STUDY ON THE ROLE OF IL-6/IL-6R/GP130 SIGNAL TRANSDUCTION PATHWAY IN NEUROIMMUNOMODULATION

Shuangyan Zheng[1, 2, #], Chunrong Zeng[1, 2, #], Menglan Liu[1, 2], Panyi Xiang[1, 2], Lin Wang[1, 2], Zuoxiao Li[1, 2, *]
[1]Department of Neurology, the Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan, 646000, China - [2]Neurological Diseases and Brain Function Laboratory, Luzhou, Sichuan, 646000, China
[#]These authors contributed equally to this work as co-first author

ABSTRACT

Objective*: To analyze the role of the IL-6/IL-6R/gp130 signal transduction pathway in neuroimmunomodulation.*

Methods: *IL-6 knockout mice (experimental group) and normal mice were divided into a non-immune group, immune 2d group, immune 4d group, immune 6d group or immune 8d group, with eight mice in each group. To compare the expression of IL-1β between mice with varying immune status, the immunohistochemistry method was used to measure the expression of IL-1β in the central brain region of mice. Then, the ELISA method recorded the expression of IL-1β and TNF-α in the peripheral blood of mice to study the role of the IL-6/IL-6R signaling pathway in the neuroimmunomodulatory network.*

Results: *The expression level of IL-1β in the central brain region of mice in the experimental group with varying immune status, was lower than that of normal mice, however this difference was only significant when the immune 4d and immune 6d experimental mice were compared to normal mice (P<0.05). As expected, expression of IL-1β in the central brain regions in non-immune experimental mice was lower than that of the normal group (P<0.05). Although expression of IL-1β in the peripheral blood of mice with varying immune status in the experimental group, was markedly lower than that of normal mice, this difference was only significant in the immune 8d group (P<0.01). As expected, the expression level of IL-1β in the central brain region of mice in the non-immune group was also significantly lower than that of the normal group (P<0.05). The expression level of TNF-α in the peripheral blood of mice with varying immune status in the experimental group was significantly higher than that recorded in the immune 2d group (P<0.05). There was no statistically significant difference in the expression level of TNF-α in the peripheral blood of mice between the non-immune group and the normal group (P>0.05).*

Conclusion: *The expressions of IL-1β and TNF-α in IL-6 knockout mice under varying immune status were reduced to different degrees. It is speculated that the loss of IL-6/IL-6R signal influences regulation of the peripheral immune system, which is involved in a variety of complex physiological function transmissions and plays an important role in neuroimmunomodulation.*

Keywords: *IL-6/IL-6R/gp130 signal pathway, neuroendocrine system, immune system.*

DOI: 10.19193/0393-6384_2021_4_355

Received *March 15, 2020;* ***Accepted*** *October 20, 2020*

Introduction

In recent years, the relationship between the neuroendocrine system and the immune system has received increasing attention. There is substantial nerve distribution throughout the immune organs. The nervous system interacts with immune cell receptors through the pituitary endocrine pathway, which is composed of neuroendocrine, neurotransmitters and neuropeptides. The nervous system regulates the lymphoid organs and lymphocytes transmit immune information to the central nervous system through lymphoid factors, forming a bidirectional regulatory ring[(1)]. The neuroendocrine system regulates immune response in the body by releasing neurotransmitters and neuropeptides to affect the function of the immune system. The immune system and immune reaction processes have varying degrees of influence on the function of the neuroendocrine system. The neuroendocrine system and immune system interact

and influence each other to maintain homeostasis and resist the invasion of harmful substances[2, 3]. During this process, cytokines released by the immune system play a major signaling role. Cytokines are a group of important immunomodulatory peptides that are produced by various types of cells; they affect the secretion of hormones and the growth and development of endocrine glands.

Cytokines play an important role in immune system inflammation, nervous system pain, sleep, mood and other processes[4]. A large number of studies have shown that neuroendocrine cells can directly or indirectly affect the action of cytokines, which can affect the release of hormones in the hypothalamic pituitary gland or inhibit hormone secretion[5, 6]. Interleukin 6 (IL-6) is a recognized intra- and cross-system signaling pathway. In the present study, IL-6 knockout mice were used to model various states of immunity in order to analyze the role of the IL-6/IL-6R/gp130 signal transduction pathway in neuroimmunomodulation.

