Production and Expression of Gro-α and RANTES by Peripheral Blood Mononuclear Cells Isolated from Patients with Kawasaki Disease and Measles

We investigated whether the production and gene expression of Gro-α and RANTES in Kawasaki disease differ in measles. Forty-two samples from 14 patients in different clinical stages of Kawasaki disease, eight samples from 8 patients in the acute stage of measles and seven samples from 7 healthy children were collected. The present study was performed using ELISA and RT-PCR for the productions and gene expression of the chemokines. The production of Gro-α was markedly elevated during the acute stage of measles compared with Kawasaki disease. Moreover, the expression of Gro-α was increased in every case of measles, but not in Kawasaki disease. The production of RANTES was elevated in the acute stage of both diseases when compared to the healthy control. However, the plasma RANTES level did not change significantly according to the clinical stages of Kawasaki disease. A correlation between the production and gene expression of RANTES and Gro-α was not found in Kawasaki disease. These results suggest that Kawasaki disease differs from measles with regard to Gro-α production and expression, but not RANTES. Gro-α might play an important role in the acute stage of measles, however not in Kawasaki disease. Further studies are needed to clarify the efficacy of Gro-α as a marker in measles.

Key Words: Mucocutaneous Lymph Node Syndrome; Vasculitis; Measles; Chemokines; RANTES

INTRODUCTION

Kawasaki disease, first described by Kawasaki in 1967, is an acute febrile illness of early characterized by prolonged fever, polymorphous skin rash, conjunctival injection, oral mucosal changes, erythematous induration of hands and feet, and cervical lymphadenopathy (1). Although the clinical and epidemiological features suggest an infectious origin, its etiology remains unknown. In the acute stages, it can be very difficult to differentiate it from other febrile infectious diseases. Especially, Kawasaki disease shares some clinical findings with measles such as high fever, conjunctivitis, skin rash, and so forth. It is important to differentiate these diseases in the acute febrile stage in measles endemic areas.

Numerous studies have demonstrated the interaction between leukocytes and vascular endothelial cells likely contributes to the pathogenesis of Kawasaki disease, and vascular lesions are infiltrated by large numbers of mononuclear cells in response to chemotactic stimuli (2-4).

Recently, Wong and associates (5) have been concerned about chemokines (6), a newly identified family of proinflammatory cytokines in Kawasaki disease. So it is important to understand the factors that may recruit and activate leukocytes to the regions of vasculitis in this disease (7-16). However, comparing the profiles of these chemokines between Kawasaki disease and measles have not been studied yet.

In the present study, we compared the production and gene expressions of chemokine; Gro-α (growth-related oncogene-α) and RANTES (regulated upon activation in normal T cells expressed and secreted) (7-16). The plasma RANTES level did not change significantly according to the clinical stages of Kawasaki disease. A correlation between the production and gene expression of RANTES and Gro-α was not found in Kawasaki disease. These results suggest that Kawasaki disease differs from measles with regard to Gro-α production and expression, but not RANTES. Gro-α might play an important role in the acute stage of measles, however not in Kawasaki disease. Further studies are needed to clarify the efficacy of Gro-α as a marker in measles.

MATERIALS AND METHODS

Study population

The study cohort were composed of three groups. The first group included 14 consecutive patients (10 males, 4 females; mean age, 35.6 months, range 11 to 83 months) who met the diagnostic criteria for Kawasaki disease as described elsewhere (1). All patients received high dose single intravenous gamma globulin (IVIG) infusion (Liv-gamma 2 g/kg in 10 to 12 hr) in combination with oral aspirin (Rhonal 60-100 mg/kg divided into 3 equal doses) treatment within the first 10 days of illness (acute phase) and were prospectively evaluated at
Table 1. Baseline characteristics of study groups

| Characteristics | Kawasaki disease | Measles | Healthy control |
|-----------------|------------------|---------|----------------|
| Cases (male/female) | 14 (10/4) | 8 (3/5) | 7 (4/3) |
| Age (range, months) | 35.6∗ (11-83) | 28.1∗ (7-128) | 34.3 (8-55) |

∗p<0.05.

Yeungnam University Hospital, Dae-Gu, Korea. All patients underwent serial blood sampling and echocardiographic evaluation. The blood sampling and echocardiographic study was done according to the disease course as follows; acute stage, before treatment with IVIG and aspirin, subacute stage, 7 to 10 days after intravenous gamma globulin treatment, and convalescent stage, 30 to 40 days after the onset of the Kawasaki disease. Normal ranges for coronary artery size defined according to diagnostic criteria of the Research Committee on Kawasaki disease, Ministry of Health and Welfare, Japan (17). In younger than 5 yr, internal lumen diameters of 3 mm or more, and in aged 5 yr or older, internal lumen diameter of 4 mm or more is considered coronary artery dilatation. Three patients were found to have dilatations of the coronary arteries. In all of them, the echocardiographic findings became normal during one year of follow-up. None of the patients died.

The second group included 8 samples from eight patients (3 males, 5 females; mean age, 28.1 months, ranges from 7 to 128 months) in the acute febrile stage of measles. And 7 healthy children (4 males, 3 females; mean age, 34.3 months, ranges from 8 to 55 months) who had no apparent underlying diseases composed the third group (Table 1).

