Veterinary Immunology and Immunopathology 166 (2015) 145–150

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm

Short communication

Lipopolysaccharide stimulation upregulated Toll-like receptor 4 expression in chicken cerebellum

Abdur Rahman Ansari[a,b], Le Wen[a], Hai-Bo Huang[a], Ji-Xiang Wang[a], Xi-Yao Huang[a], Ke-Mei Peng[a], Hua-Zhen Liu[a,*]

[a] Department of Anatomy, Histology and Embryology, College of Animal Science and Veterinary Medicine, Huazhong Agricultural University, Wuhan, Hubei 430070, China
[b] Section of Anatomy and Histology, Department of Basic Sciences, College of Veterinary and Animal Sciences (CVAS), Jhang, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan

ARTICLE INFO

Article history:
Received 20 February 2015
Received in revised form 4 May 2015
Accepted 19 May 2015

Keywords:
Cerebellum
Chicken
Innate immunity
Lipopolysaccharide
Toll-like receptor 4

ABSTRACT

Toll-like receptors (TLRs) play crucial roles in innate and adaptive immune responses to invading pathogens. TLR4 is responsible for the recognition of bacterial lipopolysaccharide (LPS) in different parts of central nervous system of many vertebrates. To better understand the functions of TLR4 in cerebellum of chicken, present study was designed to identify the cell types that express TLR4 during postnatal stages as well as the changes in its expression in response to LPS challenge. For this purpose, cerebella were collected from chicken aged 1, 14 and 40 days ($n = 7$ in each group) to analyze TLR4 distribution pattern. The cerebella from 14 chickens injected with LPS or sterilizing saline were also collected at Day 14 ($n = 7$ in each group) to investigate changes in TLR4 expression. This expression was analyzed by immunohistochemistry using an anti-TLR4 antibody. TLR4 was constitutively expressed in the Purkinje cell layer, pia mater, neurons in medulla and blood vessels in the cerebellum and LPS stimulation significantly up-regulated TLR4 expression on Day 14 in the chicken cerebellum. This study provides evidence that neurons in chicken cerebellum can express TLR4 in vivo and suggests that these neurons may play an important role in initiating a defense reaction via activation of TLR4.

1. Introduction

A comprehensive communication network is present between central nervous system (CNS) and the immune system (Quan and William, 2007). Microglia are the major type of resident immune cells in brain that play crucial role in inflammatory response (Colton and Wilcock, 2010) and constitute about 10–20% of the parenchyma in CNS (Steel et al., 2014). In the host innate immunity, pattern recognition receptors (PRRs) act as basic components that mostly activate the microglia. PRRs recognize pathogen-associated molecular patterns (PAMPs), which are associated with the pathogenic microbes. Toll-like receptors (TLRs) belong to PRRs subfamily that largely expressed in many of the cells in CNS, especially in microglia. To date, 10 functional TLRs have been identified in humans (Kawai and Akira, 2010; Facci et al., 2014) and chicken (Kannaki et al., 2015) and as many as 13 in mouse (Reuven et al., 2014).

TLRs are traditionally related to the immunity, however recent studies found that they act as mediators of CNS plasticity and regulate the cognitive role in the absence of a pathogen derived ligand. Recently, endogenous activation of TLRs is getting attention, because it is well known that TLRs are not only implicated in eliciting pathogen trigged

* Corresponding author. Tel.: +86 27 87286970; fax: +86 27 87280408.
E-mail address: lhz219@mail.hzau.edu.cn (H.-Z. Liu).

http://dx.doi.org/10.1016/j.vetimm.2015.05.004
0165-2427/

immune responses, but also involved in physiological and metabolic processes as well as in certain pathological conditions unrelated to infection (Okun et al., 2011).

The expressions of chicken TLRs have been described on immune cells as well as in adult tissues and embryo (Kannaki et al., 2015). TLRs 1–10 have been expressed at detectable levels in human brain (Bsibsi et al., 2002) and TLRs 1–9 in isolated microglia in mouse (Olson et al., 2004). The expression of mRNA for many of the TLRs has been detected at relatively low levels in normal uninfected mouse brain (Mishra et al., 2006). Low-level constitutive mRNA expression of TLRs 2, 4, 5 and 9, was also detected in astrocytes however, after stimulation with definite TLR ligands, there was an increase in their expressions (Bowman et al., 2003).

