Severe Lymphopenia and Related T-cell Immunity in an Avian Influenza A (H7N9)-Infected Patient

Nan-Nan Zhang[1,2,3,4], Yi Zhang[1,2,3], Jin-Gen Xia[1,2,3], Min Li[1,2,3], Xu Huang[1,2,3], Ruo-Yang Zhang[1,2,3], Qing-Yuan Zhan[1,2,3,4]

[1]Center for Respiratory Diseases, China-Japan Friendship Hospital, Beijing 100029, China
[2]Department of Pulmonary and Critical Care Medicine, China-Japan Friendship Hospital, Beijing 100029, China
[3]National Clinical Research Center for Respiratory Diseases, Beijing 100029, China
[4]Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100730, China

To the Editor: Following H7N9 infection, the host lymphocyte immunity plays an antiviral role.[1] Furthermore, low frequencies of T-cells correlate with disease severity.[2] Herein, we present a H7N9-infected patient with life-threatening lymphopenia (only 0.06×10^9/L in the peripheral blood), which we have barely ever seen before. We also measured the proportions of T-cell subpopulations in the blood and bronchoalveolar lavage fluid (BALF).

Without any exposure to live poultry, a 17-year-old boy became sick with the onset of anuria, increased creatinine level, fever, and chills after a lithotripsy on May 25, 2017, at a hospital of Hebei Province in China. When his urinary symptoms improved, he developed a high fever and dyspnea and was transferred to our hospital on June 3. The patient was diagnosed with acute respiratory distress syndrome, and tracheal intubation and mechanical ventilation were initiated. Soon thereafter, he was confirmed to be positive for the H7N9 virus through a commercial real-time polymerase chain reaction assay, which uses defective virus with H7 and N9 fragments and normal saline as the positive and negative controls, respectively. The sensitivity is 1.0×10^3 copies/ml. This kit has been verified with influenza A virus H1N1 (2009), seasonal influenza A virus H1 subtype and H3 subtype, avian influenza virus H5N1, influenza B virus, etc., and no crossover will occur. After confirmation, the therapy was immediately modified with the addition of oseltamivir (150 mg, bid). On June 6, the patient developed bilateral lung focal ground-glass opacities and consolidation. Therefore, venovenous extracorporeal membrane oxygenation (ECMO) was initiated. Subsequently, the drug regimen was revised with tigecycline, cefotaxime, amikacin, ciprofloxacin, and peramivir. Fortunately, with continuous ECMO therapy for 77 days, antibiotic and antiviral therapy, and other supportive treatments, the patient recovered and was discharged from the hospital on day 145 of hospitalization.

Of note, the patient developed severe lymphopenia (0.06×10^9/L) on June 6. We therefore sought to evaluate his T-cell subpopulations, including Th1/Tc1, Th2/Tc2, Th9/Tc9, Th17/Tc17, and Treg, in BALF and blood using flow cytometry. The surface markers included anti-human CD3-Percp-cy5.5, CD4-APC-H7, CD8-BV510, CD25-BV421, and programmed cell death-1 (PD-1)-PE (BD Biosciences, San Jose, California, USA). The intracellular cell staining involved fixation and permeabilization as described,[3] followed by staining with anti-human interferon-γ-Alexa 488, interleukin (IL)-4-BV711, IL-9-PE, and IL-17-BV650 (BD Biosciences). Data were acquired using Fortessa (BD Biosciences). As expected, we found that the overall frequencies of T-cell subgroups were conspicuously reduced in blood compared to the corresponding controls, who were age- and gender-matched healthy donors without any medical conditions we recruited, and the BALF profiles paralleled that observed in the blood. However, the PD-1 expression on $CD4^+$ and $CD8^+$ T-cells was elevated compared with that in controls [Table 1], suggesting that the patient was highly immunocompromised with T-cell exhaustion. Interestingly, the lymphocyte compartment showed a reversal (1.0×10^9/L) when the H7N9 virus test returned negative on June 19. The PD-1 expression on $CD4^+$ and $CD8^+$ T-cells was lower than on the day when the severe lymphopenia occurred. Moreover, a slightly higher proportion of T-cell subpopulations were found in the case patient than in the controls who were immunocompetent, regardless of preexisting immunosuppression [Table 1]. Overall, these implied that the patient might have experienced a relatively enhanced T-cell immune activation.

