Original Article

Cytokine levels are associated with the severity of varicella infections

Meijun Hao[1]*, Xiaojun Wang[2]*, Jingpei Du[3], Lifeng Liu[2], Yanmei Jiao[2], Hao Wu[2], Jiasheng Zheng[1], Wei Li[2]

[1] *Intervention Therapy Center of Liver Diseases, Beijing You'an Hospital, Capital Medical University, Beijing, China*
[2] *Center for Infectious Diseases, Beijing You'an Hospital, Capital Medical University, Beijing, China*
[3] *Department of Infectious Diseases, Third Teaching Hospital, Xinxiang Medical College, Xinxiang, Henan, China*

*These authors contributed equally to this work

Abstract

Introduction: Varicella is a highly contagious disease. Epidemics of varicella are seen every year globally and present a threat to public health, especially in China and other developing countries.

Methodology: Clinical and laboratory findings of 865 varicella patients admitted to Beijing You'an Hospital, China, between January 2011 and December 2013 were collected and analyzed. Patients with isolated complication were grouped as SI (skin infection, n = 132) and LD (liver damage, n = 89). Two hundred and one patients without complications were grouped as control (mild group). Levels of T-cell subtypes and eight serum cytokines and were also tested. Levels of IFNγ and IL-6 were monitored prospectively in another 12 grouped patients.

Results: SI was complicated in 21.7% (188/865) of varicella cases, and LD was complicated in 16.8% (145/865). The rates of SI and LD in varicella patients increased rapidly in the past three years. No laboratory findings were associated with SI or LD (all $p > 0.05$). IL-6 and IFNγ levels were correlated with amniotic membrane extract (AME) ($p = 0.044$ and $p = 0.038$). Their levels peaked at day 1 of admission, and then started to decline.

Conclusions: The incidence of serious complications has become more common in recent years. IL-6 and IFNγ may possibly be used as early serum markers for identifying patients at risk of developing complications such as skin infections in varicella.

Key words: varicella; chickenpox; clinical features; cytokines; T-cell subtypes.

J Infect Dev Ctries 2015; 9(2):190-196. doi:10.3855/jidc.5255

(Received 05 May 2014 – Accepted 28 August 2014)

Introduction

Varicella (also named chickenpox) is an acute and highly infectious disease caused by the varicella zoster virus (VZV) [1]. Most infected patients are between one and nine years of age. The incidence rate of varicella is around 10–20/1,000 people per year [2]. Although generally considered a mild disease in children, varicella can also have serious complications and detrimental sequelae [2]. Death can also occur in severe cases [2,3]. The most common complications of varicella include skin infection and liver damage [2].

The vaccine against varicella was developed almost 20 years ago and has been used worldwide, which led to a sharp decline in the incidence of varicella within a few years [2,3,4]. In China, the varicella-zoster vaccine is not included in the national immunization program, and is only available commercially.

Despite good vaccination coverage around the world, major outbreaks have occurred in many countries, including developed countries such the United States, England, and Korea [2,4-6]. In China, the vaccine has been available in the private sector for a decade and its overall vaccine effectiveness (VE) is over 83% [7]. The persistent occurrence of varicella cases have brought global attention to the efficacy of the vaccines and dynamic changes of the disease epidemiology. Careful evaluation is required to adopt effective public health measures.

Clinical diagnosis based on typical skin rash and fever is not very difficult in populations with poor vaccine coverage; however, there is currently no way to predict or make an early diagnosis for severe cases with complications such as skin infection and liver damage, due to poor understanding of the molecular mechanisms of VZV pathogenesis [8]. The question remains about how to make accurate and early diagnosis and to avoid the development of serious

complications. Certain aspects of host immune responses to VZV infection may be used in diagnosis, such as T-cell subtypes in cellular immunity and different cytokine levels. Cytokines are key mediators in inflammatory processes during viral infections. Their levels have been found to be associated with VZV infection. VZV infection is known to be able to affect host IL-1 level, which is one of the key proinflammatory mediators in response to viral infections [9]. Cellular responses to VZV infections have also been studied and have been proven to be critical [10-13].

