Short Communication

Alteration in Serum Levels of Inflammatory Cytokines during Parainfluenza Virus Type 1 Infection in Patients with Severe Multiple Disabilities

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SUMMARY: An epidemic of parainfluenza virus type 1 (PIV1) infection occurred in a hospital ward housing patients with severe motor and intellectual disabilities. Twenty-three infected patients exhibited persistent high fever for 4–16 days and decreased lymphocyte counts. One-half of the symptomatic patients had increased blood monocyte counts and the other half progressed to bronchitis or pneumonia. We also compared levels of 27 cytokines in the sera of 21 patients during the acute and normal phases of infection. Cytokine levels were measured with a bead immunoassay performed using the Luminex Multiplex System. Serum levels of interleukin (IL)-1Ra, C-C-motif chemokine (CCL) 2, and C-X-C-motif chemokine (CXCL) 10 significantly increased during the acute phase. In contrast, the serum level of CXCL8 decreased slightly. These results suggest the involvement of monocytes/macrophages and respiratory epithelial cells in the initial stage of PIV1 infection. A previous report using nasal wash samples also found a significant increase in levels of CXCL10 during the acute phase. Hence, CXCL10 may be a useful marker of a cytokine storm produced upon viral infection. However, alterations in levels of IL-1Ra, CCL2, and other cytokines differed between the 2 studies, suggesting that the cytokine profile produced systemically at viral infection is different from that produced at mucosal sites. Further analysis is required to clarify the mechanisms underlying cytokine production during PIV1 infections.

Human parainfluenza virus (PIV) is commonly identified as a causative agent of respiratory tract infections (1). Among the 4 types of PIV, types 1 (PIV1) and 3 belong to the genus *Respirovirus*, and types 2 and 4 belong to the genus *Rubulavirus*. Reportedly, PIV1 and 3 cause infections mainly in the lower respiratory tract, whereas PIV2 and 4 mainly infect the upper respiratory tract. In particular, PIV1 frequently infects epithelial cells of the respiratory tract and induces lower respiratory tract infections, such as bronchitis and pneumonia, especially in immunocompromised hosts, children, and the elderly (1,2).

Severe viral infections are known to induce cytokine storms, which impact the progression of infectious diseases (3). During a cytokine storm, interferons (IFNs), interleukins (ILs), chemokines, colony-stimulating factors (CSFs), and tumor necrosis factors (TNFs) are produced in varying amounts, resulting in various immune responses. In addition, infections by the same virus have been reported to induce cytokine storms with different cytokine profiles (2,4). Therefore, specific cytokine responses to individual infectious disease remain under investigation.

Here, we report an epidemic of PIV1 infection that occurred in a hospital ward housing patients with severe multiple disabilities. We analyzed the clinical characteristics of PIV1 infection and changes in serum levels of cytokines in symptomatic patients.

In a 60-bed ward at Ehime Medical Center (Ehime, Japan), 23 patients (14 men and 9 women) aged 18–68 years (average age, 39.8 years), had high temperatures over a period of 1 month (April 7–May 9, 2012). Most patients also exhibited clinical symptoms of cough, sputum production, nasal discharge, and wheezing. The highest temperature and clinical symptoms of each patient were recorded daily. Blood and urine tests and chest radiography were performed as necessary. Residual sera collected during the symptomatic (acute) and recovery (2–3 months later, normal) phases were stored at -80°C until analyzed for cytokine content. Nasal swabs were collected from 12 randomly selected symptomatic patients during the acute phase of infection and were stored in culture medium (UTM Kit; Copan, Brescia, Italy) at -80°C until analyzed for the presence of respiratory tract viruses. The level of C-reactive protein (CRP), white blood cell (WBC) count, and lymphocyte and monocyte ratios were measured in the hospital laboratory. The mean and standard deviation (SD) values for each blood cell type were as follows: WBC count (cells/ μL): (male) $5,860 \pm 1,460$, (female) $4,930 \pm 1,190$; lymphocyte ratio (%): (male) 34.5 ± 7.9 , (female) 34.2 ± 7.2 ; monocyte ratio (%): (male) 5.1 ± 1.2 , (female) 4.9 ± 1.2 . We considered values in excess

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of the mean $\pm$ 2SD value to be outside of normal limits.

We screened nasal swabs for the genomes of respiratory tract viruses (PIV1, PIV2, PIV3, metapneumovirus, respiratory syncytial virus, coronavirus, rhinovirus, adenovirus, and enterovirus) by performing reverse transcription-polymerase chain reaction (RT-PCR) with primer pairs suitable for each virus (5–10). For the detection of PIV1, PIV2, and PIV3, 3 primer pairs were used (5). The presence of PIV was confirmed by cell culture using Vero E6 cells. The nucleotide sequences of the hemagglutinin neuraminidase (HN) gene of PIV1 were determined by RT-PCR using the primer pair (CAGAATTAATCAGACAAGAAGTRATATCAAG and GGTTRATTTCAACAATRTGGAAGCAGTA) and sequencing was performed using an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) (11). The HN gene sequences of PIV1 isolates were compared by phylogenetic tree analysis with the neighbor-joining method.