Materials and methods

Experimental reagents and instruments

The following reagents were utilized:

• Normal saline (Shanghai Yuanye Biotechnology Co., Ltd.);

• Rabbit anti-mouse IL-1β antibody (Shanghai Hengfei Biotechnology Co., Ltd.);

• Purified human serum γ globulin IgG (Shanghai Yubo Biotechnology Co., Ltd.);

• A DAB chromogenic kit (Beijing Chreagen Biotechnology Co., Ltd.);

• Xylene (Shanghai Yaji Biotechnology Co., Ltd.);

• Neutral gum (Shanghai Jinsui Biotechnology Co., Ltd.);

• APES (Beijing Fubo Biotechnology Co., Ltd.);

• A mouse IL-1β ELISA kit (Wuhan Fine Biotechnology Co., Ltd.);

• A mouse TNF-α kit (Shanghai Zhenyu Biotechnology Co., Ltd.).

The following experimental instruments were utilized:

• A -4°C refrigerator (Guangzhou Kezhilan Instrument Co., Ltd.);

• A -20°C refrigerator (Shanghai Xinyu Biotechnology Co., Ltd.);

• An optical electron microscope (Guangzhou Mingmei Optoelectronic Technology Co., Ltd.);

• An electronic balance (Dongguan Pubiao Experimental Equipment Technology Co., Ltd.);

• A microplate reader (Shanghai Zuofei Laboratory Equipment Co., Ltd.);

• A constant temperature magnetic stirrer (Shanghai Chenlian Biotechnology Development Co., Ltd.);

• A microwave oven (Galanz Electrical Appliance Factory, Shunde, China);

• An electric thermostatic incubator (Wuhan Chundu Biotechnology Co., Ltd.).

Establishment and grouping of experimental animal models

IL-6 knockout mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd; normal mice were purchased from Beijing Baiao Innovation Technology Co., Ltd. Subjects were 40 IL-6 knockout mice and 40 normal mice; they were kept in cages with an average temperature of 22 to 28oC, 65% to 75% humidity, kept in an alternate day-night 12 h cycle with a common diet and free drinking water. IL-6 knockout mice (experimental group) and normal mice were equally divided into five groups: a non-immune group, immune 2d group, immune 4d group, immune 6d group and immune 8d group; each group contained eight mice.

The purified human serum γ globulin IgG was dissolved in sterile saline and mixed with Freunds Complete Adjuvant to prepare the water-in-oil emulsion. All mice except for the non-immune group, were injected with a 0.1ml water-in-oil emulsion in the tail, blood and brain samples were then taken on days 2, 4, 6 and 8 for subsequent experiments.

Detection indicators

The immunohistochemistry method was used to detect the expression of IL-1β in the central brain region of mice with varying immune status. Sections of brain tissue were placed at room temperature for resuscitation and then submerged in a 0.01M citrate-sodium citrate repair solution. Brain tissue was then repaired with microwave heating for three rounds lasting 7min, 4min and 4min, and then left to cool to room temperature.

Tissue sections were then balanced with distilled water for three minutes, followed by two rounds of balancing with a 0.05M buffer solution. The primary antibody diluent was prepared with a 0.05M PBS buffer solution, mixed and dropped into brain tissue sections, and then incubated at 37°C for two hours.

Sections were then removed and washed with a PBS buffer. The secondary antibody mixture was prepared with a 0.05M PBS buffer that was mixed and dropped into brain tissue sections, and then incubated at 37°C for one hour.

Sections were then removed and rinsed with a PBS buffer. Horseradish peroxidase-labeled avidin was diluted with a 0.05M PBS buffer, mixed and dropped into brain tissue sections, then incubated at 37°C for one hour; sections were then removed and rinsed with a PBS buffer. A concentrated DAB kit was used for color development. First, 1ml of reagent A was added with one drop of reagent B; after mixing evenly, they were dropped onto brain tissue sections and incubated at room temperature for eight minutes. Next, the sections were dehydrated using an alcohol gradient and were sealed with neutral gum. Following drying, the sections were observed under an optical microscope and photographed.

IL-1β and TNF-α expression in the peripheral blood of mice was recorded using the ELISA method. First, a wash buffer was used to wash the 96-well ELISA plate coated with antibodies; the plate was washed twice, for three minutes each time. The prepared standard substance and serum samples were then added to each well, followed by the diluted biotin-binding substance; the plate was then incubated with oscillation at room temperature for two hours. Liquid in the 96-well plate was then dried and the plate was rinsed three times using a wash buffer, for three minutes each time. Next, diluted avidin-HRP conjugate was added to each well and then incubated with oscillation at room temperature for one hour. Again, liquid in the 96-well plate was dried and the plate was rinsed three times using a wash buffer, for three minutes each time.

