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Age-Related Regional Difference of Interleukin-1 Expression in Rat Brain after Lipopolysaccharide Treatment

Aging is associated with altered immune responses including dysregulation of cytokine production. Of cytokines, interleukin-1 (IL-1) family has been primarily involved with central nervous system. To evaluate the age-related different response of IL-1 family following peripheral administration of lipopolysaccharide (LPS), immunohistochemical study of IL-1β and IL-1 receptor expression was performed on Sprague-Dawley rat brain. Experimental animals were divided into four groups; saline-treated young (3-5 months) and old (over 24 months), and LPS-treated young and old groups. After intraperitoneal (i.p.) injection of LPS, three to five rats within each group were killed at 1, 2, 4, 8 and 16 hr. After fixation in 4% neutral buffered formalin, the brain slices were paraffin-embedded. Immunohistochemical staining using labelled streptavidin biotin was performed. The results showed that IL-1β immunoreactivity was seen in the endothelial cell of pons in both LPS-treated young and old rats, with slightly longer persistency in old group. IL-1RI immunoreactivity appeared initially in the neurons of cerebral cortex in LPS-treated old group, compared with predominantly the cerebellum in LPS-treated young group. In conclusion, our study shows that there is age-related, different neuronal localization of IL-1RI expression at different points of time after LPS treatment.

Key Words: *Interleukin-1; Immunohistochemistry; Lipopolysaccharides; Aging; Rats; Brain*

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INTRODUCTION

Aging is associated with altered immune responses including dysregulation of cytokine production (1). For example, studies have reported increases in interleukin-1 (IL-1), IL-1 receptor antagonist, and transforming growth factor-β production with aging (2-8). Often, fever may be blunted or even absent in elderly patients with infection (9), which means that aging is also associated with alteration in the physiological host responses to inflammatory or infectious stimuli.

Of cytokines, IL-1 family has been primarily involved with central nervous system.

The IL-1 family currently comprises at least two known agonists, IL-1α and IL-1β. Most studies to date suggest that IL-1β plays the major role in the brain and in neurodegeneration (10). IL-1β is a proinflammatory cytokine that induces fever physiologically (11-13). It is reported that constitutive expression of IL-1 in the brain of normal healthy animals is extremely low. However, IL-1 expression is actively induced by peripheral administration of lipopolysaccharide (LPS) (14), which is a component of the cell wall of gram-negative bacteria and plays a key role in the induction of brain-mediated illness symptoms.

Two distinct subtypes of IL-1 receptor have been identified and cloned, the type 1 receptor (IL-1RI) and the type 2 receptor (IL-1RII) (15). Recent studies suggest that the IL-1RI is primarily responsible for mediating the biological effects of IL-1 (15). However, most studies to evaluate age-related different response of IL-1 family expression in central nervous system have been based on mRNA level.

In the present study, with immunohistochemical method, we investigated the IL-1β and IL-1RI expression of young and old Sprague-Dawley rat brain following peripheral administration of LPS to evaluate the age-related different response.

MATERIALS AND METHODS

Animals and LPS injection

Experimental animals were male young (3-5 months)

and old (over 24 months) Sprague-Dawley rats were used. They were housed individually and maintained ad libitum on powdered rat foods and tap water. The animals were cared for in accordance with the guidelines of Korean Academy of Medical Sciences. Experimental rats were divided into four groups, saline-treated young and old, and LPS-treated young and old rats. Three to five rats were decapitated at 1, 2, 4, 8 and 16 hr after intraperitoneal injection of LPS in a dosage of 2.5 mg/kg dissolved in 0.9% saline, and the brains were quickly collected. Each brain was immediately placed in 4% neutral buffered formalin overnight. Then the brain was coronally sectioned with 3 mm-thickness to four slices at −5, −2, +2, +5 mm relative to bregma and the slices were paraffin-embedded.