LPS in high doses cause endotoxic shock and death on the other hand, low quantities of LPS lead to sickness behavior in mice (Palin et al., 2009). Systemic injections of LPS in high doses are also reported to trigger the innate immunity in the CNS, particularly in meninges and the parenchyma. After systemic LPS stimulation, the circulating cytokines in the brain vasculature may lead to the activation of endothelial cells. LPS binds to TLR4 and exerts its effects via one of two downstream signaling pathways; myeloid differentiation factor 88 (MyD88)-dependent pathway and MyD88-independent pathway and finally both of these pathways lead to IFN-β production and IFN-inducible genes expression (Thomson et al., 2014).

The cellular distribution of TLR protein expressions were also described in different CNS cells from normal and infected mice as well as leukocytes infiltrating the brain upon parasitic infection and the up-regulation of all of the tested TLRs have been found except TLR5 (Mishra et al., 2006). The analysis of TLRs related gene expression indicates the association of these receptors with plasticity and neurogenesis of the CNS during brain development. TLR4 expression is present in embryonic stages and its appropriate level is maintained in mice brain throughout adulthood (Lathia et al., 2008; Kaul et al., 2012).

Most of the studies related to the distribution and expression patterns of TLR4 during normal conditions and infection have been performed on insects, mammals and rodent species. Although TLR4 has been reported in the brain as well as in other tissues of chicken (Leveque et al., 2003), however the in vivo distribution and expression pattern of TLR4 in chicken brain, especially cerebellum is still unclear. Therefore, the present investigation was performed to study the distribution pattern and expression changes in TLR4 in the chicken cerebellum under normal conditions and after LPS injection to better understand the function of TLR4 in the CNS of chicken.

2. Materials and methods

2.1. Animals

In total, thirty-five 1-day-old broiler chicks (Cobb strain) were purchased from Zhengda chicken breeding company (Wuhan, China). The chickens were reared under conventional housing conditions without any vaccinations. Out of the 35 broiler chickens, 14 one-day-old were intraperitoneally injected with LPS (LPS from Salmonella ATCC 13076, Sigma, 1 mg/kg, 1.25 mg/ml) or the same dose of sterile saline (0.75%, 0.8 ml/kg). The dose rate of LPS 1 mg/kg body weight is reported to be sufficient to induce the immune response in chicken (Subedi et al., 2007). All the animal procedures were conducted according to protocols approved by the Animal Care and Use Committee for Biological Studies, Hubei Province, PR China.

2.2. Tissue collection and preparation

Cerebella from chickens were collected at Days 1, 14 and 40 of age ($n = 7$ in each group) to analyze the distribution pattern of TLR4 in normal conditions, whereas the cerebella from 14 chickens injected with LPS or sterile saline were harvested at Day 14 ($n = 7$ in each group) to investigate changes in the expression of TLR4. All the collected tissues were fixed in 4% paraformaldehyde solution in phosphate-buffered saline (PBS) for 24 h, dehydrated and then embedded in paraffin wax. After that, 4-μm tissue sections were cut using a Leica microtome (Nussloch Gmbh, Germany) and mounted on polylysine-coated slides (Boster Corporation, China). All the sections were dried overnight at 37 °C.

2.3. Immunohistochemical staining

The immunohistochemical staining (IHC) was performed by using the anti-TLR4 antibody. After tissue sections were deparaffinized with xylene and rehydrated with decreasing concentrations of ethanol; the sections were then soaked in 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. PBS was used in all the steps during IHC however, antigen retrieval was achieved by microwaving the slides for 22 min (6 min at 700 W followed by 16 min at 116 W) in citrate acid buffer (pH 6.0). Sections were then reacted with 5% normal goat serum (Thermo Scientific, Rockford, USA) for 10 min at 37 °C to prevent any non-specific reactions, and incubated with a rabbit polyclonal antibody to human TLR4 (1:100 dilution; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) at 4 °C overnight. This antibody to TLR4 was raised against a peptide near the C-terminus of human TLR4, and has previously been used to identify TLR4 in chickens (Huang et al., 2014). After incubation with the primary antibody, sections were washed in PBS three times, and incubated for 20 min with goat anti-rabbit IgG antibody (Thermo Scientific, Rockford, USA) labeled with peroxidase. Slides were washed three times in PBS again and stained with diaminobenzidine tetrahydrochloride (DAB) stain (Thermo Scientific, Rockford, USA). Counterstaining was done with hematoxylin. Negative control sections were stained using the same method, but without using the primary antibody.

2.4. Semi-quantitative analysis for the TLR4 protein in chick cerebella

Five serial sections from each cerebellum were examined under a light microscope (BH-2; Olympus, Japan)