalitis

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HIGHLIGHTS

- Acute EMCV infection leads to a highly reproducible neuronal degeneration in mouse cerebellum.
- IDO1 is upregulated in activated microglia within the EMCV-induced neurodegenerative lesions.
- IDO1 expression may affect EMCV-induced neuronal degeneration in cerebellum.

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ABSTRACT

Indoleamine 2,3-dioxygenase1 (IDO1) is the rate-limiting enzyme in the kynurenine pathway that converts L-tryptophan to L-kynurenine. Encephalomyocarditis virus (EMCV) can cause acute myocarditis in various animals including mice. Previously, IDO1 has been reported to have an important immunomodulatory function in immune-related diseases. However, the pathophysiological roles of IDO1 following acute viral infection of central nervous system are not fully understood. We observed that acute EMCV infection leads to a highly reproducible neuronal degeneration in mouse cerebellum. The goal of this study is to determine tissue/cell-specific and time-dependent expressions of IDO1 during acute EMCV infection in mouse cerebellum. IDO1 was up-regulated in microglia, which was recognized to be activated morphologically and positive for ionized calcium-binding adapter molecule 1 (Iba-1), a protein expressed in microglia, within EMCV-induced cerebellar lesions showing neuronal degeneration although the very weak expression of IDO1 is detected only in cytoplasm of Purkinje cells. No GFAP immunostaining was observed in EMCV-induced cerebellar lesions although many reactive astrocytes surrounding the lesions showed strongly positive immunostaining for GFAP 10 days after the viral inoculation. Thus, IDO1 expression may affect EMCV-induced neuronal degeneration in cerebellum.

1. Introduction

Indoleamine 2,3-dioxygenase 1 (IDO1) is the rate-limiting enzyme for the tryptophan catabolism to kynurenine. IDO1 has long been of interest because it catabolizes L-tryptophan, leading to increases in the levels of metabolites such as kynurenine and quinolinic acid [1]. Growing evidence indicates that some of these metabolites are involved in the neurotoxicity associated with several brain disorders, such as Huntington's [2], Parkinson's [3] and Alzheimer's [4] diseases, as well as in hepatic encephalopathy [5] and glutaryl-CoA dehydrogenase deficiency [6]. However, the pathophysiology of the brain damage in these neurodegenerative disorders is not completely understood. Kwidzinski et al. reported that the temporal and local increase in IDO expression in inflamed tissue is involved in reducing autoimmune CNS inflammation. Some trp degradation products can be toxic not only to T cells, but also to neurons [7]. Therefore, time limitation of IDO activity within the CNS appears to be important to prevent the

Abbreviations: IDO, Indoleamine 2,3-dioxygenase; TUNEL, Terminal dUTP-biotin nick end labeling; EMCV, Encephalomyocarditis virus.

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neuronal damage that occurs under long-term expression (e.g., during SIV/HIV encephalitopathy).

Encephalomyocarditis virus (EMCV), which is a small non-enveloped single-stranded RNA virus, can cause acute myocarditis in various animals including mice. EMCV infection in mice is an established model for viral myocarditis, dilated cardiomyopathy, and congestive heart failure [8]. Hoshi et al. [9] also reported that IDO1 has an important role in acute EMCV-induced myocarditis. Furthermore, in addition to myocarditis but also EMCV infection induces encephalitis in many mammalian species [10]. Of note, EMCV infection primarily affects the cerebral hemispheres and thalamus [11]. The cerebellum was generally spared in viral infections; however, stable degenerative lesions in cerebellum after EMCV infection were detected in our preliminary experiment (unpublished observations). The previous study has reported the cerebellar lesions induced by EMCVs in mice [12] at 7 days and 28 days after inoculation; however, our study is the first report to describe the time-course at 0, 2, 4, 7 and 10 days after EMCV inoculation for virus-induced cerebellar lesions.

Of note, it has been reported that IDO is detectable only in microglia and astrocytes [13], not in neurons [14]. However, more recently IDO has been reported as being present in human fetal neurons [15] and in murine hippocampal neurons, where levels are upregulated by interferon (IFN) [16]. We have reported that transient forebrain ischemia in gerbils produces tissue/cell-specific and time-dependent expressions of IDO in hippocampal CA1, cingulate cortex and hypothalamus, which was not related to glial cell activation [17]. Thus, exact localization of IDO in central nervous system still remains to be established.

The objective of this study was firstly, to investigate the localization of IDO1 within EMCV-induced cerebellar lesions showing neuronal degeneration in the acute phase, and secondly to demonstrate the time-course changes of IDO1 expression in this model.