Immunohistochemical staining

Immunohistochemical staining using labelled streptavidin biotin method was performed. The primary antibodies used in this study were IL-1β (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) and IL-1RI (Santa Cruz Biotechnology). Formalin-fixed, paraffin-embedded tissues were cut with 6 μm-thickness. After deparaffinized and rehydrated with graded alcohol, sections were first incubated in 0.3% H_2O_2 for 15 min, and then in normal goat serum for 30 min, followed by incubation with primary antibodies (1:20) overnight at 4℃. After several washes in Tris buffer, sections were incubated in biotinylated secondary antibody for 1 hr, then in streptavidin peroxidase for 1 hr. Peroxidase reaction was carried out with 3-3'-diaminobenzidine as chromogen. Sections were mounted on silane-coated slides, air dried, and then were dehydrated in graded ethanol and finally covered with Permount. Control sections were processed in parallel with test specimens except for the incubation with primary antibody.

RESULTS

Expression of IL-1β

The distribution of IL-1β immunoreactive cells is summarized in Table 1. Neither saline-treated young nor old rat brain showed recognizable immunoreactive cells throughout the entire brain. LPS-treated old rat brain showed IL-1β immunopositivity on the endothelial cell, particularly in the pons after 1, 2 and 4 hr (Fig. 1). The initial immunoreactivity in both experimental groups could not be detected at 8 hr after LPS injection. Compared with LPS-treated young rat, the positive immunoreaction for IL-1β was detected earlier and lasted longer in LPS-treated old rat. Neurons and glial cells were not reactive in either LPS-treated young or old rat brain.

Expression of IL-1RI

Positive immunoreactivity against IL-1RI was detected diffusely on the endothelial cells, glial cells and choroid plexus in all experimental groups, regardless of LPS treatment (data not shown). However, regional variation was observed as to the distribution of immunoreactive neurons (Table 2). Both saline-treated young and old groups

Table 1. Distribution of IL-1β immunoreactive cells after LPS injection

Group		Time after injection (hr)			
		1	2	4	8
Normal saline	Young	(-)	(-)	(-)	(-)
	Old	(-)	(-)	(-)	(-)
Lipopolysaccharide	Young	(-)	E (PO)	(-)	(-)
	Old	E (PO)	E (PO)	E (PO)	(-)

E, endothelial cell; PO, pons

Table 2. Distribution of IL-1RI immunoreactive neurons after LPS injection

Group		Time after injection (hr)			
		1	2	4	8
Normal saline	Young			PO, Cbr (±)	
	Old			PO, Cbr (±)	
Lipopolysaccharide	Young	CbII	CbII	CbII	Cbr, CbII (±)
	Old	Cbr	Cbr	Cbr, CbII (±)	Cbr, CbII

Cbr, cerebral cortex; CbII, cerebellum; PO, pons

showed strong immunopositivity for IL-1RI particularly on neurons of the pons (Fig. 2), whereas there was a few neurons with the immunopositivity in the cerebral cortex. IL-1RI immunoreactivity appeared initially on Purkinje cells of cerebellum in LPS-treated young group (Fig. 3), followed by the neurons of cerebral cortex. The reverse was observed in LPS-treated old group, in which the immunoreactivity appeared first on neurons of cerebral cortex (Fig. 4), and then Purkinje cells of cerebellum.

DISCUSSION

This study demonstrates the age-related difference in neuronal localization of IL-1RI expression in rat brain after LPS treatment, although there was no significant difference as to the IL-1β expression. IL-1RI immunoreactivity appeared initially on the neurons of cerebral cortex in LPS-treated old rat brain, compared to the Purkinje cells of cerebellum in LPS-treated young rat

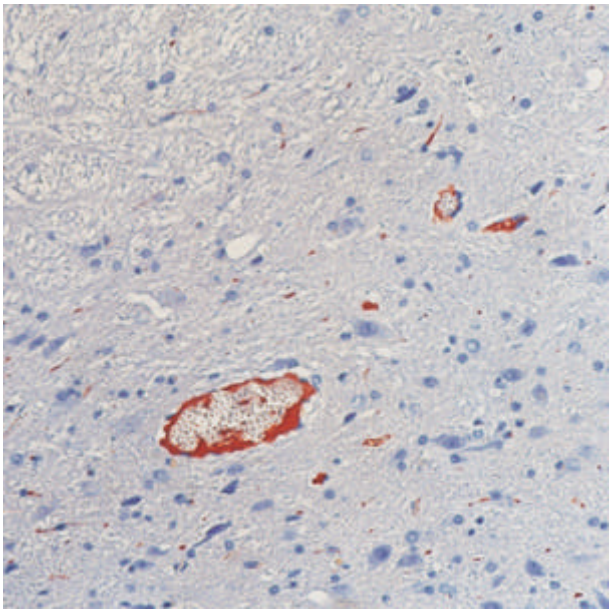

Fig. 1. IL-1β immuoreactive endothelial cells of pons in old rat brain at 4 hr after LPS injection (×200).

