

Original Article

Kinetic analysis of the immunity in a pregnant patient infected with avian influenza H7N9

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Abstract: Background: Human infection with avian influenza A H7N9 has emerged in China since February, 2013. The immunologic changes in pregnant women infected with H7N9 are not known. Objective: To report the clinical data and kinetic changes of immunity in a pregnant woman infected with H7N9 virus in Zhenjiang, Jiangsu, China. Methods: The clinical data were collected and immunity status was monitored in this patient. Results: H7N9 virus became undetectable in sputum from 14 days since onset of symptoms after effective antiviral therapy with oseltamivir and symptomatic/supporting treatments. The symptoms and signs in this patient gradually improved from 15 days since onset of symptoms. Peripheral lymphocytes initially decreased and gradually increased. The percentage of CD4+ T cells increased since 16 days after onset of symptoms. The kinetic changes of cytokines including IFN- γ , IFN- α , TNF- α , IL-10 and TGF- β 1 matched the development and recovery of illness. Her family members, including her parents exposed to H7N9 positive materials in poultry market, were H7N9 negative. Conclusions: Our results indicate that pregnant women are susceptible to H7N9 virus and H7N9 infection in pregnant women is curable without significant impact on fetus. Kinetic changes of pro-inflammatory and anti-inflammatory cytokines play a role in the pathogenesis and clinical outcome in the pregnant patient with H7N9 infection.

Keywords: H7N9, cytokine, pregnancy

Introduction

A novel avian influenza virus A (H7N9) infection has been described in eastern China during February and March 2013 [1]. A total of 131 human infections have been reported till May 17, of which 36 (27%) cases were fatal [2]. Although the outbreak of H7N9 infection seems to be controlled because only few new cases were reported, there is a worry about the pandemic of H7N9 infection. The increased risk of influenza for the pregnant patients has been noted in three epidemics: mortality was noted to be as high as 49% and 50% during the 1918 and 1957 epidemics, and the infection rate of pregnant patients was 10% during 2009 H1N1 pandemic [3, 4]. Furthermore, women in the second and third trimesters have higher mortality and morbidity rates than first trimester women [5]. Pregnant women are susceptible to influenza virus due to physiologic changes that affect hormone, respiratory and cardiovascular

systems [3]. The immune system altered to tolerate a genetically foreign fetus also results in the increased risk for influenza infection and complications [6]. The morbidity and mortality of H7N9 infection in pregnant women are not yet known. There were two pregnant women infected with H7N9 including in this report [7]. Here we report a pregnant patient infected with H7N9 and analyze the kinetic characteristics of immune status.

Material and methods

Patient and associated examination

A 25-year-old woman pregnant for 23⁺⁶ weeks was hospitalized in the Department of Respiratory Medicine, Affiliated People's Hospital of Jiangsu University, on April 5, 2013 with a history of cough, sore throat, myalgia and irregular fever for 7 days. The patient denied the history of contact with sick persons or live poultry,

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Table 1. Laboratory measurements in the patient infected with H7N9

Time since onset of symptoms	9d	10d	14d	16d	23d	31d	52d	Normal range
White blood cell ($\times 10^9/L$)	7.1	16.0	11.0	11.4	7.1	6.5	6.8	4.0-10.0
Lymphocyte ($\times 10^9/L$)	0.5	0.8	0.18	0.11	0.21	0.24	0.26	0.8-4.0
Monocyte ($\times 10^9/L$)	0.1	0.2	0.07	0.05	0.08	0.08	0.06	0.1-1.0
Neutrophil ($\times 10^9/L$)	6.5	15.0	0.74	0.83	0.70	0.62	0.67	1.6-7.5
Platelet ($\times 10^9/L$)	222	-	299	351	361	173	190	100-300
Prothrombin time (s)	11.00	-	10.30	11.70	11.30	-	-	9.00-13.30
Activated thromboplastin time (s)	33.00	-	27.10	27.30	29.70	-	-	20.00-40.00
D-dimer ($\mu g/L$)	1.28	-	9.56	7.38	4.24	-	-	0.10-0.55
Total bilirubin ($\mu mol/L$)	15.30	-	9.10	15.00	15.00	7.30	6.90	2.10-17.30
aspartate aminotransferase (U/L)	54.00	-	113.00	53.00	26.00	37.00	48	0.00-40.00
Alanine transaminase (U/L)	54.00	-	63.00	49.00	48.00	48.00	62	0.00-40.00
Glutamine amino transpeptidase (U/L)	45.00	-	123.00	99.00	105.00	100.00	62	5.00-40.00
Urea (mmol/L)	1.50	-	-	8.00	9.20	2.70	2.30	2.10-7.50
Creatinine ($\mu mol/L$)	30.00	-	-	57.00	59.00	40.00	41.00	35.00-135.00
Potassium (mmol/L)	2.60	-	4.11	4.35	4.00	3.51	-	3.50-5.10
Sodium (mmol/L)	128.00	-	142.60	139.60	131.50	132.30	-	135.00-145.00
Lactate dehydrogenase (U/L)	344.00	-	-	681.00	267.00	200.00	207.00	110.00-220.00
Creatinine kinase (U/L)	271.00	-	-	72.00	11.00	16.00	67.00	10.00-110.00
Hydroxybutyric acid dehydrogenase (U/L)	-	-	-	597.00	247.00	180.00	-	110.00-240.00