The TMB substrate solution was then added to the 96-well plate, it was incubated at room temperature in the dark for 10 minutes; the termination solution was then added to terminate the color development reaction. The OD value at 450nm was determined using a microplate reader.

Statistical methods

Data were analyzed using SPSS 21.0. Measurement data were expressed as mean ± standard deviation ($\bar{x}\pm s$).

Mean differences between two groups were assessed for statistical significance using t-tests, while Analysis of Variance (ANOVA) was used to compare the means of multiple groups. $P<0.05$ indicated statistically significant difference.

Results

Integral optical density values of IL-1β in the central brain region of mice with varying immune status

The expression level of IL-1β in the central brain region of mice with varying immune status in the experimental group was substantially lower than that of normal mice; this difference was statistically significant between the immune 4d group and the immune 6d group ($P<0.05$). The expression level of IL-1β in the central brain region of mice in the non-immune group of experimental mice was also lower than that of the normal group ($P<0.05$). Results are shown in Figure 1.

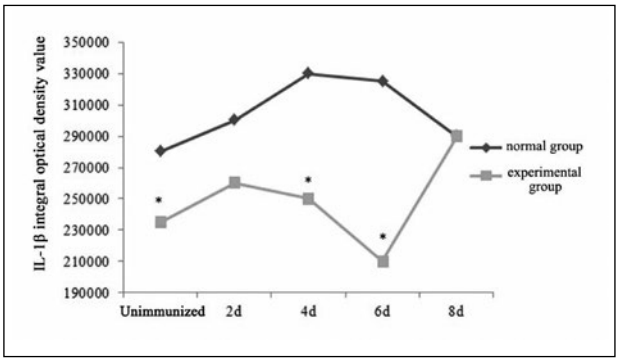

Figure 1: Expression trend of integrated optical density values of IL-1β in the central brain regions of mice with varying immune status.
*Notes: *P<0.05 compared with normal mice in the same group.*

Expression level of IL-1β in the peripheral blood of mice with varying immune status

The expression level of IL-1β in the peripheral blood of mice with varying immune status in the experimental group was markedly lower than that of normal mice, and this difference was statistically significant in the immune 8d group ($P<0.01$). The expression level of IL-1β in the peripheral blood of mice in the non-immune group was also significantly lower than that of the normal group ($P<0.05$). Results are presented in Table 1 and Figure 2.

Groups	Non-immune	Immune 2d	Immune 4d	Immune 6d	Immune 8d
Experimental	30.93±2.09*	29.37±3.10	28.69±1.38	27.53±1.28	28.06±1.17*
Normal	28.57±2.14	28.61±0.93	29.96±2.12	28.54±2.03	33.24±1.37
t	2.232	0.664	1.420	1.190	8.132
P	0.043	0.517	0.177	0.254	<0.001

Table 1: Expression level of IL-1β in the peripheral blood of mice with varying immune status.
*Notes: *P<0.05 compared with normal mice in the same group.*

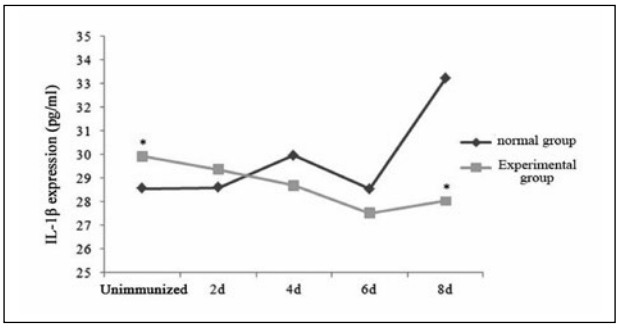

Figure 2: Expression level of IL-1β in the peripheral blood of mice with varying immune status.

Expression level of TNF-α in the peripheral blood of mice with varying immune status

As expected, the expression level of TNF-α in the peripheral blood of mice with varying immune status in the experimental group was higher than in the immune 2d group, and this difference was statistically significant (P<0.05). There was no statistically significant difference in the expression level of TNF-α in the peripheral blood of mice between the non-immune group and the normal group (P>0.05). Results are illustrated in Table 2 and Figure 3.