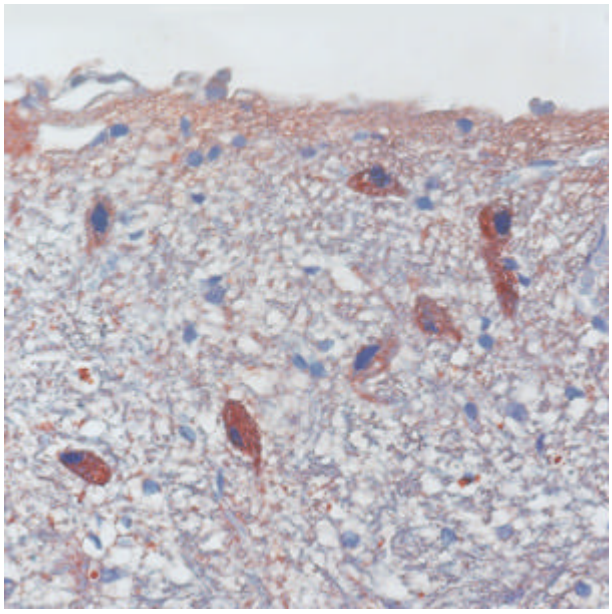

Fig. 2. IL-1RI immunoreactive neurons of pons in old rat brain at 2 hr after LPS injection (×200).

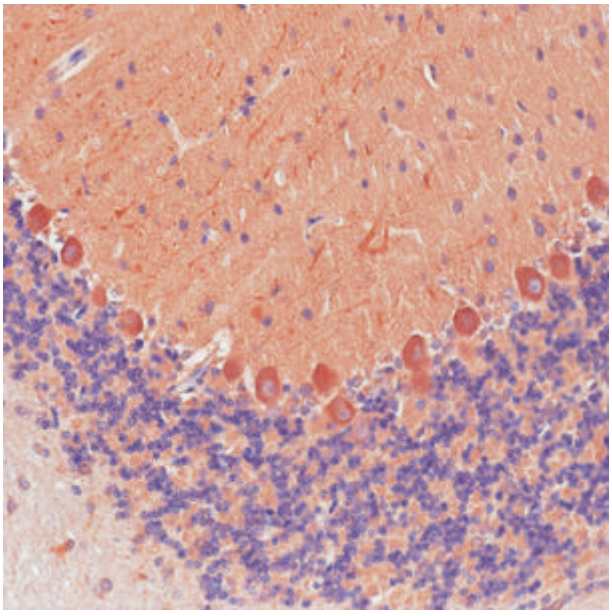

Fig. 3. IL-1RI immunoreactive Purkinje cells in young rat brain at 2 hr after LPS injection (×200).

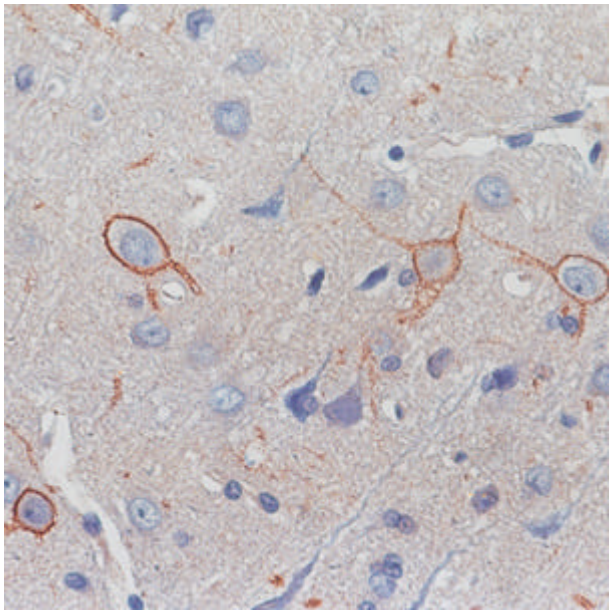

Fig. 4. IL-1RI immunoreactive neurons of cerebral cortex in old rat brain at 2 hr after LPS injection (×400).

brain.