-: not detected.

but her parents were workers in the live poultry market. Physical examination on admission revealed stable vital signs apart from body temperature high at 39.9°C. This study was approved by the Institutional Review Board of the Affiliated People's Hospital of Jiangsu University.

RNA extraction and real-time RT-PCR

RNA was extracted from throat-swab or sputum specimens with Roche High Pure Viral RNA Kit (Cat. No. 11858882001) according to the manufacturer's instructions. Real-time RT-PCR assays for H7N9 virus were performed to verify the viral subtypes with the Influenza virus A Real Time RT-PCR Kit (Cat. No. R-0309-02, Shanghai ZJ Bio-Tech Co, Ltd, China) and Avian influenza virus (H7N9) real-time RT-PCR Kit (Cat. No. RR-0051-02, Shanghai ZJ Bio-Tech Co., Ltd, China) according to the manufacturer's instructions.

Lymphocyte subsets assay with flow-cytometry

Blood was obtained from this patient to identify percentages and absolute counts of the lymphocyte subsets with BD Multitest IMK Kit (Cat No: 340503) using flow-cytometry according to the manufacturer's instructions.

Analysis of cytokines with ELISA

Plasma was collected in different days since onset of symptoms. We monitored twenty different cytokines and chemokines (IL-2, sll-2R, IL-6, IL-8, IL-10, IFN- α , IFN- γ , TNF, VEGF, TGF- β 1, bFGF, M-CSF, GM-CSF, MCP-1, MIP-1 α , PARC, MIGF, RANTES, IGF-1, and TGF- β 1) in the 9d, 14d, 16d, 23d, and 31d since onset of symptoms with ELISA (Invitrogen, R&D, Senxiong biotech) according to the manufacturer's instructions.

Results

Laboratory measurements

The laboratory measurements of sequential blood specimens were listed in **Table 1**. Laboratory tests showed a low level in lymphocytes before 23 days since onset of symptoms, an increase in glutamine amino transpeptidase after 14 days since onset of symptoms. Elevated levels of aspartate aminotransferase, creatine kinase, and lactate dehydrogenase were observed (**Table 1**). Total peripheral lymphocyte counts were low at presentation and gradually increased to peak 6 days after admission. However, lymphocytes decreased and

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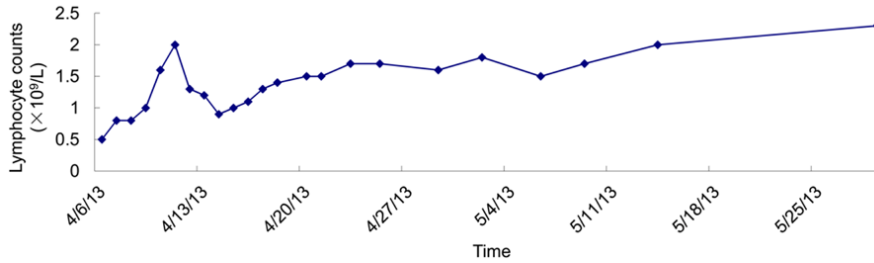


Figure 1. Dynamic change of peripheral lymphocytes in the pregnant patient with H7N9 infection.

then increased to the second peak on May 28 again (**Figure 1**).