The objective of this study was to summarize the clinical and laboratory data of a group of varicella patients admitted to Beijing You'an Hospital, China, and to evaluate the possible correlation that may exist between serum markers and disease severity. Patients with skin infection and/or liver damage were defined as severe cases, since these are the most common complications of varicella infections seen in practice. This study aimed to characterize the current clinical features, epidemiology, and dynamic changes of varicella infections in China. In addition, we sought to identify potential early serum marker(s) of disease severity, so that prophylactic measures could be taken to reduce the incidence of serious complications.

Methodology

Case definition

Varicella infection was defined as either the isolation of the virus from at least one site (throat swab, blood, stool, cerebrospinal fluid [CSF], or other), or a fourfold rise in varicella IgG antibody titer in acute and convalescent sera, with a negative bacterial culture. Skin infection (SI) was defined as a disease characterized by bacterial skin infections with or without positive bacterial culture. Liver damage (LD) was defined as elevated liver function parameters from blood tests.

Study population

The study population comprised 865 patients who met the case definition described above. The patients were consecutively admitted to Beijing You'an Hospital, Capital Medical University (Beijing, PR China) between January 2011 and December 2013. Patients were grouped into three categories based on disease severity. The first group, mild, included varicella patients without SIs or LD; the second group, SI, included varicella patients complicated with SI only; the third group, LD, included varicella patients complicated with LD only.

Data source

All parameters, except kinetic level changes of IL-6 and IFNγ, included in the investigation were collected by reviewing patients' medical records, which were preserved in the medical record library and the medical computerized database at Beijing You'an Hospital, Capital Medical University. The patient records were retrospectively examined for the primary set of data, which included demographic characteristics (age and sex), clinical parameters (signs and symptoms), laboratory values (hematologic, biochemical, and microbiological findings), radiologic data, patient's status at discharge (recovered or died), admission and discharge dates, and length of hospital stay (LOS). For kinetic level changes of IL-6 and IFNγ, 12 typical patients (four from each of mild, SI, and LD groups) were selected.

The ethics committee of Beijing You'an Hospital approved this study, which covered the retrospective analysis of the 865 records and the additional study of cytokine levels in 12 patients. Parents or caretakers of all 865 participants, including 12 patients for cytokine research, gave written informed consent on behalf of their participating children for their children's information to be stored and used for research. Human experimentation guidelines of PR China were followed in this study.

Cytokine level determination

The blood samples of all patients for cytokine determination were collected at the time of admission, and cytokine levels were tested by two trained technicians immediately. From the 865 selected cases, 12 typical patients were selected, and blood samples were collected on day 0, day 1, day 2, day 3, and day 4 after admission. The plasma was harvested within 30 minutes of venipuncture at 37°C from ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood samples and stored at -70°C until analyzed. The Bio-Plex Human 8-Plex kit (Bio-Rad, Hercules, USA) was used to detect IL-2, IL-4, IL-6, IL-8, IL-10, IFNγ, GM-CSF, and TNFα levels on a Luminex200 xMAP analyzer system (Luminex, Austin, USA), according to the manufacturer's instructions.

Statistical analysis

Proportional data were tested using the Chi-square test or Fisher's exact test. Continuous data were tested using the Student's *t* test. The Mann-Whitney *U* test was used for nonparametric data that did not have a

normal distribution. All analyses were performed using SPSS software, version 11.0. A p value < 0.05 was considered to be significant.

Results

Patient characteristics and clinical and laboratory findings

In this study, data of 865 hospitalized patients with varicella infection admitted in Beijing You'an Hospital between January 2011 and December 2013 were collected. The average time from the appearance of either fever or rash to hospital admission was 2.22 ± 2.15 days. Hospital stay ranged from 2 to 12 days, with an average of 7.36 ± 5.99 days. All patients in this study recovered and were discharged. No patients died.