Lymphocytopenia is an important feature of H7N9 infection.[2,4] The lymphocyte count in our patient was only 0.06×10^9/L with a significant reduction, which is rarely seen in H7N9-infected patients. However, no evidence of monocytosis (monocyte count:

Address for correspondence: Prof. Qing-Yuan Zhan, Center for Respiratory Diseases, China-Japan Friendship Hospital, Beijing 100029, China
Department of Pulmonary and Critical Care Medicine, China-Japan Friendship Hospital, Beijing 100029, China
National Clinical Research Center for Respiratory Diseases, No. 2, East Yinghua Road, Chaoyang District, Beijing 100029, China
Peking Union Medical College, Chinese Academy of Medical Sciences, Beijing 100730, China
E-Mail: doctormtxyy@163.com

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Table 1: Percentages of T-cell subsets of healthy controls versus patient (%)

T-cell subsets	Controls* versus patient			
	Severe lymphopenia		Lymphocyte reversal	
	Blood	BALF	Blood	BALF
IFN-γ				
$CD4^+$	13.23 ± 1.49 versus 1.03	15.47 ± 4.72 versus 1.69	13.23 ± 1.49 versus 16.9	15.47 ± 4.72 versus 21.2
$CD8^+$	23.43 ± 3.79 versus 0.82	33.33 ± 6.20 versus 1.06	23.43 ± 3.79 versus 30.3	33.33 ± 6.20 versus 36.1
IL-4				
$CD4^+$	0.35 ± 0.07 versus 0.14	1.613 ± 0.54 versus 0.26	0.35 ± 0.07 versus 0.52	1.61 ± 0.54 versus 1.84
$CD8^+$	0.71 ± 0.24 versus 0.21	1.66 ± 0.724 versus 0.18	0.71 ± 0.24 versus 0.71	1.66 ± 0.72 versus 1.58
IL-9				
$CD4^+$	0.87 ± 0.28 versus 0.48	3.82 ± 0.77 versus 1.98	0.87 ± 0.28 versus 1.46	3.82 ± 0.77 versus 11.6
$CD8^+$	1.03 ± 0.13 versus 0.34	4.45 ± 0.74 versus 0.82	1.03 ± 0.13 versus 1.34	4.45 ± 0.74 versus 11.1
IL-17				
$CD4^+$	1.12 ± 0.36 versus 0.32	1.96 ± 1.00 versus 0.51	1.12 ± 0.36 versus 1.10	1.96 ± 1.00 versus 3.11
$CD8^+$	0.75 ± 0.18 versus 0.14	1.59 ± 0.76 versus 0.19	0.75 ± 0.18 versus 1.56	1.59 ± 0.76 versus 2.55
PD-1				
$CD4^+$	14.6 ± 1.12 versus 40.2	17.4 ± 1.19 versus 45.5	14.6 ± 1.12 versus 23.1	17.4 ± 1.19 versus 31.1
$CD8^+$	13.8 ± 3.29 versus 30.0	17.6 ± 2.25 versus 35.6	13.8 ± 3.29 versus 17.3	17.6 ± 2.25 versus 25.4
Treg	7.10 ± 0.48 versus 1.11	10.4 ± 3.16 versus 2.54	7.10 ± 0.48 versus 10.3	10.4 ± 3.16 versus 11.2

*Data of the healthy donors (n = 3) are presented as mean ± SD. BALF: Bronchoalveolar lavage fluid; IFN-γ: Interferon-γ; IL-4: Interleukin-4; IL-9: Interleukin-9; IL-17: Interleukin-17; PD-1: Programmed cell death-1; Treg: Regulatory T-cell; SD: Standard deviation.

0.01×10^9/L) accompanying the lymphocytopenia was found as reported previously,[4] which might be due to the transient exhaustion of the immune response. T-cells might undergo an acute exhaustion that is mediated by an overexpression of PD-1 in response to the H7N9 virus and previous urinary pathogens in the patient, which were consistent with that in H1N1 infection.[5] These factors might lead to the deleterious immunological response and increased host susceptibility to secondary infection. Importantly, the BALF T-cell profiles parallels those in blood; but they are in a relatively higher levels compared with those in blood. This is mostly because T-cells can migrate from the blood to the lungs and become involved in the injurious immunity in the lung. Thus, our investigation has revealed, for the first time, a patient with critical H7N9 infection, with severe lymphopenia and T-cell subpopulation immune dysregulation.

Declaration of patient consent

The patient and the healthy donors have provided written consent. Written informed consent was obtained for publication and any accompanying images. Our study was approved by the Ethics Committee of China-Japan Friendship Hospital (No. 2017-12).

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Conflicts of interest

There are no conflicts of interest.

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