

Production of Chemokines in Kawasaki Disease, Henoch-Schönlein Purpura and Acute Febrile Illness

We compared the production of three chemokines; interferon- γ -inducible protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1) and growth-related oncogene- α (Gro- α) that attracts monocytes or neutrophils, or both, in peripheral blood at acute stage of Kawasaki disease (n=29), Henoch-Schönlein purpura (n=15) and acute febrile illnesses (n=12). The production of the chemokines was assayed by ELISA. The plasma levels of IP-10 were markedly elevated in Kawasaki disease (538.6 ± 336.4 pg/mL) and acute febrile illnesses (417.1 ± 262.2 pg/mL) compared with in Henoch-Schönlein purpura (58.7 ± 95.7 pg/mL) ($p < 0.05$). The MCP-1 levels were elevated in Kawasaki disease (443.0 ± 473.1 pg/mL) and acute febrile illnesses (328.6 ± 261.1 pg/mL) compared with in Henoch-Schönlein purpura (82.9 ± 79.0 pg/mL) ($p < 0.05$). The Gro- α levels were elevated only in acute febrile illnesses (134.3 ± 153.6 pg/mL) compared with in Kawasaki disease (31.8 ± 22.1 pg/mL) or Henoch-Schönlein purpura (29.4 ± 53.3 pg/mL) ($p < 0.05$). According to these results, monocytes may play an important role in Kawasaki disease. In acute febrile illnesses, both monocytes and neutrophils may play an important role. By contrast, Henoch-Schönlein purpura may not be associated with the role of monocytes and neutrophils. Further studies using a larger number of cases are needed.

Key Words : *Mucocutaneous Lymph Node Syndrome; Kawasaki Disease; Purpura, Schönlein-Henoch; Acute Febrile Illness; Measles; Gastroenteritis; Pneumonia; Tonsillitis; Mumps; Exanthem Subitum; Chemokines, CC; Chemokines, CXC; Chemokines*

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INTRODUCTION

Kawasaki disease, first described by Dr. Tomisaku Kawasaki in Japan in 1967, is acute febrile vasculitis in childhood characterized by prolonged fever, polymorphous skin rash, non-purulent conjunctivitis, oral mucositis, erythematous induration of extremities, and cervical lymphadenopathy (1).

The differential diagnosis includes scarlet fever, toxic shock syndrome, measles, drug reaction, viral infection and other vasculitis syndromes. It can be difficult to differentiate from other acute febrile illnesses, because of some clinical and epidemiologic features support an infectious origin. From the histopathological aspects, Kawasaki disease is vasculitis in the small and medium-sized blood vessels (2, 3), similar to Henoch-Schönlein purpura, which is the most commonly encountered vasculitis syndrome in childhood. Especially, the interaction between leukocytes and vascular endothelial cells contributes to pathogenesis of these diseases (4, 5). So it is important to understand the factors that may recruit and activate leukocytes to the regions of vasculitis in both diseases.

Chemokine is one of the most important factors in this process (6). Recently, many studies investigated chemokines to understand the factors that may recruit and activate leukocytes to the regions of vasculitis in several diseases (7-11).

In the present study, we compared the production of three chemokines; interferon- γ -inducible protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1) and growth-related oncogene- α (Gro- α) in peripheral blood from patients with Kawasaki disease, Henoch-Schönlein purpura and other acute febrile illnesses, which overlap each other in some clinical or pathological findings.

MATERIALS AND METHODS

Study population

All patients were prospectively evaluated at the Department of Pediatrics, Yeungnam University Hospital, Daegu, Korea, between February 2001 and May 2003. The patients were composed of three groups: the first group included 29 patients (16 males and 13 females; mean age, 2.43 yr, range 6 months to 7 yr) at the acute febrile stage of Kawasaki disease. The second group included 15 patients (11 males and 4 females; mean age, 6.07 yr, range 2 to 14 yr) at the acute stage of Henoch-Schönlein purpura. The third group included 12 patients (6 males and 6 females; mean age, 3.4 yr, range 1 month to 8 yr) at the acute febrile stage of illnesses, such as

measles (3 cases), acute gastroenteritis (2), pneumonia (2), pharyngotonsillitis (2), and one each case of mumps, exanthem subitum, and urinary tract infection (Table 1).

Sampling

Peripheral blood sample (5 mL) were drawn from the patients at acute stage, and were centrifuged at 2,000 rpm/min for 30 min. Plasma samples were frozen at -70°C for the ELISA assay.

ELISA assay

ELISAs for IP-10, MCP-1 and GRO- α were performed according to the manufacturer's instruction using the kits from the R&D systems (Minneapolis, MN, U.S.A.). Briefly, standards and patient plasma were incubated in anti-human IP-10 (or MCP-1 or Gro- α) coated plates, 100 μL per well, at room temperature for 2 hr. Then the plates were further incubated with 200 μL of prepared biotinylated antibody at room temperature for 1 hr, and then 200 μL of tetramethylbenzidine substrate solution was added for development in darkness at room temperature for 20 min. The reaction was stopped by adding of 50 μL of stop solution. The absorbance was measured at 450 nm for IP-10, MCP-1 and GRO- α .