Of the patients, 389 (45.0%) were male, and 476 (55.0%) were female. The male-to-female ratio was about 1:1.22. The ratio did not show any significant differences among patients with different complications, thereby indicating that gender is not a risk factor for complications. The average age of varicella patients was 11.34 ± 8.99 years. The patients' ages ranged from 1 to 37 years of age; 17.8% of the patients were over 17 years of age. From 2011 to 2013, the ratio of patients over 17 years of age fluctuated without significant change (18.1% in 2011; 17.6% in 2012; 18.5% in 2013).

Skin rash was the most common clinical finding in varicella patients, seen in all (100%) patients. The most common sites of skin rash included head/face (98.8%, 855/865), trunk (93.9%, 812/865), extremities (91.2%, 789/865), and mouth (76.1%, 658/865). Malaise (92.9%, 804/865) and fever (80.8%, 699/865) were also commonly seen in varicella. Neurological complication was rare, and only 2.5% (22/865) of patients showed ataxia (Table 1). A significant number of patients had a fever higher than 39°C upon admission, but no predictive value of high fever on disease severity was found (with or without complications; p > 0.05).

Of the 865 varicella patients, 201 (23.2%) had no complications and were defined as mild. There were 188 (21.7%) cases complicated with skin infections, and 145 (16.8%) with liver damage. There were no gender differences found among patients with skin infections or liver damage. Within the past three years, the incidence rates of common complications such as skin infections and liver damages in varicella patients have changed significantly. Among the varicella virus-infected patients, the rates of skin infections and liver damage were both sharply elevated. The incidence of liver damage even doubled in 2012 compared with 2010 (Figure 1).

To further characterize the effects of each distinct complication, data from 201 mild cases, 132 with skin infection only cases, and 89 with liver damage only cases were collected and analyzed. Patients with multiple complications were excluded. Statistical analysis also demonstrated that white blood cell, neutrophil, and lymphocyte counts/percentages and other routine blood test results did not show any significant differences among different groups of patients (all p > 0.05) (Table 2).

Table 1. Clinical characteristics of varicella patients (n = 865)

	Case number (n)	Percentage (%)
Rash	865	100
Head/face	855	98.8
Trunk	812	93.9
Extremities	789	91.2
Mouth	658	76.1
Malaise	804	92.9
Fever	699	80.8
Drowsiness	430	49.7
Headache	293	33.9
Abdominal pain	278	32.1
Skin infection	188	21.7
Cough	187	21.6
Constipation	146	16.9
Enlarged lymph nodes	101	11.7
Chills	76	8.8
Nausea	67	7.7
Vomiting	57	6.6
Diarrhea	37	4.3
Ataxia	22	2.5

Figure 1. Incidence rates of skin infections and liver damages in varicella. Mild: varicella patients without any complications; SI: varicella patients complicated with skin infections; LD: varicella patients complicated with liver damage.

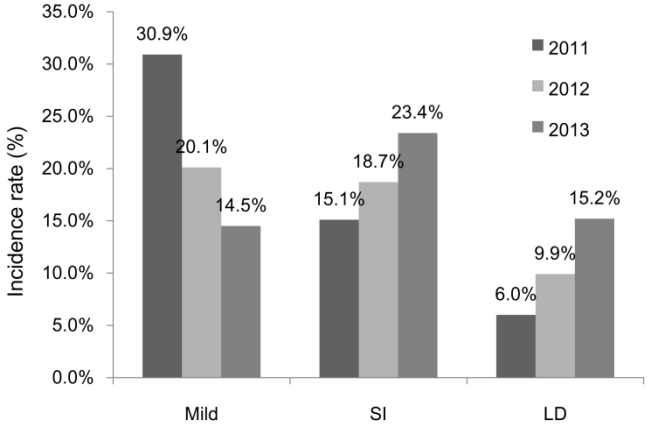


Table 2. Laboratory findings (mean ± SD [median])