Serum levels of 27 cytokines were estimated, which included platelet-derived growth factor bb, IL-1b, IL-1Ra, IL-2, IL-4, IL-5, IL-6, IL-7, C-X-C-motif chemokine (CXCL) 8 (IL-8), IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, eotaxin, fibroblast growth factor-basic, G-CSF, GM-CSF, IFN- γ , CXCL10 (IP-10), C-C-motif chemokine (CCL) 2 (MCP-1), CCL3 (MIP-1 α), CCL4 (MIP-1 β), CCL5 (RANTES), TNF- α , and vascular endothelial growth factor. Levels of cytokines were measured using a bead immunoassay with the Bio-Plex Pro Human Cytokine GI 27-Plex Panel (Bio-Rad, Hercules, CA, USA) on the Luminex Multiplex System (LX 200; Luminex Co., Austin, TX, USA) according to the manufacturer's instructions. Cytokine levels are expressed in pg/mL. Statistical analysis was performed

using the Student's *t*-test.

The study protocol was approved by the Ethics Committee of the National Hospital Organization Ehime Medical Center and conformed to the provisions of the Declaration of Helsinki. Appropriate informed consent was documented for all subjects.

Normal body temperature for the patients ranged between 36°C and 37°C, which the exception of 1 patient (patient 18) with a normal body temperature was of <35°C. In this PIV1 epidemic, 22 patients developed a body temperature of >37°C for 4–16 days (average, 11 days), and 15 patients (65%) had a temperature of >39°C. Patient 18 also developed a temperature of >37°C (Fig. 1).

Clinical findings of the patients are shown in Table 1. Most patients exhibited various symptoms, such as cough, sputum, nasal discharge, and wheezing, and in 11 patients (48%), disease progressed to bronchitis or pneumonia accompanied by high levels of CRP during the acute phase. The WBC count was greater than the mean $\pm$ 2SD value in 7 patients (30%) in the initial stage of the acute phase. However, the lymphocyte ratio was >2SD below the mean in 14 patients (61%) and the monocyte ratio was greater than the mean $\pm$ 2SD value in 13 patients (57%). However, the association among these 3 values remained unclear.

PIV1 was detected by RT-PCR and cell culture in nasal swabs obtained from 10 patients. No viruses were detectable in 2 patients. We obtained the HN gene sequences in 8 of the 10 PIV1 isolates; sequencing was unsuccessful for 2 of the isolates. Phylogenetic analysis of the PIV1 HN gene sequences obtained in this study showed that all sequences belonged to the same PIV1 subtype, suggesting that only a single PIV1 subtype had

Fig. 1. Duration of high fever in patients during the epidemic of PIV1-infection. Each box shows the highest patient temperature on each day, and the darker box shows higher temperature as shown in the explanatory boxes. * denotes the day on which blood was collected for cytokine studies.

Table 1. Clinical findings for all 23 patients infected with PIV1

Patient no.	PIV1 detection	Symptom	Lower respiratory tract infection	Blood collection (days)	CRP (mg/dl)	WBC (/μl)	Lymphocyte (%)	Monocyte (%)
1	+	cough	—	1	1.21	6,020	16.4	21.9
2	ND	nasal discharge	—	0	5.05	11,620	16.6	6.5
3	ND	cough, nasal discharge	bronchitis	0	0.96	6,300	14.8	4.4
4	+	cough, nasal discharge	—	1	1.44	3,630	22.3	12.7
5	+	cough, nasal discharge	bronchitis	0	8.98	8,490	10.5	8.4
6	+	—	—	1	0.90	8,440	23.7	7.3
7	ND	nasal discharge, wheezing	—	1	0.81	9,420	18.3	7.2
8	+	cough, nasal discharge	bronchitis	5	5.30	9,980	5.7	4.6
9	—	cough	bronchitis	0	2.62	6,830	12.6	15.7
10	+	cough	—	0	2.26	8,080	7.4	13.7
11	ND	—	—	6	0.70	3,990	26.3	11.5
12	+	cough, nasal discharge	bronchitis	1	1.46	5,080	40.0	14.6
13	ND	—	—	3	3.25	13,420	11.5	6.0
14	+	cough	—	2	3.91	7,980	35.8	18.8
15	—	sputum	bronchitis	1	5.22	6,190	35.7	10.3
16	+	cough	—	0	2.62	11,300	15.9	8.8
17	+	—	—	2	0.44	6,140	18.7	6.7
18	ND	cough, nasal discharge	pneumonia	6	7.34	11,290	12.0	4.7
19	ND	cough	bronchitis	5	2.47	6,040	46.5	8.4
20	ND	cough, sputum	—	1	0.38	16,090	25.8	3.6
21	ND	sputum	pneumonia	1	2.09	6,930	9.8	9.2
22	ND	cough	pneumonia	0	0.60	6,290	14.1	8.1
23	ND	cough, sputum	pneumonia	0	3.82	5,710	6.6	4.1

The dates of blood collection are shown as days after onset of symptoms in the column “Blood collection (days).”