Groups	Non-immune	Immune 2d	Immune 4d	Immune 6d	Immune 8d
Experimental	233.34±24.13	262.21±24.02*	247.11±21.52	244.39±14.16	241.05±18.41
Normal	220.05±12.29	223.15±9.69	235.40±25.08	268.07±39.58	255.42±31.09
t	1.388	4.265	1.002	1.593	1.125
P	0.187	0.001	0.333	0.133	0.280

Table 2: Expression level of TNF-α in the peripheral blood of mice with varying immune status.
*Notes: *P<0.05 compared with normal mice in the same group.*

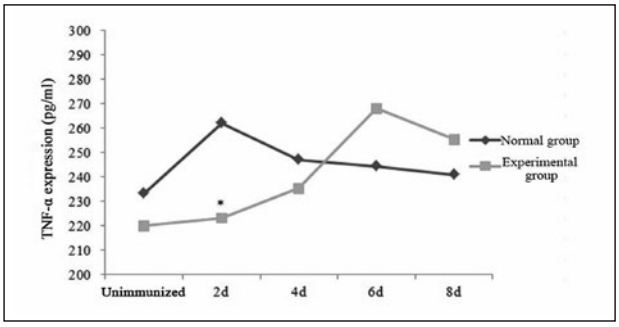

Figure 3: Expression level of TNF-α in the peripheral blood of mice with varying immune status

Discussion

As immune system functions consist of surveillance, defense and regulation, understanding its complex processes is an important to progress in clinical treatment. The immune system can resist invasion of foreign pathogenic microorganisms and maintain the stability of the body's internal environment and physiological balance. As important regulatory signals in the immune system, cytokines generally regulate cell growth and differentiation by binding to corresponding receptors, they also participate in the functional regulation at various stages of immune response[7]. Activating system signals expressed by immune active cells can complete the internal regulation of the immune system and activate the immune signal transduction network of the central nervous system. According to neuro-immuno-endocrine network theory, nerve conduction pathways may be important for the transmission of immune information[8]. In this theory, cytokines are considered to regulate the regeneration and differentiation of neurons and glial cells, the maturation and remodeling of synapses and other structures, and regulate functional activities during the development and remodeling of the central nervous system. Abnormalities in this process will affect neural networks related to neural functions, promoting nervous system diseases[9].

IL-6 is mainly produced by macrophages. As a systemic inflammatory factor, IL-6 regulates the role between the immune system and the nervous system. As a key cytokine in the nervous system, and as an independent factor, it can regulate the occurrence and development of nervous system diseases. L is multipotent and multifunctional in the immune system; it is mainly involved in the clearance of infectious pathogens, the promotion of tissue healing, the differentiation and development of hematopoietic cells, the induction of acute immune response, and physiological function transmission of the nervous system[10]. In terms of signal transduction, the IL-6 signal pathway can be divided into the classical signal pathway and the cross pathway. IL-6 binds to membrane receptors in the classical signal transduction pathway and is only expressed in macrophages, T cells, neutrophils and in other specific types of cells[11]. IL-6 is composed of two IL-6, IL-6R, and a gp associated molecule, 130 (gp130). IL-6 binds to IL-6R to produce an IL-6/IL-6R complex. Relevant data showed that IL-6R was only expressed in monocytes, T cells, B cells, while gp130 was widely expressed in the heart, liver, kidney, spleen, brain, and that IL-6 may up-regulate the expression of gp130. IL-6/IL-6R complex binds to gp130, which is responsible for signal transduction on the cell membrane, to produce homologous dimer gp130, which activates

the intracellular JAK-STAT and MAPK pathways[12]. IL-6 recruits JAK to the gp130 binding site through gp130 and activates JAK to phosphorylate STAT3 and dimer in the nucleus, thus playing the role of transcription factor and regulating the expression level of various cytokines associated with acute phase response and inflammation[13]. IL-6/IL-6R signal transduction pathway imbalance can promote a variety of inflammatory diseases and other serious diseases, and has important biological activities in the body. IL-6TS can lead to the downregulation of IL-6 and the sIL-6R-dependent tumor suppressor gene Maspin, which is closely related to the occurrence and development of chronic inflammatory diseases and various tumors. IL-6 is closely related to other cytokines involved in immune response and cross-system signaling. Stromal cells and osteoblasts secrete IL-6 in the presence of IL-1 and TNF-α[14]. Relevant data showed that IL-1 was an effective promoter to induce the production of IL-6, and that the expression level of IL-6 could be significantly increased in eye socket connective tissue fibroblasts treated with IL-1[15].