	Mild (n = 201)	SI (n = 132)	LD (n = 89)
WBC (10^9/L)	8.2 ± 2.9 (7.0)	12.7 ± 5.8 (11.2)	8.2 ± 3.9 (6.9)
Neutrophils (%)	57.9 ± 16.5 (59.1)	79.9 ± 25.1 (82.5)	60.9 ± 23.1 (68.0)
Lymphocytes (%)	39.9 ± 21.3 (35.8)	22.9 ± 29.3 (37.7)	42.3 ± 29.3 (40.0)
HB (g/dL)	122.2 ± 17.9 (121.3)	127.1 ± 19.1 (128.9)	125.5 ± 25.2 (131.1)
PLT (10^9/L)	242.7 ± 102.1 (255.1)	245.3 ± 102.2 (249.1)	254.1 ± 92.2 (242.7)
CK (IU/L)	279.4 ± 456.8 (98.0)	298.2 ± 567.2 (199.2)	278.0 ± 512.1 (178.3)
CK-MB (IU/L)	18.1 ± 11.0 (15.2)	16.1 ± 13.2 (15.9)	17.1 ± 9.9 (15.9)
LDH (IU/L)	254.1 ± 105.8 (229.1)	301.8 ± 121.1 (267.6)	277.3 ± 159.3 (256.3)
Serum amylase (IU/L)	95.1 ± 35.1 (116.0)	103.3 ± 34.7 (93.0)	91.3 ± 40.9 (94.9)

SI: isolated skin infection; LD: isolated liver damage

Table 3. Counts of T-cell subgroups in varicella patients (mean ± SD [median])

	Mild (n = 201)	SI (n = 132)	LD (n = 89)
CD4+ T cells	962 ± 365 (931)	1,108 ± 385 (1,125)	1,013 ± 439 (977)
CD8+ T cells	419 ± 193 (378)	584 ± 394 (594)	499 ± 187 (449)
CD3+ T cells	1,208 ± 321 (1211)	1,342 ± 645 (1,392)	1,501 ± 554 (1,432)

SI: isolated skin infection; LD: isolated liver damage

Table 4. Plasma cytokine levels of varicella patients (mean ± SD [median])

Cytokine (pg/mL)	Mild (n = 201)	SI (n = 132)	LD (n = 89)
IL-2	2.9 ± 1.7 (3.0)	6.1 ± 3.2 (5.1)	4.9 ± 1.9 (4.2)
IL-4	0.9 ± 0.9 (0.8)	1.2 ± 0.6 (1.2)	1.0 ± 0.8 (0.9)
IL-6	20.1 ± 10.0 (11.9)	27.6 ± 12.9 (24.2)*	17.2 ± 14.4 (20.2)
IL-8	33.1 ± 19.9 (24.2)	30.0 ± 22.2 (33.7)	29.8 ± 31.1 (17.8)
IL-10	8.3 ± 4.9 (9.2)	19.5 ± 11.1 (17.1)	12.2 ± 12.6 (13.4)
IFNγ	69.1 ± 56.2 (71.4)	133.3 ± 62.8 (102.1)*	79.9 ± 53.8 (88.2)
GM-CSF	7.7 ± 7.2 (7.1)	9.2 ± 7.3 (8.8)	8.2 ± 7.9 (7.9)
TNFα	13.2 ± 13.1 (14.2)	15.5 ± 10.4 (16.3)	18.8 ± 10.2 (12.4)

*$p < 0.05$; SI: isolated skin infection; LD: isolated liver damage

T-cell levels of varicella patients

The absolute counts of lymphocytes in mild patients and patients with different isolated complications did not differ significantly (Table 2). However, there may have been some differences among different immune phenotypes in T-cell subgroups between mild patients and patients with isolated complications. As shown in Table 3, CD3+, CD4+, and CD8+ cell counts were further analyzed. The numbers of all these three T-cell subtypes seemed elevated in patients with complications compared with patients in the mild group; however, statistical analysis failed to show any significant difference among the groups.

Plasma cytokine levels of varicella patients

Cytokines are key mediators of host immune responses to all viral and bacterial invasions. The levels of IL-2, IL-4, IL-6, IL-8, IL-10, IFNγ, GM-CSF, and TNFα in all three groups of subjects were therefore tested upon admission, and the results are presented in Table 4.

All eight tested cytokines were statistically correlated to each other (all $p < 0.05$). There seemed to be an activation of a well-connected cytokine network during varicella virus infection. A significant portion of patients showed elevated levels of IL-6 and IFNγ (55.0%, 476/865 and 24.4%, 211/865, respectively). The elevation of IL-2, IL-4, IL-8, GM-CSF, and TNFα levels were minimal.