Virological PCR detection

Two throat-swab specimens and two sputum specimens were obtained from patient on 9 days, 10 days, 14 days and 15 days since onset of symptoms, respectively. All samples were tested for H7N9 with real-time RT-PCR. H7N9 virus was detected positive in throat swab specimens on both 9 days and 10 days since onset of symptoms, and then became undetectable in sputum specimens on both 14 days and 15 days since onset of symptoms (**Table 2**). The family members of the pregnant women were H7N9 negative, but the materials from the chicken cage and discharges in the live poultry market where her parents worked were H7N9 positive.

Lymphocyte subsets assay

Peripheral lymphocyte subsets were observed during H7N9 infection. CD4⁺ T cell percentage increased on 16 days and 18 days since onset of symptoms (**Table 3**).

Cytokines assay

Five cytokines (TGF- β 1, Rantes, MIGF, INF- γ , and M-CSF) significantly increased on 9 days since onset of symptoms, among which two cytokines (TGF- β 1 and Rantes) progressively increased, MIGF maintained the high levels from 9 days to 31 days since onset of symptoms, M-CSF increased to the highest level on 16 days since onset of symptoms and then decreased, and INF- γ gradually decreased after 9 days since onset of symptoms. IL-10 slightly increased on 9 days since onset of symptoms and then gradually decreased (**Figure 2**).

Eight cytokines (b-FGF, IL-8, IL-6, INF- α , IL-2, TNF- α , MIP-1 α , and MCP-1) increased on 14 days or 16 days since onset of symptoms, among which TNF- α continued to increase after 16 days since onset of symptoms, IL-8 and

MCP-1 significantly decreased on 31 days since onset of symptoms, four cytokines (bFGF, IL-6, IL-8, and MIP-1) began to decrease on 31 days since onset of symptoms (**Figure 2**).

There was no significant change in the levels of other six cytokines (GM-CSF, IGF-1, VEGF, sIL-2R, PARC, and sICAM-1) compared to normal ranges (**Figure 2**).

Discussion

During physiologic pregnancy, although the number of peripheral lymphocytes is not changed, it is commonly accepted that the mother must maintain tolerance of the fetal semi-allograft while not suppressing her own immune system and exposing herself and the fetus to infection. Induction of Th2-secreted cytokines (IL-4, IL-6, and GM-CSF) and suppression of Th1-secreted cytokines (IL1, IL-2, IL-12, INF- γ , and TNF- α) play critical roles in pregnancy outcome [8]. However, these immunologic alterations may induce a state of increased susceptibility to influenza virus. Pregnant women were more likely to be hospitalized with an acute cardiopulmonary illness during influenza epidemics compared with non-pregnant women [9]. Furthermore, Pregnant women had increased rates of morbidity and mortality from influenza infection [9, 10]. Data during 2009 H1N1 epidemic showed pregnant women were at increased risk of hospitalization, admission to the intensive care unit, death and other severe outcome [10, 11]. Although the effect of H5N1 infection in pregnant women is poorly known, 4 of 6 pregnant women with H5N1 infection died and 2 women who survived had spontaneous abortion [12]. The family members of the pregnant woman infected with H7N9 virus had no symptoms and negative virus detection, also suggesting the susceptibil-

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Table 2. Virological PCR detection in the patient infected with H7N9

specimen	throat swab (shallow)	throat swab (deep)	Sputum	Sputum
Time since onset of symptoms (days)	9d (2013-04-6)	10d (2013-04-7)	14d (2013-04-11)	15d (2013-04-12)
H7 gene (cycle threshold values)	30	27	-	-
N9 gene (cycle threshold values)	30	28	-	-

-: not detected.

Table 3. Lymphocyte subtype assay in the patient infected with H7N9

Time since onset of symptoms(days)	12d (2013-04-9)	16d (2013-04-13)	18d (2013-04-15)	51d (2013-05-8)	Normal range
T Cell (%)	71	85↑	80	82	50-84
CD4/CD8	1.96	0.82	2.76	1.23	0.82-2.78
B Cell (%)	9	6	6	3↓	5-18
CD3+CD4+ T Cell (%)	45	65↑	58↑	43	27-51
CD3+CD8+ T Cell (%)	23	17	21	35	15-44
NK Cell (%)	10	6	12	14	5.6-30.9

↑: increased; ↓: decreased.

ity to H7N9 virus in pregnant population. The morbidity and mortality of H7N9 infection is not yet known.