The results of this study showed that the expression levels of IL-1β and TNF-α in the peripheral blood of mice with varying immune status in the experimental group were significantly lower than those of normal mice in some groups ($P<0.05$). Statistically significant differences in IL-1β and TNF-α in the central nervous system and peripheral blood of IL-6 gene knockout mice compared to normal mice, both under various immune states, supports IL-6 as a key influencer in the central nervous system and throughout the body as a multipotent public signal between systems.

In conclusion, expression levels of IL-1β and TNF-α in IL-6 knockout mice were reduced to different degrees under varying immune conditions. It is considered that the absence of IL-6/IL-6R signal influences regulation of peripheral immune system function, which is involved in a variety of complex physiological function transmissions, and plays an important role in the process of neuroimmunomodulation.

References

1) De Angelis E, Pecoraro M, Rusciano MR, Ciccarelli M, Popolo A. Cross-talk between neurohormonal pathways and the immune system in heart failure: A review of the literature. Int J Mol Sci 2019; 20: 1698.
2) Sears CG, Sears L, Zierold KM. Sex differences in the association between exposure to indoor particulate matter and cognitive control among children (age 6-14 years) living near coal-fired power plants. Neurotoxicol Teratol 2020; 78: 106855.
3) Agorastos A, Bozikas VP. Gut microbiome and adaptive immunity in schizophrenia. Psychiatriki 2019; 30: 189-192.
4) Berczi I. The Neuroimmune Supersystem (NISS). Adv Neuroimmune Biol 2018; 7: 3-12.
5) Spichak S, Dinan TG, Cryan JF. Gut–neuroimmune interactions: The unexpected role of the immune system in brain development. Biochem 2019; 41: 36-41.
6) Korneva EA, Perekrest SV, Shainidze KZ, Novikova NS, Pugach VA, et al. Effects of restraint stress on lipopolysaccharide-induced reactions of orexinergic system. Adv Neuroimmune Biol 2017; 6: 131-138.
7) Merlini E, Iannuzzi F, Calcagno A, Bai F, Trunfio M, et al. Peripheral and cerebrospinal fluid immune activation and inflammation in chronically HIV-infected patients before and after virally suppressive combination antiretroviral therapy (cART). J Neurovirol 2018; 24: 679-694.
8) Fiandra L, Capetti A, Sorrentino L, Corsi F. Nanoformulated antiretrovirals for penetration of the central nervous system: State of the art. J Neuroimmune Pharmacol 2017; 12: 17-30.
9) Limanaqi F, Biagioni F, Busceti CL, Ryskalin L, Soldani P, et al. Cell clearing systems bridging neuro-immunity and synaptic plasticity. Int J Mol Sci 2019; 20: 2197.
10) Johnston PA, Sen M, Hua Y, Camarco DP, Shun TY, Lazo JS, et al. HCS campaign to identify selective inhibitors of IL-6-induced STAT3 pathway activation in head and neck cancer cell lines. Assay Drug Dev Technol 2015; 13: 356-376.
11) Gao Y, Yang J, Cai Y, Fu S, Zhang N, et al. IFN-γ-mediated inhibition of lung cancer correlates with PD-L1 expression and is regulated by PI3K-AKT signaling. Int J Cancer 2018; 143: 931-943.
12) Graham A, Charles M. A Role for soluble IL-6 receptor in osteoarthritis. J Funct Morphol Kinesiol 2017; 2: 27-35.
13) Cole AJ, Fayomi AP, Anyaeche VI, Bai S, Buckanovich RJ. An evolving paradigm of cancer stem cell hierarchies: Therapeutic implications. Theranostics 2020; 10: 3083-3098.
14) Kirchmeyer M, Hamdorf M, Minoungou NWE, Rose-John S. Modulation of the IL-6-signaling pathway in liver cells by miRNAs targeting gp130, JAK1, and/or STAT3. Servais FA, Mol Ther Nucleic Acids 2019; 16: 419-433.
15) Dehghani B, Hashempour T, Hasanshahi Z. Interaction of human herpesvirus 8 viral interleukin-6 with human interleukin-6 receptor using in silico approach: The potential role in hhv-8 pathogenesis. Curr Proteom 2020; 17: 107-116.

Corresponding Author:
Zuoxiao Li
Email: qsi26t@163.com
(China)