Among mild, SI, and LD groups, cytokine levels were further measured, compared, and analyzed. All cytokine levels did not show significant changes between patients with different disease severity (all $p > 0.05$, SI or LD groups vs. mild group), except for IL-6 and IFNγ. Compared to those for patients in the mild group, the levels of IL-6 and IFNγ in patients in the SI group were elevated significantly ($p = 0.044$ and $p = 0.038$, respectively). However, this difference was not observed between the mild group and the LD group ($p > 0.05$) (Table 4)

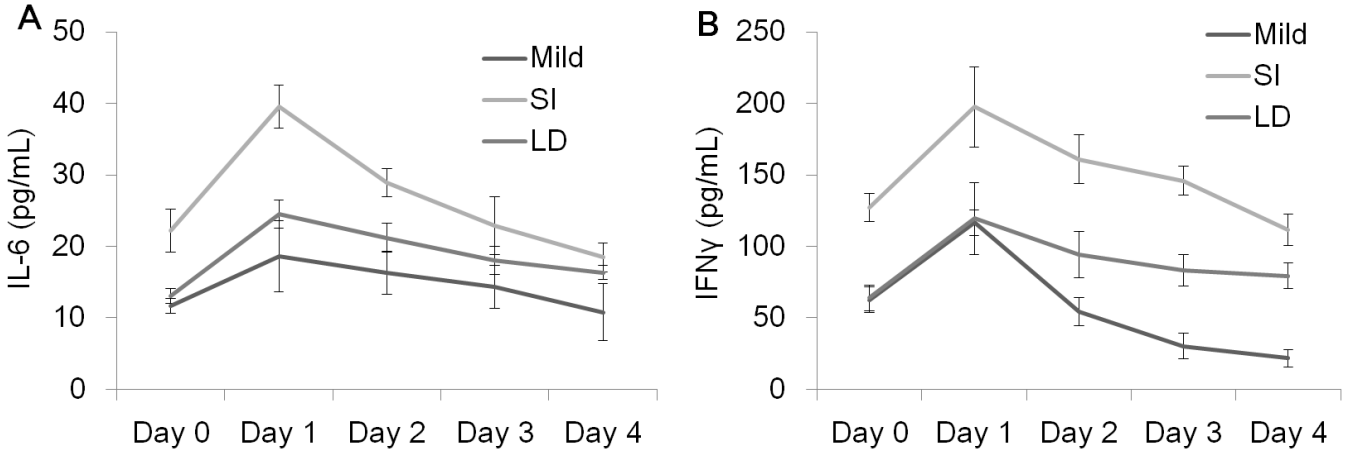
Kinetic changes of IL-6 and IFNγ in patients with severity differences

Varicella infection could be progressive, and the cytokine levels change along with disease progression. The kinetic changes in IL-6 and IFNγ levels within a small group of patients with or without different complication(s) were therefore prospectively monitored. In each group (mild, SI, and LD) of varicella patients, four typical patients were tested for IL-6 and IFNγ levels daily. Dynamic changes of IL-6 and IFNγ levels are shown in Figure 2. In all three groups, the levels of IL-6 and IFNγ peaked at day 1 after admission and then started to decline. The cytokine levels in varicella virus-infected patients with SI were consistently higher than those in the other two groups of patients. These results confirm that IL-6 and IFNγ levels are associated with the severity of varicella and also change along with the progression of the disease.

Discussion

Varicella is highly contagious illness caused by the varicella zoster virus, a type of human herpes virus. Even though there is a commercially available vaccine

Figure 2. Kinetic changes of IL-6 and IFNγ in varicella patients with or without different complications. IL-6 (**A**) and IFNγ (**B**) levels were measured at day 0, 1, 2, 3, and 4 after admission. Mild: varicella patients without any complications; SI: varicella patients complicated with skin infections; LD: varicella patients complicated with liver damage.



against VZV infection, the infection rates are still high in many regions of the world [3,14]. After increased use of the vaccine in recent years, the epidemic features of varicella have changed. Our study indicates that between 2011 and 2013, the percentage of mild cases dropped greatly, while the rate of severe cases with complications, especially skin infections and liver damage, were elevated significantly (Figure 1). This indicates that the severity of varicella cases in China may be elevated. Although deaths are still very rare, subsequent serious sequelae such as neurological defects are alarming and must be taken into consideration with appropriate concerning steps.

