The Study of IL-10 Expression and Immunologic Mechanism in the Brain Tissue of Mice with Herpes Simplex Virus Encephalitis*

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ABSTRACT Objective: To investigate the IL-10 expression in the brain tissue of mice with herpes simplex encephalitis and the possible immunologic mechanism. Methods: Mice were randomly divided into 4 groups with 7 mice in each: normal control group, HSV-1 infection group, acyclovir treated group, and dexamethasone treated group. The mice in last three groups were inoculated with HSV-1 solution to make the models of HSE. The expressions of IL-10 of the mice brain tissues in each group were compared by using immunohistochemisty. The mice were observed carefully and the score of neurological injury of the mice was assessed. Pearson Correlations was used to describe the correlations of cytokines and neurological injury score. Results: The neurological injury score was the highest in HSV-1 infection group than that in the other groups, and the dexamethasone treated group was the lowest than others (P<0.05). The neurological injury score in acyclovir treated group was between the scores in HSV-1 infection group and dexamethasone treated group. After HSV-1 infection, IL-10 was significantly increased compared with that in normal control group (P<0.05). The production of IL-10 in acyclovir treated group decreased compared with HSV-1 infection group (P<0.05), and examethasone treated group decreased significantly compared with that in HSV-1 infection group and acyclovir treated group (P<0.05). IL-10 and neurological injury score were positive correlation (r=0.82, P<0.05). Conclusions: IL-10 may inhibit the production of inflammatory cytokines by microglia and protect CNS against the immune injury to regulate the size of the immune response.

Key words: Herpes simplex encephalitis; Acyclovir; Glucocorticoid; IL-10

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Introduction

Herpes simplex virus encephalitis (HSE) is a life-threatening consequence of HSV infecting the central nervous system (CNS), with high mortality and serious morbidity even by appropriate antiviral treatment among survivors[1]. HSE is a sporadic encephalitis with an annual incidence of HSE was between 1/250 000 and 1/ 1 000 000 [2, 3]. The mechanism by which HSV-1 infection causes HSE has not been clear yet.. This study was to explore possible immune mechanisms in HSE after the treatment of acyclovir and dexamethasone and to guide the clinical treatment.

1 Materials and Methods

1.1 Materials

1.1.1 Mice Kunming mice were purchased from Qingdao Insititute For The Control Of Drug Products, and they were 3-week old male mice, weighing from 11 to 13 g. The mice were divided into 4 groups randomly: normal control group, HSV-1 infection group, ACV treated group and dexamethasone treated group. Each group had 7 mice.

1.1.2 Viruses HSV-1 strains were a kind gift from Microbiology Department of Qingdao Medical Collage, and it was titrated after its growth in Vero cells. The virus was stored in freezer at -80 ℃ until required.

1.2 Methods

1.2.1 Inoculation and intervention of the Mice Mice were anesthetized with 10% chloral hydrate (3.5 ml/kg), and inoculated with HSV-1 solution to make the models of HSE. The HSV-1 infection group, acyclovir treated group, and dexamethasone treated group were inoculated intracranially with 100TCID50 (10-2/l) viral solution 20uL. Normal control group were infused into culture solution of HSV-1 20μL. The mice in acyclovir treated group and dexamethasone treated group were fed with ACV (350 mg/kg) once a day for 6 days on the first day after the postinoculation. The mice in dexamethasone treated group were anesthetized with dexamethasone (2 mg/kg) on the third day after the postinoculation.

1.2.2 Neurological injury score Twenty eight mice were observed and the symptoms of neurological injury were recorded from 0 to 10 by score(Table 1).

1.2.3 Immunohistochemisty Two mice were died in HSV-1 infection group, one was on the 4th day, and the other was on the 5th day. One mouse of acyclovir treated group was died on the 5th day. The brains of them were preserved in 10% formaldehyde. The other mice were deep anesthetized on the 6th day after inoculation, and sacrificed by cardiac perfusion of 0.9% Nacl and 10% formaldehyde at room temperature. Their brains were preserved and prepared for immunohistochemisty.

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Table 1 Neurological injury score

	0	1	2	3
Behavior	Spontaneous exploratory behavior	Move when it was stimulated	Don't move	
Gait	Normal gait	Ataxia	Crawling	Don't move
Eating	Eating	Can't eat		
Drinking	Drinking	Can't drink		
The response of pain	Movable	Head or trunk movements	Limbs curled up	No response to painful stimulus

Note: 0 no neurological injury, 1-3 slightly neurological injury, 4-6 midrange neurological injury, 7-10 severe neurological injury

1.3 Statistical analysis

A one-way ANOVA was used for multiple comparisons. Data were shown as mean ± standard error. Pearson Correlations was used to describe the correlations of cytokines and neurological injury score. Statistical significance was set at $P<0.05$.

2 Results

2.1 The production of IL-10 in brain tissue

After HSV-1 infection, IL-10 was significantly increased comparing with normal control group ($P<0.05$). The production of IL-10 in acyclovir treated group was decreased comparing with HSV-1 infection group ($P<0.05$), and dexamethasone treated group was decreased significantly comparing with HSV-1 infection group and acyclovir treated group ($P<0.05$)(Table 2).

2.2 Neurological injury score

The neurological injury score was the highest in HSV-1 infection group, and the lowest in dexamethasone treated group($P<0.05$). The neurological injury score in acyclovir treated group was between the scores in HSV-1 infection group and dexamethasone treated group(Table 2).