Both innate and adaptive immune responses are initiated to play against influenza. The production of antiviral and inflammatory cytokines and chemokines are involved in such host immune responses. Elevated blood levels of IFNs, TNF- α , MCP-1, MIP-1, RANTES, IL-2, IL-6, IL-8, IL-10, and IP-10 were observed in individual patients with H5N1 infections [13-16]. However, the induction of the human innate immune response to H7N9 virus infection has not been well characterized. Recently, serum levels of six cytokines and chemokines were found to increase in patients with H7N9 infection [3].

Both the percentage and absolute counts of peripheral blood lymphocytes decreased in the second and third trimester [17, 18]. The kinetic changes of peripheral lymphocytes in this pregnant woman suggest the presence of early lymphocytopenia which is similar with non-pregnant patients [7]. Although the percentage of T helper cells remained unchanged [17, 18], CD4 + T cells were induced after H7N9 infection and hypercytokinemia was observed in this patient. Although Th1 cytokine response is suppressed during pregnancy, it still maintains the capacity of a defensive response in the context of infection [8]. IFN- γ is rapidly induced to release by Th1 cells after H7N9 infection. The early increase of IFN- γ appears to activate the antiviral function via recruiting and activating macrophages, NK cells, and T cells to perform their

effector functions, including producing immunoregulatory and antiviral monokines and cytokines [19]. The time from the onset of illness to the first negative result of H7N9 test after treatment was 13 days, which was consistent with a recent report [7]. While H7N9 virus was depleted, INF- γ level gradually decreased and the clinical manifestations were gradually improved. Recently, it has been found that the inflammatory response is played out over time in a reproducible and organized way after an initiating stimulus [20]. The delayed increase of IFN- α expression might be involved in the removal of residual viruses. TNF α , also secreted by Th1 cells, is the best known and most intensely studied of the pro-inflammatory cytokines and plays an important role in viral infections. TNF α had been considered as an early-phase cytokine after avian influenza infection [14, 2, 22]. However, plasma level of TNF α was not elevated in this patient before 14 days since onset of symptoms, which was consistent with the results of H5N1 infection [14, 23]. It is also considered that TNF α is dispensable for influenza viral clearance [24]. The continued late-phase elevation of TNF α level was observed in this patient, which may be implicated in the pathogenesis of chronic lung inflammation and fibrosis [25, 26]. IL-2 is slightly decreased in healthy pregnant women compared with non-pregnant women [27]. In this pregnant patient, IL-2 significantly decreased, which was similar to the observation in those individuals infected with H1N1 virus [28].

IL-10 is one of anti-inflammatory cytokines produced by Th2 cells. It suppresses the release of

