Comparative study on five kinds of cytokines in the tissues of Chinese giant salamander (*Andrias davidianus*) of different developmental stages

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Abstract. By using of the double antibody sandwich method of ELISA, the activities of five cytokines including IL-2, IL-4, IFN-α, IFN-β and TNF-α from the blood serum, liver, intestine and spleen at two developmental stages of Chinese giant salamander (*Andrias davidianus*) were determined to analyze the distribution of the cytokines. The result indicated that five cytokines were found in these four tissues, while their activities were different in different tissues and different ages. The highest activity of IL-2 and IL-4 was all present in blood serum of two different ages. The activity of IFN-α was the highest in blood serum of 1-year-old and in spleen of 2-year-old, respectively. The activity of IFN-β was also highest in blood serum of two different ages. The activity of TNF-α was highest in liver of two different ages. Thus, this study provides convincing reference for blood serum and liver as the most important distribution area of Chinese giant salamander.

1. Introduction

Cytokines belong to kinds of small molecular polypeptides with biological activity synthesized and secreted by a variety of activated cells, and it involved in intercellular signal transduction and interaction [1]. According to the structure and biological function, cytokines can be divided into interleukin, interferon, colony-stimulating factor, chemokine, growth factor and tumor necrosis factor [2]. As an important medium of vertebrates intracellular and extracellular communication, cytokines participate in the regulation of hematopoiesis, inflammatory response, damaged tissue repair and host immune defense response [2-3].

In aquatic animals, the immune defense mechanism consists of specific and non-specific immune responses. The non-specific immune responses of aquatic animals include the skin mucus barrier, the phagocytosis of reticular endothelial system and some cytokines in body fluids or tissues [4-5].

Interleukin (IL) is an important cytokine closely related to the non-specific immune regulation function, and its typical members include IL-1, IL-2, IL-4, IL-6 and so on [1]. Previous studies have shown that many types of IL were found in aquatic vertebrates and its play a regulatory role in the immune response [4,6]. Interferon type I (IFN-I) is an important secretory cytokine with broad-spectrum
antiviral activity and immunomodulatory ability in aquatic vertebrates, and which mainly including IFN-α and IFN-β [7-8]. Tumor necrosis factor-α (TNF-α) is a cytokine produced by activated phagocytes, lymphocytes and other immune cells invaded by pathogens [9]. As an important cytokine, TNF-α plays an important role in regulating host defense and inflammatory response of aquatic vertebrates against bacteria and virus infection [10-11].

Chinese giant salamander (*Andrias davidianus*) is a unique and rare aquatic amphibian species in China, and which has been listed in the Class II national protected species and CITES Appendix I [12]. In recent years, with the rapid development of culture, the disease problem has caused huge economic losses to breeding industry of Chinese giant salamander [13]. Cytokines such as IL, IFN-1 and TNF-α with immune activity play an important role in the immune defense response of aquatic vertebrates against pathogen infection [2]. In addition, Chinese giant salamander (*Andrias davidianus*) has important evolutionary and physiological characteristics from aquatic to terrestrial [12], so it is of great theoretical significance to study the role of cytokines in the immune defense response of Chinese giant salamander (*Andrias davidianus*). In this paper, the activity and distribution of five cytokines (IL-2, IL-4, IFN-α, IFN-β and TNF-α) in blood serum, liver, intestine and spleen at two different developmental stages of Chinese giant salamander (*Andrias davidianus*) were determined, which might provide theoretical support for the immune defense mechanism and healthy breeding of Chinese giant salamander (*Andrias davidianus*).

2. Material and Methods

2.1. Experimental materials
Chinese giant salamander (*Andrias davidianus*) was collected from Luanchuan in Henan Province, the three tails of 1-year-old and 2-year-old Chinese giant salamander were chosen, respectively. The Chinese giant salamander were not feed before the experiment, and water temperature was kept at about 20°C.

2.2. Preparation of tissue supernatant
After anesthetized and killed with MS-222 (a common fish anesthetics), the Chinese giant salamander (*Andrias davidianus*) were dissected by sterilized scissors. 0.3ml of blood from heart was collected and centrifuged at 4°C for 10 minutes to harvest blood serum. The tissues (liver, intestine and spleen) were cut about 0.1g weight added 1ml sterile 0.7% NaCl solution to homogenize in ice bath, and then these three tissues homogenate was centrifuged at 4°C for 20 min to collect supernatant.