Statistical analysis

Data were expressed as mean \pm standard deviation. The significance of differences was assessed by the Mann-Whitney test. A p -value < 0.05 was considered significant.

RESULTS

Plasma levels of IP-10

The plasma levels of IP-10 were markedly elevated in the patients with Kawasaki disease (538.6 ± 336.4 pg/mL) and those with acute febrile illnesses (417.1 ± 262.2 pg/mL) compared with in the Henoch-Schönlein purpura group (58.7 ± 95.7 pg/mL) ($p < 0.05$) (Table 1, Fig. 1). There was no statistical significance between Kawasaki disease and acute febrile illnesses.

Table 1. The characteristics of study group

	KD	HSP	AFI
Case (No.)	29	15	12
Sex (M/F)	16/13	11/4	6/6
Age (range)	2.43 yr (6 m-7 yr)	6.07 yr (2-14 yr)	3.14 yr (1 m-8 yr)

KD, Kawasaki disease; HSP, Henoch-Schönlein purpura; AFI, Acute febrile illnesses.

Plasma levels of MCP-1

The MCP-1 plasma levels were elevated in the patients with Kawasaki disease (443.0 ± 473.1 pg/mL) and those with acute febrile illnesses (328.6 ± 261.1 pg/mL) compared with Henoch-Schönlein purpura group (82.9 ± 79.0 pg/mL) ($p < 0.05$) (Table 2, Fig. 2). In addition, the plasma levels of MCP-1 were not elevated in 8 patients with Kawasaki disease, with less than 100 pg/mL.

Plasma levels of Gro- α

The Gro- α levels were elevated only in the patients with acute febrile illnesses (134.3 ± 153.6 pg/mL) (Table 2, Fig. 3). As in the MCP-1, the Gro- α level was extremely low in 2 patients with pharyngotonsillitis among the patients with acute febrile illnesses with 3.12 pg/mL and 6.10 pg/mL, respectively.

DISCUSSION

The chemokine (chemotactic cytokine) is a large family of cytokines composed of small pro-inflammatory peptides that regulate trafficking, activation and proliferation of myeloid, lymphoid, melanocytes, and vascular endothelial cells. The chemokines are divided into four groups; C, C-C, C-X-C and C-X₃-C, based on the number of amino acids between the first two cysteines (6).

The selective attraction of leukocytes in response to chemokines may be determined by the expression of specific chemokine receptors on their cell surface. For example, neutrophils express receptors for C-X-C chemokines, but not for C-C chemokines. In general, C-X-C chemokines attract neutrophils, but not monocytes, while C-C chemokines do the reverse (6).

The chemokine IP-10 is the only member of C-X-C chemokines that attracts monocytes and T lymphocytes. The IP-10 secretion has been detected in macrophages, endothelial cells, keratinocytes and activated T cells in response to a number of activating stimuli, such as IFN- α and TNF α . IP-10 is involved in delayed type hypersensitivity and participates in the pathogenesis of infection and shock, such as acute Lassa fever (10), and canine endotoxemia (11). In addition, it has been shown to be associated with inflammatory responses of immune-mediated diseases, such as rheumatoid arthritis, graft

Table 2. The plasma level of chemokines (pg/mL)

	KD	HSP	AFI
IP-10	538.6 ± 336.4	58.7 ± 95.7	417.1 ± 262.2
MCP-1	443.0 ± 473.1	82.9 ± 79.0	328.6 ± 261.1
Gro- α	31.8 ± 22.1	29.4 ± 53.3	134.3 ± 153.6

KD, Kawasaki disease; HSP, Henoch-Schönlein purpura; AFI, Acute febrile illnesses.

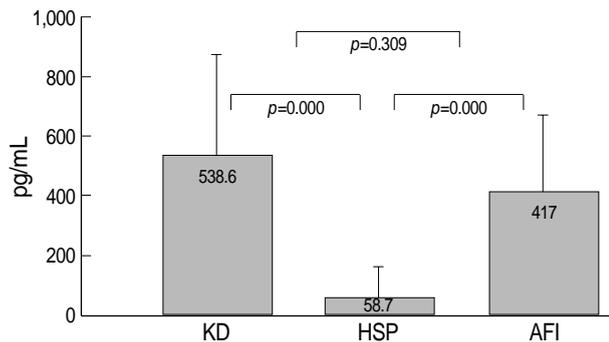


Fig. 1. The chemokine IP-10 levels in plasma. KD, Kawasaki disease; HSP, Henoch-Schönlein purpura; AFI, Acute febrile illnesses.

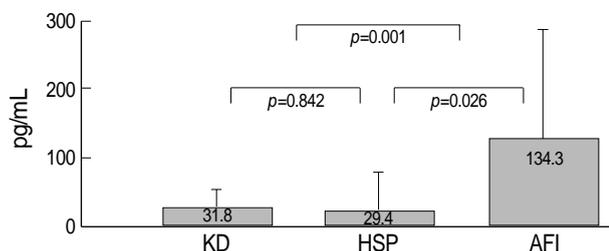


Fig. 3. The chemokine Gro-α levels in plasma. KD, Kawasaki disease; HSP, Henoch-Schönlein purpura; AFI, Acute febrile illnesses.

rejection and tuberculosis. In the present study, the plasma level of IP-10 was markedly elevated in Kawasaki disease and acute febrile illnesses compared with Henoch-Schönlein purpura.