+, detected; —, not detected; ND, not done.

spread throughout the ward (Fig. 2). The 8 sequences were registered in the DNA Data Bank of Japan (DDBJ) under the accession nos. AB897869–897876.

Serum samples were successfully obtained from 21 patients in both the acute and normal phases. Serum levels of 27 cytokines were determined and compared between the 2 phases. Levels of most cytokines were not significantly different between phases, with the exception of IL-1Ra, CCL2 (MCP-1), CXCL8 (IL-8), and CXCL10 (IP-10) (Table 2 and Fig. 3). In the acute phase, levels of IL-1Ra, CCL2 (MCP-1), and CXCL10 (IP-10) were higher, whereas the level of CXCL8 (IL-8) was lower. Only 3 cytokines, IL-1Ra, CCL2 (MCP-1), and CXCL10 (IP-10), showed statistically significant differences in levels between phases. In particular, CXCL10 (IP-10) showed the most significant increase during the acute phase. There was no correlation between changes in levels between phases among 4 cytokines (Table 2) or with WBC counts and ratios of lymphocytes and monocytes.

In this study, PIV1 was found to spread easily among immunocompromised housed in hospital wards and frequently resulted in lower respiratory tract infections, indicating the importance of the implementation of methods to prevent the spread nosocomial infections of PIV1. The present study results are similar to those of reported outbreaks of metapneumovirus infection (12).

During viral infections, inflammatory cytokines are released from various cells, including monocytes/macrophages, lymphocytes, neutrophils, epithelial cells, endothelial cells, and fibroblasts (3). Initially, virus-infected cells produce cytokines that stimulate other cells, resulting in the accumulation of inflammatory

Table 2. Changes in the concentrations of 4 cytokines (folds increase)

Patient no.	IL-1Ra	CCL2 (MCP-1)	CXCL8 (IL-8)	CXCL10 (IP-10)
1	1.3	2.44	0.85	10.03
3	1.25	1.96	1.31	6.26
4	0.98	1.26	0.21	5.97
5	1.18	1.04	0.18	15.2
6	1.24	1.26	2.56	3.11
7	1.57	2.52	0.09	17.98
8	1.28	0.96	1.18	3.25
9	1.62	0.96	0.68	2.89
10	2.86	5.57	0.63	25.91
11	2.15	1	0.51	7.46
12	1.29	5.31	0.61	7.33
13	1.76	1.3	0.46	1.01
14	1.08	1.23	0.27	13.92
15	0.97	1.79	2.09	1.42
16	0.71	0.98	0.1	4.77
17	1.5	0.93	1.2	3.26
18	1.61	2.45	2.76	18.17
19	1.62	1.44	2.55	4.1
20	2.7	3.76	1.4	15.61
21	1.61	1.71	0.93	11.97
22	0.9	3.75	0.76	5.97
Mean	1.48	2.08	1.02	8.84
SD	0.54	1.4	0.83	6.7

Each number shows by how many times the levels increased in the acute phase (concentration in acute phase/concentration in normal phase).

Serum Cytokines in PIV1 Infection

Fig. 2. Phylogenetic tree based on the sequences of HN genes from PIV1 isolated from patients. The results for 8 of the 10 isolates are shown; sequencing failed for 2 of the isolates.

cells at the infection site, and then in the increased production of various other cytokines (i.e., a cytokine storm). However, these responses are known to differ by infection of individual pathogens (3).

In this study, there were statistically significant changes in levels of IL-1Ra, CCL2, and CXCL10, which are mainly produced by monocytes/macrophages and respiratory epithelial cells. The increased production of these cytokines may be related to changes in the ratios of lymphocytes and monocytes during the initial stage of the acute phase of disease (13,14).

In spite of higher levels of IL-1Ra, CCL2, and CXCL10 during the acute phase, levels of CXCL8 (IL-8) were slightly lower. CXCL8 (IL-8) has been

reported as a potent neutrophil chemotactic factor (15,16). Hence, the slight decrease in CXCL8 expression during the acute phase may induce simultaneous changes in the number of blood neutrophils.