2.3. Determination of cytokine activity
According to the manufacturer’s instructions, the activities of five cytokines (IL-2, IL-4, IFN-α, IFN-β and TNF-α) were determined by using the double antibody sandwich method of ELISA Kit (Shanghai Yubo Bioengineering Institute, China).

2.4. Data analysis
The significance was analyzed by Student’s t-test and SPSS 13.0. Capital letters represented activity of different tissues at the same age group, lowercase represented activity of same tissue at different age groups. Adjacent letters represented a significant difference ($P<0.05$), interval letters represented a very significant difference ($P<0.01$).

3. Results and Discussions
IL-2 and IL-4 were produced by activated monocyte macrophages and lymphocytes, which can directly promote B cells proliferation and differentiation [14-15]. In aquatic vertebrates, the activity of IL-2 has been found [4,14,16], but the detection of IL-4 activity is rarely reported. Guo found that IL-2 activity in the supernatant of spleen cell culture of grass carp was lower than that of Chinese soft shelled turtle
In addition, the activity of IL-2 in grass carp and Chinese soft shelled turtle over 1-year-old was significantly higher than that of grass carp and Chinese soft shelled turtle seedlings [17]. In this paper, the results showed that the activity of IL-2 and IL-4 were all highest in blood serum of different ages of Chinese giant salamander (Figure 1 and Figure 2), which indicated that blood plasma was the most important distribution area of IL-2 and IL-4 in different ages of Chinese giant salamander.

In vertebrates, IFN-α and IFN-β was mainly synthesized by mononuclear phagocytes and fibroblasts, respectively [8,18]. At present, IFN has been detected from all kinds of aquatic vertebrates [4]. This experimental result showed that the highest activity of IFN-α was found in blood serum of 1-year-old and in spleen of 2-year-old Chinese giant salamander, respectively (Figure 3 and Figure 4). The highest activity of IFN-β was all present in blood serum of different ages of Chinese giant salamander. This result suggested that blood serum was also the main distribution area of IFN-α and IFN-β in Chinese giant salamander.

**Figure 1.** The separation and activities of IL-2 in Chinese giant salamander at different ages

**Figure 2.** The separation and activities of IL-4 in Chinese giant salamander at different ages
Blood is the liquid tissue existing in the circulatory system of aquatic vertebrates, and which contains a large number of immune related cells including red blood cells, lymphocytes, mononuclear phagocytes, granulocytes and so on, and it plays an important role in humoral regulation, immune defense and maintaining the stability of the internal environment [19-20]. Previous studies have also shown that immune related lymphocytes and mononuclear phagocytes can produce and secrete a large number of cytokines such as IL and IFN [8,14]. This experiment results also showed that blood serum was the main distribution place of IL-2, IL-4, IFN-α and IFN-β in Chinese giant salamander.

In aquatic vertebrates, TNF-α is mainly produced and secreted by macrophages, lymphocytes and fibroblasts. Previous studies have found that TNF-α can be detected in fish and amphibians [10-11, 21-22]. From this result, the activity of TNF-α was all highest in liver of different ages of Chinese giant salamander (Figure 5). This result showed that liver was the main distribution area of TNF-α in Chinese giant salamander. According to Fang [23], the liver of Chinese giant salamander is an important digestive and detoxification organ contained a large number of red blood cells, fibroblasts and phagocytes, which may be closely related to hematopoiesis and immune function. This is also consistent with Fang's [23] finding that the liver was rich in immune related cells that can produce TNF-α in Chinese giant salamander.
From the results of this study, the high activity of IL-2, IL-4 and IFN-β was all present in blood serum of two different ages. The activity of IFN-α was higher in blood serum of 1-year-old and in spleen of 2-year-old, respectively. The higher activity of TNF-α was found in liver of two different ages. In addition, five kinds of cytokines were distributed mainly in blood serum and liver of Chinese giant salamander.

4. Conclusion
In conclusion, the results indicated that five cytokines were found in these four tissues, while their activities were different in different tissues and different developmental ages. In addition, this study provided convincing reference for blood serum and liver as the most important distribution area of five kinds of cytokines in Chinese giant salamander.

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