Table 2 Expression of IL-10 and the neurological injury score in different group ($\bar{x}\pm s$)

Group	n	IL-10	Score
Normal control group	7	2.71± 1.25	0.00± 0.00
HSV-1 infection group	7	6.28± 1.38*	6.80± 2.67*
Acyclovir treated group	7	4.85± 1.07#*	4.57± 2.70*#
Dexamethasone treated group	7	3.14± 1.57#△	2.29± 0.95*#△

Note: *$P<0.05$, compare with normal control group ;#$P<0.05$, compare with HSV-1 infection group ;
△$P<0.05$, compare with dexamethasone treated group

2.3 The correlations of IL-10 and neurological injury score

IL-10 and neurological injury score had a positive correlation ($r=0.82$, $P<0.05$).

3 Discussion

Herpes simplex virus encephalitis (HSE) is a disease that mainly caused by HSV-1 infection. The HSV-1 infects sensory neurons proximal from the site of primary infection. Virions are then transported by retrograde axonal transport to neuronal cell bodies of Central Nervous System (CNS), injuring of neuronal cell. The factors which lead to the injury of neuronal cells are not only the direct infection of HSV-1, but the inflammatory cytokines produced in the immune response after the HSV-1 infected.

Microglia is the resident macrophages of the Central Nervous System CNS and the key innate immune cell against foreign invasions within the CNS. Microglia releases several inflammatory cytokines like TNF-α, IL-6 etc, while the high level of TNF-α for a long time always suggests a bad prognosis of HSE [4,5]. These cytokines enhance natural killer (NK) cell and NK-cell subset expansion and natural kill function[6,7]. Glial cells can cause CNS damage widespreadly after HSV-1 infection [8-10].

IL-10 can be produced by different cell types in humans and mice, including monocytes, macrophages, Th2 cells . IL-10 can directly inhibit the function of cytokine produced by T cells such as IL-2, IFN-α, and prevent the maturation of some immune cells. IL-10 is an immune-modulatory cytokine, regulating the activity of NK cells，and inhibiting the production of inflammatory cytokines by microglia to regulate the size of the adaptive immune response during early infection [11].

This study inoculated the mice with HSV-1 solution to make

the models of HSE. The score of neurological injury was assessed, and the expression of IL-10 in each group was compared by using immunohistochemisty to investigate the possible immune mechanisms in HSE. The slight symptom of CNS damage was found on 3th day, and the symptoms were becoming more and more serious. Serious symptoms of HSV-1 group were observed on 6th day and the production of IL-10 was higher than any other group since inoculated ($P<0.05$). These results suggest that the resident cells of brain can cause a severe inflammatory response after HSV-1 infection without effective therapy, leading to CNS damage and IL-10 increased. Ejrnaes, Stacey had studied that IL-10 increased rapidly following several acute viral infections in Serum [12, 13], as this study in the brain tissue. While the mechanism by which IL-10 mediates in early viral infection is not understood. Maybe it is associated with the function of anti-inflammatory and immunosuppression. In this study, IL-10 and neurological injury score were positive correlation, showing that IL-10 as a kind of cytokine which can keep the balance of the immunity reaction.

ACV is a potent and selective inhibitor of herpes virus DNA replication through the intermediate product-ACV triphosphate[14]. It is an established antiviral drug, was known to inhibit indoleamine 2,3-dioxygenase (IDO), and this is one mechanism to inhibit herpes virus DNA replication [15-17]. Compared with that in the HSV-1 group, the clinical symptoms improved and the decrease of IL-10 was sighnificantly ($P<0.05$) in antiviral group.

Glucocorticoid (GCs), which is known for immunosuppressive and anti-inflammatory drugs, has been shown to inhibit the gene expression and production of many cytokines which are known to induce inflammatory or immunologic responses. GCs can make proinflammatory signal transduction pathways and gene expression decreased to avoid exaggerated immune reaction. It also dysregulates NK cell function by reduce promoter accessibility which modifies the histone acetylation status. At last it decreases the expression of effector proteins necessary to make the full functional activity of NK cells [18]. After the viral infection, GCs have been documented to upregulate the expression of IL-10[19,20]. Study showed that the expression of the IL-10 gene which was induced by glucocorticoid was mediated by the transcription factor STAT3[21]. This study inferred that in the period of acute inflammatory response, the therapy of adaptive GCs or IL-10 can improve the neurological injury symptom of patients.

IL-10 regulates inflammatory responses by microglia and other cells to protect CNS against the immune injury. And GCs in combination with antiviral therapy can improve the clinical symptom of patients.

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IL-10 在单纯疱疹病毒性脑炎小鼠脑中的表达及其机制的研究 *

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摘要 **目的**:研究在单纯疱疹病毒性脑炎小鼠脑组织中 IL-10 的表达并探讨其可能的免疫学机制。**方法**:小鼠被随机分为四组,每组 7 只:空白对照组,病毒感染组,阿昔洛韦组和地塞米松组。后 3 组的小鼠被接种 HSV-1 病毒液制造单纯疱疹脑炎模型。我们用免疫组化的方法比较各组脑炎小鼠脑组织中 IL-10 的表达。观察小鼠神经损伤的表现,行神经症状评分。并采用 Pearson 相关分析神经症状评分与 IL-10 表达的相关性。**结果**:病毒感染组神经损伤最重,神经症状评分最高,阿昔洛韦组次之,地塞米松组最低(P<0.05)。IL-10 在 HSE 小鼠脑组织中的表达较正常对照组明显上调(P<0.05),病毒感染组较阿昔洛韦组高(P<0.05),病毒感染组和阿昔洛韦组较地塞米松组增高(P<0.05)。神经症状评分与 IL-10 的表达呈正相关(r=0.82, P<0.05)。**结论**:IL-10 可能抑制小胶质细胞炎性因子的产生,减轻神经细胞的损伤等介导宿主在 HSE 中的免疫应答作用。

关键词:单纯疱疹病毒性脑炎;阿昔洛韦;糖皮质激素;IL-10

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