Patients with varicella can have a spectrum of clinical manifestations. In our study, skin rash was seen in all the patients. Fever was also very common, as were digestive tract complaints. This could be due to liver damage, gastroenteritis, or elevated intracranial pressure (Table 1). All of these findings were consistent with previous reports on varicella [1,2].

Cytokines are important mediators in many cellular signaling pathways involved in immune responses. IL-10 may have protective effects during inflammation by inhibition of IL-1, IL-6, IL-8, and TNFα and reduction of reactive oxygen intermediates [15]. It could be activated in almost all inflammatory responses. In our study, IL-10 level was not correlated with complication development ($p > 0.05$). IL-6 is secreted by innate immune cells such as macrophages and dendritic cells. It also could be produced by non-leukocytes such as fibroblasts, endothelial cells, and astrocytes [16]. Elevated IL-6 concentration is associated with severe inflammatory diseases and malignancies [17]. IFNγ is a pleiotropic cytokine that is mainly produced by Th1 cells, cytotoxic CD8+ T cells, and NK cells. It is critical for both innate and adaptive immunity, and could be activated by viral infections [18]. Our findings demonstrated that elevation of IL-6 and IFNγ levels measured upon admission was related to SI, a common and severe complication in varicella patients (Table 4). Data on dynamic changes of these two cytokines also supported the possible association that may exist between IL-6 and IFNγ and disease severity and complications. In addition, our results showed that IL-6 and IFNγ peaked early, on day 1 of admission (Figure 2). Previous publications have shown the critical roles of IL-6 and IFNγ in viral infections such as in mumps [19-22], results that are consistent with our findings in varicella infections. These cytokines may be used as serum markers to make early diagnosis of skin infections or liver damage in varicella and to take early measures during the course of the disease. Further studies with larger populations of patients are required to confirm this finding.

Conclusions

Our study suggests that the incidence of serious complications has become more common in recent years, and that IL-6 and IFNγ may be used as early serum markers for patients at risk of developing complications such as skin infections in varicella.

Acknowledgements

This work was supported by the National Twelve-five Project for Control and Prevention of Major Infectious Diseases [2014ZX10001002-001-002], Twelve-five Key Science and Technology Five Year Plan of China [2012BAI15B08], Beijing Natural Science Foundation of China [7142078, 7132077], National Natural Science Foundation of China [81371332], Beijing Key Laboratory [BZ0089], and Beijing Municipal Administration of Hospitals Clinical Medicine Development of Special Funding Support [ZY201401].

References

1. Arvin AM (1996) Varicella-zoster virus. Clin Microbiol Rev 9: 361-381.
2. Heininger U, Seward JF (2006) Varicella. Lancet 368: 1365-1376.
3. Macartney K (2014) Prevention of varicella: time for two-dose vaccination. Lancet 383: 1276-1277.
4. Andrei G, Snoeck R (2013) Advances in the treatment of varicella-zoster virus infections. Adv Pharmacol 67: 107-168.
5. Oh SH, Choi EH, Shin SH, Kim YK, Chang JK, Choi KM, Hur JK, Kim KH, Kim JY, Chung EH, Lee SY, Park SE, Cha S, Kim KN, Ma SH, Eun BW, Kim NH, Jo DS, Choi BY, Kim SA (2014) Varicella and Varicella Vaccination in Korea. Clin Vaccine Immunol 21: 762-768.
6. Gould D (2014) Varicella zoster virus: chickenpox and shingles. Nurs Stand 28: 52-58.
7. Wang Z, Yang H, Li K, Zhang A, Feng Z, Seward JF, Bialek SR, Wang C (2013) Single-dose varicella vaccine effectiveness in school settings in China. Vaccine 31: 3834-3838.
8. Zerboni L, Sen N, Oliver SL, Arvin AM (2014) Molecular mechanisms of varicella zoster virus pathogenesis. Nat Rev Microbiol 12: 197-210.
9. Nour AM, Reichelt M, Ku CC, Ho MY, Heineman TC, Arvin AM (2011) Varicella-zoster virus infection triggers formation of an interleukin-1beta (IL-1beta)-processing inflammasome complex. J Biol Chem 286: 17921-17933.
10. Haberthur K, Engelmann F, Park B, Barron A, Legasse A, Dewane J, Fischer M, Kerns A, Brown M, Messaoudi I (2011) CD4 T cell immunity is critical for the control of simian varicella virus infection in a nonhuman primate model of VZV infection. PLoS Pathog 7: e1002367.