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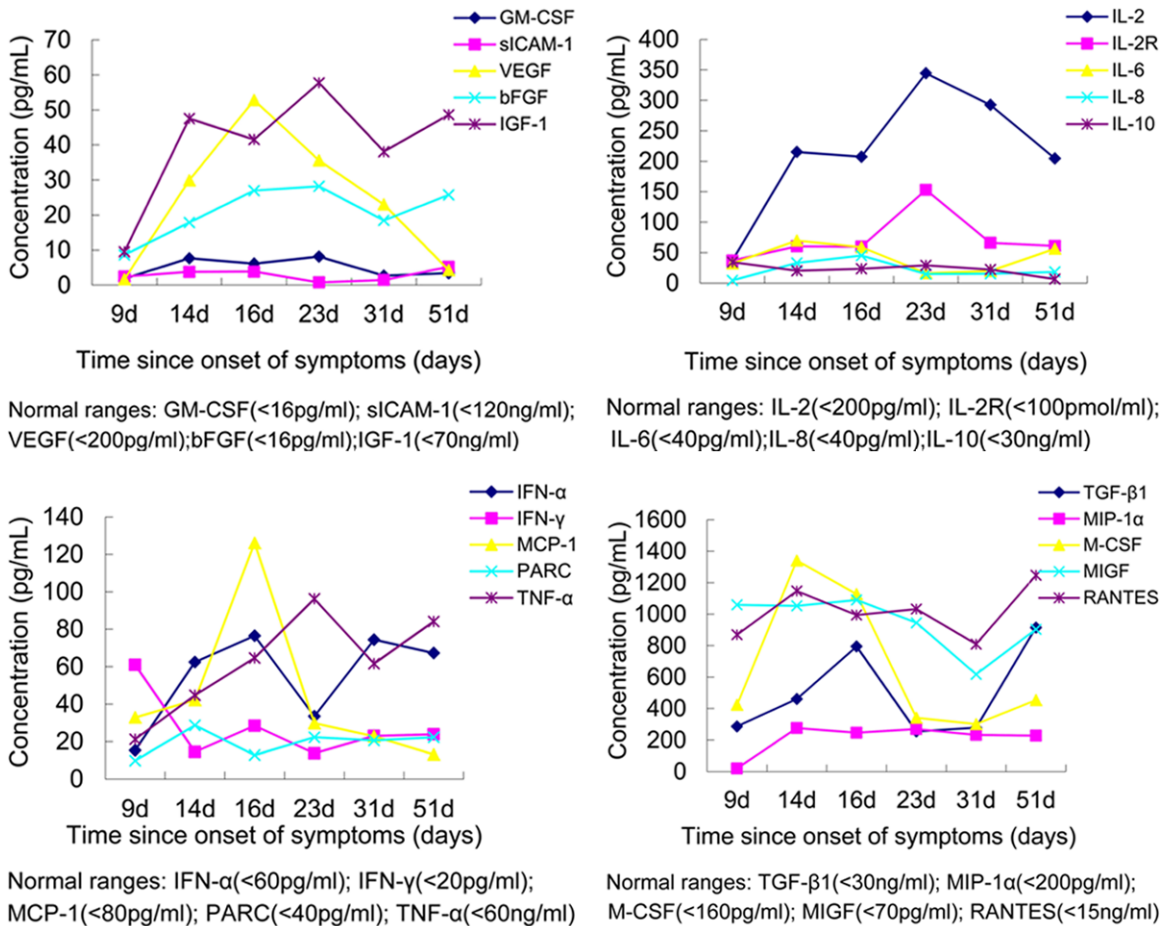


Figure 2. Plasma cytokine and chemokine changes in the patient with H7N9 infection.

pro-inflammatory mediators from monocytes/macrophages and therefore inhibits the LPS- and IFN- γ -induced secretion of TNF- α , IL-1 β , IL-6, IL-8, G-CSF, and GM-CSF [29, 30]. Although IL-10 is released from first and second trimester [31], the dynamic changes of IL-10 level in this pregnant patient indicated that IL-10 increased in acute phase of H7N9 infection. The early increase of IL-10 in this patient may have a critical role in the immune balance.

TGF- β 1 is a Th3-type anti-inflammatory mediator involved in the immune system by controlling several aspects of inflammatory responses, T cell differentiation, B cell isotype switching and tolerance [32]. There is no notable alteration in serum level of TGF- β 1 in normal pregnant women [29]. The acute release of TGF- β 1 in this patient might play a role in immune homeostasis. Persistent elevation of TGF- β 1 seems to be involved in the promotion of lung fibrosis through recruitment and activa-

tion of monocytes and fibroblasts, induction of ECM, and stimulation of angiogenesis [25, 33].

In conclusion, our results indicate that pregnant women are susceptible to H7N9 virus and H7N9 infection in pregnant women is curable without significant impact on fetus. Kinetic changes of pro-inflammatory and anti-inflammatory cytokines play a role in the pathogenesis and clinical outcome in the pregnant patient with H7N9 infection.

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Disclosure of conflict of interest

None to declare.