2. Materials and methods

2.1. Viral inoculation

A myocarditic variant of EMCV was generously provided by Dr. Seto (Keio University, Tokyo, Japan). The virus was stored at −80 °C in Hanks' balanced salt solution with 0.1% bovine serum albumin (BSA) until use. Mice were inoculated intraperitoneally with 500 plaque forming units (pfu) of EMCV in 0.1 mL of saline. Six-week-old male C57BL/6J mice (Japan SLC Inc., Hamamatsu, Japan) were used for inoculation and housed in an isolated room on 12 h light/dark cycles (8.00 am/8.00 pm) at 22 °C, and were given free access to food and water. The day of virus inoculation was defined as day 0 in the current study. The experiments were performed in accordance with the institutional guidelines of Gifu University.

2.2. Tissue preparation

At 2, 4, 7 and 10 days after viral inoculation, animals ($n = 5$) were perfused transcardially with saline and then with phosphate-buffered 10% formalin. Brains were removed and processed for paraffin embedding. Three micrometer coronal sections were cut, mounted on slides and then used for hematoxylin and eosin (HE) staining.

2.3. Immunohistochemistry

For the immunohistochemical assay, sections were deparaffinized with xylene and rehydrated through a series of graded alcohol solutions. Endogenous peroxidase activity was blocked by incubation in 0.3% hydrogen peroxidase in methanol for 30 min, and then the sections were heated in 0.1 M citrate buffer (pH 6.0) using the Pascal® heat-induced target retrieval system (DAKO, Carpinteria, CA). Nonspecific binding sites were blocked using 2% BSA in phosphate-buffered saline (PBS) for 1 h at room temperature. Sections were incubated with rabbit anti-mouse IDO1 antibody (antibody specific to mouse IDO1was generated by the peptide [H]-CMKPSKKKPTDGDKS-[OH]; 1:400) [18], rabbit anti-Iba 1 (019-19471, WAKO chemicals; 1:1000), mouse anti-GFAP (M0761, DAKO; 1:100) and anti-Ki-67 (TEC-3, DAKO, 1:100) at 4 °C overnight. For anti-rabbit and anti-rat antibodies, biotinylated anti-rabbit IgG (16-15-06, KPL, 1:250) and biotinylated anti-rat IgG (E0468, DAKO, 1:200) were applied as secondary antibodies, respectively, and then streptavidin peroxidase (SA-5704, Vector Laboratories) were used. For anti-mouse antibodies, we used a Mouse 2 Mouse HRP Ready-To-Use Kit (MTM001, ScyTek Laboratories) according to the manufacturer's protocol. The peroxidase binding sites were detected by staining with 3,3′-diaminobenzidine (DAB) in 0.05 M Tris–EDTA buffer, pH 7.6. Finally, counterstaining was performed using Mayer's hematoxylin.

2.4. TUNEL assay

TUNEL staining was performed as described previously [19] with some modification of the method of Gavrieli et al. [20]. After incubation with 20 μg/mL proteinase K (Sigma), the serial sections used as HE staining were immersed in TDT buffer (30 mM Trizma base, pH 7.2, 140 mM sodium cacodylate, 1 mM cobalt chloride). TDT (3333566, Roche Diagnostics) and biotinylated dUTP (1093070, Roche Diagnostics) were diluted in TDT buffer and sections were then incubated with these reagents at 37 °C for 1 h. The sections were covered with streptavidin peroxidase (P0397, DAKO), and stained with DAB as a substrate for the peroxidase. Finally, counterstaining was done using Mayer's hematoxylin.

2.5. RNA extraction and RT-PCR analysis

At 12 and 24 h, 2, 4 and 7 days after viral inoculation, brains were removed, and total RNA was extracted from the cerebellum with Isogen (317-02503, Nippon Gene) and determined by the absorbance at 260 nm. Reverse transcription polymerase chain reaction (RT-PCR) was carried out with mRNA Selective PCR Kits (RR025A, Takara Biochemicals). The following oligonucleotide primer pairs were synthesized: EMCV sense, 5′-GTCGTGAAGG AAGCAGTTCC-3′; antisense, 5′-CACGTGGCTTTTGGCCGCAGAGGC-3′; GAPDH sense, 5′-ACGACCCCTTCATTGACCTCAACT-3′; antisense, 5′-ATATTTCTCGTGGTTCACACCCAT-3′. The PCR products were analyzed by agarose gel electrophoresis with ethidium bromide staining. The optimum number of cycles was determined experimentally for each gene product and to verify uniform amplification.

2.6. Statistical analysis

RT-PCR experiment was performed at least three times. Data were presented as mean ± SD. Differences between groups were considered statistically significant according to one-way ANOVA followed by the Tukey-tests. P-values <0.05 were defined statistically significant.

3. Results

3.1. HE staining, immunohistochemistry for Iba-1, GFAP and Ki-67 and TUNEL staining

Time-course of HE staining for histomorphological examination, immunohistochemistry for Iba-1 and GFAP expression, and TUNEL