11. Ouwendijk WJ, Mahalingam R, de Swart RL, Haagmans BL, van Amerongen G, Getu S, Gilden D, Osterhaus AD, Verjans GM (2013) T-Cell tropism of simian varicella virus during primary infection. PLoS Pathog 9: e1003368.
12. Weinberg A, Levin MJ (2010) VZV T cell-mediated immunity. Curr Top Microbiol Immunol 342: 341-357.
13. Levin MJ, Smith JG, Kaufhold RM, Barber D, Hayward AR, Chan CY, Chan IS, Li DJ, Wang W, Keller PM, Shaw A, Silber JL, Schlienger K, Chalikonda I, Vessey SJ, Caulfield MJ (2003) Decline in varicella-zoster virus (VZV)-specific cell-mediated immunity with increasing age and boosting with a high-dose VZV vaccine. J Infect Dis 188: 1336-1344.
14. Marin M, Guris D, Chaves SS, Schmid S, Seward JF (2007) Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 56: 1-40.
15. Cassatella MA, Meda L, Bonora S, Ceska M, Constantin G (1993) Interleukin 10 (IL-10) inhibits the release of proinflammatory cytokines from human polymorphonuclear leukocytes. Evidence for an autocrine role of tumor necrosis factor and IL-1 beta in mediating the production of IL-8 triggered by lipopolysaccharide. J Exp Med 178: 2207-2211.
16. Hirano T (1998) Interleukin 6 and its receptor: ten years later. Int Rev Immunol 16: 249-284.
17. Rincon M (2012) Interleukin-6: from an inflammatory marker to a target for inflammatory diseases. Trends Immunol 33: 571-577.
18. Boehm U, Klamp T, Groot M, Howard JC (1997) Cellular responses to interferon-gamma. Annu Rev Immunol 15: 749-795.
19. Xu P, Luthra P, Li Z, Fuentes S, D'Andrea JA, et al. (2012) The V protein of mumps virus plays a critical role in pathogenesis. J Virol 86: 1768-1776.
20. Fujii N, Yokosawa N, Shirakawa S (1999) Suppression of interferon response gene expression in cells persistently infected with mumps virus, and restoration from its suppression by treatment with ribavirin. Virus Res 65: 175-185.
21. Fujii N, Oguma K, Kimura K, Yamashita T, Ishida S, Fujinaga K, Yashiki T (1988) Oligo-2',5'-adenylate synthetase activity in K562 cell lines persistently infected with measles or mumps virus. J Gen Virol 69: 2085-2091.
22. Ulane CM, Rodriguez JJ, Parisien JP, Horvath CM (2003) STAT3 ubiquitylation and degradation by mumps virus suppress cytokine and oncogene signaling. J Virol 77: 6385-6393.

Corresponding authors

Prof. Wei Li
Center for Infectious Diseases,
Beijing You'an Hospital, Capital Medical University
Beijing 100069, China
Phone: 086-010-83997147
Fax: 086-010-83997164
Email:vision988@126.com

Prof. Jiasheng Zheng
Intervention Therapy Center of Liver Diseases
Beijing You'an Hospital, Capital Medical University
Beijing 100069, China
Phone: 086-010-83997147
Fax: 086-010-83997164
Email: jjstigm@126.com

Conflict of interests: No conflict of interests is declared.