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References

- [1] World Health Organization. Background and summary of human infection with influenza A (H7N9) virus. http://www.who.int/influenza/human_animal_interface.
- [2] Bai T, Zhou J and Shu Y. Serologic study for influenza A (H7N9) among high-risk groups in China. *N Engl J Med* 2013; 368: 2339-2340.
- [3] Longman RE and Johnson TR. Viral respiratory disease in pregnancy. *Curr Opin Obstet Gynecol* 2007; 19: 120-125.
- [4] Louie JK, Acosta M, Winter K, Jean C, Gavali S, Schechter R, Vugia D, Harriman K, Matyas B, Glaser CA, Samuel MC, Rosenberg J, Talarico J, Hatch D; California Pandemic (H1N1) Working Group. Factors associated with death or hospitalization due to pandemic 2009 influenza A (H1N1) infection in California. *JAMA* 2009; 302: 1896-1902.
- [5] Pazos M, Sperling RS, Moran TM and Kraus TA. The influence of pregnancy on systemic immunity. *Immunol Res* 2012; 54: 254-261.
- [6] Jamieson DJ, Theiler RN and Rasmussen SA. Emerging infections and pregnancy. *Emerg Infect Dis* 2006; 12: 1638-1643.
- [7] Gao HN, Lu HZ, Cao B, Du B, Shang H, Gan JH, Lu SH, Yang YD, Fang Q, Shen YZ, Xi XM, Gu Q, Zhou XM, Qu HP, Yan Z, Li FM, Zhao W, Gao ZC, Wang GF, Ruan LX, Wang WH, Ye J, Cao HF, Li XW, Zhang WH, Fang XC, He J, Liang WF, Xie J, Zeng M, Wu XZ, Li J, Xia Q, Jin ZC, Chen Q, Tang C, Zhang ZY, Hou BM, Feng ZX, Sheng JF, Zhong NS and Li LJ. Clinical Findings in 111 Cases of Influenza A (H7N9) Virus Infection. *N Engl J Med* 2013; 368: 2277-2285.
- [8] Sykes L, MacIntyre DA, Yap XJ, Teoh TG and Bennett PR. The Th1:th2 dichotomy of pregnancy and preterm labour. *Mediators Inflamm* 2012; 2012: 967-629.
- [9] Neuzil KM, Reed GW, Mitchel EF, Simonsen L and Griffin MR. Impact of influenza on acute cardiopulmonary hospitalizations in pregnant women. *Am J Epidemiol* 1998; 148: 1094-1102.
- [10] Hartert TV, Neuzil KM, Shintani AK, Mitchel EF Jr, Snowden MS, Wood LB, Dittus RS and Griffin MR. Maternal morbidity and perinatal outcomes among pregnant women with respiratory hospitalizations during influenza season. *Am J Obstet Gynecol* 2003; 189: 1705-1712.
- [11] Mulooly JP, Barker WH and Nolan TF. Risk of acute respiratory disease among pregnant women during influenza A epidemics. *Public Health Rep* 1986; 101: 205-211.
- [12] Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza AV, Abdel-Ghafar AN, Chotpitayasonondh T, Gao Z, Hayden FG, Nguyen DH, de Jong MD, Naghdaliyev A, Peiris JS, Shindo N, Soeroso S and Uyeki TM. Update on avian influenza A (H5N1) virus infection in humans. *N Engl J Med* 2008; 358: 261-273.
- [13] Beigel JH, Farrar J, Han AM, Hayden FG, Hyer R, de Jong MD, Lochindarat S, Nguyen TK, Nguyen TH, Tran TH, Nicoll A, Touch S, Yuen KY; The Writing Committee of the World Health Organization (WHO) Consultation on Human Influenza A/H5. Avian influenza A (H5N1) infection in humans. *N Engl J Med* 2005; 353: 1374-1385.
- [14] Peiris JS, Yu WC, Leung CW, Cheung CY, Ng WF, Nicholls JM, Ng TK, Chan KH, Lai ST, Lim WL, Yuen KY and Guan Y. Re-emergence of fatal human influenza A subtype H5N1 disease. *Lancet* 2004; 363: 617-619.
- [15] Peiris JS, Cheung CY, Leung CY and Nicholls JM. Innate immune responses to influenza A H5N1: friend or foe? *Trends Immunol* 2009; 30: 574-584.
- [16] Chen Y, Liang W, Yang S, Wu N, Gao H, Sheng J, Yao H, Wo J, Fang Q, Cui D, Li Y, Yao X, Zhang Y, Wu H, Zheng S, Diao H, Xia S, Zhang Y, Chan KH, Tsoi HW, Teng JL, Song W, Wang P, Lau SY, Zheng M, Chan JF, To KK, Chen H, Li L and Yuen KY. Human infections with the emerging avian influenza A H7N9 virus from wet market poultry: clinical analysis and characterisation of viral genome. *Lancet* 2013; 381: 1916-1925.
- [17] Tallon DF, Corcoran DJ, O'Dwyer EM and Grealley JF. Circulating lymphocyte subpopulations in pregnancy: a longitudinal study. *J Immunol* 1984; 132: 1784-1787.
- [18] Iwatani Y, Amino N, Tachi J, Kimura M, Ura I, Mori M, Miyai K, Nasu M and Tanizawa O. Changes of lymphocyte subsets in normal pregnant and postpartum women: postpartum increase in NK/K (Leu 7) cells. *Am J Reprod Immunol Microbiol* 1988; 18: 52-55.
- [19] Guidotti LG and Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. *Annu Rev Immunol* 2001; 19: 65-91.
- [20] Hao S and Baltimore D. The stability of mRNA influences the temporal order of the induction of genes encoding inflammatory molecules. *Nat Immunol* 2009; 10: 281-288.
- [21] Szretter KJ, Gangappa S, Lu X, Smith C, Shieh WJ, Zaki SR, Sambhara S, Tumpey TM and