However, the relationships between the IL-1Ra, CCL2, CXCL8, and CXCL10 and the relationships between the cytokines and blood cells were unclear in this study, suggesting that other factors may influence changes in blood cell counts.

Changes in cytokine levels have been reported in nasal wash samples obtained during PIV1 infection (2). The profiles of the altered cytokines were different from those in the present study. In the nasal wash samples, levels of 4 other cytokines (CCL3, CCL4, CCL5, and

Fig. 3. Cytokine levels in sera obtained from 21 patients infected with PIV1. We selected the 4 cytokines that underwent remarkable level changes between the acute and normal phases. The mean (box) and SD (line) of each cytokine are shown. Numbers show the concentration of each cytokine (pg/ml).

CXCL9) increased during the acute phase. Although levels of CXCL10 increased, as seen in the present study, levels of IL-1Ra remained unchanged in the nasal wash samples. The most significant increase in both studies was observed in CXCL10, suggesting that it could be used as a marker of a cytokine storm during PIV1 infection as well as other major inflammatory cytokines (17). The serum level of CXCL8, which decreased in our study, showed an opposite trend in the nasal wash samples. Such differences in cytokine levels indicate that different mechanisms are possibly involved in the production/secretion of cytokines at mucosal sites.

In conclusion, further studies are required to clarify the precise mechanisms responsible for the induction of a cytokine storm during respiratory tract infections and identify differences in cytokine profiles between sera and mucosal samples.

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Conflict of interest None to declare.

REFERENCES

- Henrickson KJ. Parainfluenza viruses. *Clin Microbiol Rev.* 2003;16:242-64.
- El Feghaly RE, McGann L, Bonville CA, et al. Local production of inflammatory mediators during childhood parainfluenza virus infection. *Pediatr Infect Dis J.* 2010;29:e26-e31.
- Tisoncik JR, Korth MJ, Simmons CP, et al. Into the eye of the cytokine storm. *Microbiol Mol Biol.* 2012;76:16-32.
- Bennett BL, Garafalo RP, Cron SG, et al. Immunopathogenesis of respiratory syncytial virus bronchiolitis. *J Infect Dis.* 2007;195:1532-40.
- Echevarría JE, Erdman DD, Swierkosz EM, et al. Simultaneous detection and identification of human parainfluenza viruses 1, 2, and 3 from clinical samples by multiplex PCR. *J Clin Microbiol.* 1998;36:1388-91.
- Matsuzaki Y, Takashita E, Okamoto M, et al. Evaluation of a new rapid antigen test using immunochromatography for detection of human metapneumovirus in comparison with real-time PCR assay. *J Clin Microbiol.* 2009;47:2981-4.
- Sullender WM, Sun L, Anderson LJ. Analysis of respiratory syncytial virus genetic variability with amplified cDNAs. *J Clin Microbiol.* 1993;31:1224-31.
- Gaunt ER, Hardie A, Claas EC, et al. Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method. *J Clin Microbiol.* 2010;48:2940-7.
- Ishiko H, Shimada Y, Yonaha M, et al. Molecular diagnosis of human enteroviruses by phylogeny-based classification by use of the VP4 sequence. *J Infect Dis.* 2002;185:744-54.
- Xu W, McDonough MC, Erdman DD. Species-specific identification of human adenoviruses by a multiplex PCR assay. *J Clin Microbiol.* 2000;38:4114-20.
- Mizuta K, Saitoh M, Kobayashi M, et al. Detailed genetic analysis of hemagglutinin-neuraminidase glycoprotein gene in human parainfluenza virus type 1 isolates from patients with acute respiratory infection between 2002 and 2009 in Yamagata prefecture, Japan. *Virology.* 2011;533.
- Matsuda S, Nakamura M, Hirano E, et al. Characteristics of human metapneumovirus infection prevailing in hospital wards housing patients with severe disabilities. *Jpn J Infect Dis.* 2013;66:195-200.
- Guilliams M, Lambrecht BN, Hammad H. Division of labor between lung dendritic cells and macrophages in the defense against pulmonary infections. *Mucosal Immunol.* 2013;6:464-73.
- Schaap-Nutt A, Liesman R, Bartlett EJ, et al. Human parainfluenza virus serotypes differ in their kinetics of replication and cytokine secretion in human tracheobronchial airway epithelium. *Virology.* 2012;433:320-8.
- Mukaida N. Pathophysiological roles of interleukin-8/CXCL8 in pulmonary diseases. *Am J Physiol Lung Cell Mol Physiol.* 2003;284:L566-77.
- Kobayashi Y. The role of chemokines in neutrophil biology. *Front Biosci.* 2006;13:2400-7.
- Lui M, Guo S, Hibbert JM, et al. CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications. *Cytokine Growth Factor Rev.* 2011;22:121-30.