The chemokine MCP-1, a member of the C-C family of chemokines, attracts monocytes and lymphocyte, but not neutrophils (12-15). MCP-1 has been involved to elicit degranulation and respiratory burst in monocytes and cytokine production in these cells. In this regard, MCP-1 has been called as monocyte chemotactic and activating factor (MCAF). MCP-1 is also a potent histamine-releasing agent since it attracts monocytes that secrete supernatants, containing histamine release factors. In this study, MCP-1 was elevated in the patients with Kawasaki disease and acute febrile illnesses compared with in the Henoch-Schönlein purpura group. However, the plasma levels of MCP-1 were not elevated consistently. In 8 cases of Kawasaki disease, the MCP-1 levels were below 100 pg/mL. Further studies incorporating a larger number of cases are needed.

Gro-α is a member of C-X-C chemokines that attracts neutrophils, monocytes, basophils and lymphocytes. Gro-α contributes to the ongoing inflammatory process and neutrophil infiltration associated with various diseases (8, 16-22). In the present study, Gro-α was elevated only in acute febrile illnesses compared with in Kawasaki disease and Henoch-Schönlein purpura.

The interaction between leukocytes and vascular endothelial cells contributes to the pathogenesis of Kawasaki disease. This immune activation accompanied by elevated levels of

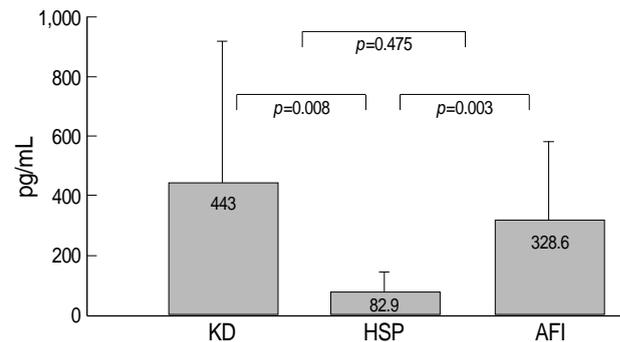


Fig. 2. The chemokine MCP-1 levels in plasma. KD, Kawasaki disease; HSP, Henoch-Schönlein purpura; AFI, Acute febrile illnesses.

various pro-inflammatory cytokines. Increased production of tumor necrosis factor α (TNF-α), interleukin (IL)-1β, IL-2, interferon (IFN)-α, IL-6, IL-8, RANTES, MCP-1, macrophage inflammatory protein-1β, (MIP-1β) and IP-10 has been detected in peripheral blood of patients with Kawasaki disease (4, 7, 8, 22-25).

Henoch-Schönlein purpura is one of the vasculitis disorders with rash, arthritis, abdominal pain and renal involvements (26). Although Henoch-Schönlein purpura has been suggested to represent an immunoglobulin A related immune complex disease, the pathogenesis has not been fully elucidated. Acute inflammation in Henoch-Schönlein purpura manifests as leukocytoclastic vasculitis on light microscopy. Although the factors that determine and mediate the severity of Henoch-Schönlein purpura remain poorly understood, proinflammatory cytokines such as IL-1, IL-6 and TNF-α (27-30) may be involved. Besbas *et al.* (28) demonstrated that TNF-α and IL-1β levels are high in the acute phase of Henoch-Schönlein purpura. Wu *et al.* (29) also demonstrated that TNF-α and IL-1β levels were high in Henoch-Schönlein purpura glomerulonephritis.

Many pro-inflammatory cytokines such as IL-1, IL-6, IL-8, TNF-α and IP-10 are involved in the pathogenesis of other febrile processes (7, 10, 31-34). Cha *et al.* observed the elevated levels of IL-6 on stimulation of *Candida albicans* (33). Valbuena *et al.* also detected elevation of MCP-1 in response to the infection of spotted fever group rickettsiae (34). We also observed Gro-α was elevated in measles, mumps and other viral infections. Interestingly, however, Gro-α was extremely low in 2 patients with pharyngotonsillitis among the acute febrile illnesses. Regrettably, the causative organisms in the patients with pharyngotonsillitis were not identified.

In conclusion, during the acute stage of Kawasaki disease, the IP-10 and MCP-1 levels were elevated. IP-10, MCP-1 and Gro-α were elevated during the other acute febrile illnesses. By contrast, none of these chemokines were elevated in Henoch-Schönlein purpura. According to these results, monocytes may play an important role in Kawasaki disease. In other acute febrile illnesses, both monocytes and neutrophils may play an important role. Henoch-Schönlein purpura may

not be associated with the role of monocytes and neutrophils. Further studies are needed to define the clear relationship between the chemokines and neutrophils in these illnesses.

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