The IL-1 family currently comprises at least two known agonists, IL-1α and IL-1β. Most studies to date suggest that IL-1β plays the major role in the brain and in neurodegeneration (10). IL-1β is a proinflammatory cytokine that induces fever (11-13). While constitutive expression of IL-1 in the brain of normal healthy animals is extremely low, IL-1 expression is actively induced by peripheral administration of LPS (14). However, it is not fully understood by what routes IL-1 produced by immune cells by relevant stimuli can reach the brain. In consideration of the ineffectiveness of IL-1 in passing the blood-brain barrier, it is hypothesized that IL-1 may be produced in the brain in response to peripheral administration of LPS (14).

Ilyn et al. revealed that LPS induced up-regulation of the expression of IL-1β in the hypothalamus, cerebellum and hippocampus by RNase protection assays (16). Van Dam et al. showed that IL-1β immunopositive cells induced by are seen in the cells of meninges, choroid plexus, in some perivascular cells, and in small stellate cells with ramifying processes, which may be ED2-positive perivascular cells or ED1-positive microglial cells (15). According to our immunohistochemical study, the IL-1β immunopositivity was seen in the endothelial cells of pons in both LPS-treated young and old rat brain, albeit a small difference in the immunoreactivity between the two groups. Our results are in part consistent with the observation by van Dam et al. With RNase protection assays to determine mRNA changes for IL-1β in young and old Long-Evans rats, Gayle et al. revealed that IL-1β induced an up-regulation of hypothalamic and midbrain IL-1β mRNA (17). However, they could not find any age-related differences in IL-1β mRNA levels that we observed.

Two distinct subtypes of IL-1 receptor have been identified and cloned. The type 1 receptor (IL-1RI), which is expressed in vivo primarily by T-cells, fibroblasts, β cells of the pancreatic islets, developing oocytes and granule cells of ovarian follicles. The type 2 receptor (IL-1RII) is principally produced by basal epithelial cells of the skin, vagina, and urethra. Recent studies suggest that the IL-1RI is primarily responsible for mediating the biological effects of IL-1 (15). The central distribution of the IL-1RI has been thoroughly characterized at the mRNA level in mouse. However, there are reports with discrepant reports as to the distribution of this receptor in rat (18). By in situ hybridization assay, Ericsson et al. revealed that IL-1RI in rat brain is distributed predominantly over barrier-related cells, including the leptomeninges, non-tanycytic portions of the ependyma, the choroid plexus, and vascular endothelium. They also found that low to moderate levels of the IL-1RI mRNA were detected in several groups of neuronal cells, including the basolateral nucleus of the amygdala, the arcuate nucleus of the hypothalamus, the trigeminal and hypoglossal motor nuclei, and the area postrema (18). Our results are in part consistent with the observation by Ericsson et al., except for the distribution of IL-1R immunoreactive neurons. Interestingly, our study showed that the different localization of IL-1RI expression between young and old rat brain is detected immediately after LPS treatment. However, it is very difficult to interpret the biologic meaning of this age-related difference of IL-1RI expression at different points of time, whether it is associated with blunted fever response observed in the elderly, or not. Carlos et al. showed that old Long-Evans rats are not defective in their capacity to develop a fever in response to brain administration of IL-1β (19), suggesting that there is a difference of age-related fever response among species. Generally, it is known that IL-1 from the circulation may have access to cells of circumventricular organs such as the organum vasculosum of the lamina terminalis, in turn, producing prostaglandins (i.e. PGE_2) that can affect thermosensitive neurons in the preoptic anterior hypothalamus and thereby inducing a febrile response. However, our results showed that IL-1RI expression immediately after LPS treatment was detected in neurons of cerebral cortex and cerebellum, not circumventricular organ. Our possible assumption of this difference is that there may be present a kind of age-related, site-specific difference of cytokine expression and local immune regulation in the brain, at least, immediately after the relevant stimuli (20). However, the biologic meaning of this age-related different localization of IL-1RI should be evaluated further.

In conclusion, our study demonstrated that there is age-related difference in neuronal localization of IL-1RI expression at different point of time after LPS treatment.

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