Immune characteristics in a pregnant patient with H7N9 infection

- Katz JM. Role of host cytokine responses in the pathogenesis of avian H5N1 influenza viruses in mice. *J Virol* 2007; 81: 2736-2744.
- [22] Baskin CR, Bielefeldt-Ohmann H, Tumpey TM, Sabourin PJ, Long JP, García-Sastre A, Tolnay AE, Albrecht R, Pyles JA, Olson PH, Aicher LD, Rosenzweig ER, Murali-Krishna K, Clark EA, Kotur MS, Fornek JL, Proll S, Palermo RE, Sabourin CL and Katze MG. Early and sustained innate immune response defines pathology and death in nonhuman primates infected by highly pathogenic influenza virus. *Proc Natl Acad Sci U S A* 2009; 106: 3455-3460.
- [23] de Jong MD, Simmons CP, Thanh TT, Hien VM, Smith GJ, Chau TN, Hoang DM, Chau NV, Khanh TH, Dong VC, Qui PT, Cam BV, Ha do Q, Guan Y, Peiris JS, Chinh NT, Hien TT and Farrar J. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat Med* 2006; 12: 1203-1207.
- [24] Damjanovic D, Divangahi M, Kugathasan K, Small CL, Zganiacz A, Brown EG, Hogaboam CM, Gauldie J and Xing Z. Negative regulation of lung inflammation and immunopathology by TNF- α during acute influenza infection. *Am J Pathol* 2011; 179: 2963-2976.
- [25] Agostini C and Gurrieri C. Chemokine/cytokine cocktail in idiopathic pulmonary fibrosis. *Proc Am Thorac Soc* 2006; 3: 357-363.
- [26] Clark IA. How TNF was recognized as a key mechanism of disease. *Cytokine Growth Factor Rev* 2007; 18: 335-343.
- [27] Szarka A, Rigó J Jr, Lázár L, Beko G and Molvarec A. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. *BMC Immunol* 2010; 11: 59.
- [28] Jost S, Quillay H, Reardon J, Peterson E, Simmons RP, Parry BA, Bryant NN, Binder WD and Altfeld M. Changes in cytokine levels and NK cell activation associated with influenza. *PLoS One* 2011; 6: e25060.
- [29] Fiorentino DF, Zlotnik A, Mosmann TR, Howard M and O'Garra A. IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 1991; 147: 3815-3822.
- [30] De Waal Malefyt R, Abrams J, Bennett B, Figdor CG and de Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 1991; 174: 1209-1220.
- [31] Hanna N, Hanna I, Hleb M, Wagner E, Dougherty J, Balkundi D, Padbury J and Sharma S. Gestational age-dependent expression of IL-10 and its receptor in human placental tissues and isolated cytotrophoblasts. *J Immunol* 2000; 164: 5721-5728.
- [32] Banchereau J, Pascual V and O'Garra A. From IL-2 to IL-37: the expanding spectrum of anti-inflammatory cytokines. *Nat Immunol* 2012; 13: 925-931.
- [33] Roberson EC, Tully JE, Guala AS, Reiss JN, Godburn KE, Pociask DA, Alcorn JF, Riches DW, Dienz O, Janssen-Heininger YM and Anathy V. Influenza induces endoplasmic reticulum stress, caspase-12-dependent apoptosis, and c-Jun N-terminal kinase-mediated transforming growth factor- β release in lung epithelial cells. *Am J Respir Cell Mol Biol* 2012; 46: 573-581.