Informed consents were obtained from the parents of the patients included in this study.

Echocardiographic assessment of coronary artery

The 2-dimensional echocardiographic study was performed with a 7.5 MHz transducer (Sequoia® CA, U.S.A.) to obtain the coronary artery dimension at the level of the aortic root in the parasternal short axis view. All patients received a single dose of chloral hydrate (80 mg/kg) orally for sedation.

Sera

Samples of venous blood were obtained from patients and controls, and were centrifuged at 2,000 rpm/min for 30 min. After the isolation of peripheral blood mononuclear cells (PBMCs), serum samples were frozen at -70°C for the ELISA assay.

Reagents

Reverse transcriptase-polymerase chain reaction (RT-PCR) kits were purchased from Perkin Elmer (Norwalk, CT, U.S.A.). Trizol solution for total RNA isolation and a 100 bp DNA ladder as molecular weight marker were obtained from Gibco/BRL (Life Technologies, Gaithersburg, MD, U.S.A.).

ELISA assay

ELISAs for RANTES and Gro-α were performed according to manufacturer’s instruction using kits from R&D systems (Minneapolis, MN, U.S.A.). Briefly, add 100 µL of standards or patient serum was incubated in anti-human RANTES (or Gro-α) coated well plates at room temperature for 2 hr. After the 200 µL of prepared biotinylated antibody was added, the plates were incubated at room temperature for 1 hr, and then the 200 µL of tetramethylbenzidine substrate solution was added and the plates were developed in the dark state at room temperature for 20 min. The reaction was ceased by adding 50 µL of stop solution. Absorbence of serum was measured at 450 nm for RANTES and Gro-α.

PBMCs isolation and total RNA isolation

PBMC were isolated by density centrifugation on a Ficoll-Hypaque gradient at 2,000 rpm for 30 min, then total RNA was isolated with the use of Trizol solution, as instructed by the manufacturer. Briefly, after the addition of 1 mL of Trizol and 200 µL of chloroform followed by centrifugation, the aqueous phase was combined with an equal volume of isopropanol. The precipitated pellet was washed with 70% ethanol, and suspended again in diethylpyrocarbonate (DEPC)-treated water.

Chemokine RT-PCR

Reverse transcription and PCR amplification were performed as described by Cha et al. (18). One µg of total RNA per sample were reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Perkin Elmer, Norwalk, CT, U.S.A.) and oligo dT priming according to the manufacturer’s instruction, at 42°C for 15 min. Amplification with specific primers was performed in a Gene Amp PCR system 9600 (Perkin Elmer) 35 cycles for 15 sec, 60°C denaturation for 30 sec, 60°C annealing for 1 min, 94°C denaturation for 1 min, 60°C annealing for 1.5 min, 72°C extension profile in case of RANTES. Amplification of mRNA for the housekeeping gene β-actin was used as internal quality standard. Amplified products were electrophoresed on 1.5–2% agarose gel stained with 0.5 µg/mL ethidium bromide. The primer sequences were as follows: β-actin (712 bp) sense; 5′-ggatccatatcaagcctcgccgagca-3′, antisense; 5′-gaagcttgggagccagctc-3′, RANTES (275 bp) sense; 5′-atgagtcgtcctccgagcagc-3′, antisense; 5′-ctagttccttcagtaagtt-3′, Gro-α (385 bp) sense; 5′-atgaactgcctggcc-3′, antisense; 5′-ggcaatgtggcagccttc-3′.

Statistical Methods

Results were presented as mean ±SD. Statistical differences were analyzed by the Mann-Whitney test. A p-value
<0.05 was considered significant.

RESULTS

Plasma levels of Gro-α

The plasma levels of Gro-α were markedly elevated during the acute stage of measles than those of the patients with Kawasaki disease and healthy control group \( (p<0.01) \) (Fig. 1). Among the patients with Kawasaki disease, it is somewhat elevated in the subacute stage, but we could not find obvious elevation of plasma Gro-α level when compared with healthy control group (Fig. 2).

Plasma levels of RANTES

The plasma levels of RANTES were more elevated in the acute stage of Kawasaki disease than measles and healthy controls (Fig. 3), but there was no statistical significance. Among the patients with Kawasaki disease, there was no obvious correlation between clinical stages of the Kawasaki disease and the plasma levels of RANTES, yet it is elevated in each stage (Fig. 4).

Expression of Gro-α and RANTES mRNA in PBMCs

The expression of Gro-α mRNA was increased in every case of measles, but was not in the acute stage of the Kawasaki disease and the control group. RANTES mRNA was ex-
pressed extensively in both diseases, but not in the control group (Fig. 5).

According to the clinical stages in Kawasaki disease, RANTES mRNA was expressed inconsistently, but Gro- mRNA was not expressed (Fig. 6).

DISCUSSION

Kawasaki disease is an acute febrile illness of early childhood (1). Although the clinical and epidemiological features suggest an infectious origin, its etiology remains unknown. Histopathological findings in Kawasaki disease indicate a vasculitis that predominantly affects the medium-sized arteries (19, 20).

The differential diagnosis includes scarlet fever, toxic shock syndrome, measles, drug reaction, viral infection, and other vasculitis syndromes. Especially, Kawasaki disease shares some clinical findings with measles such as high fever, conjunctivitis, and skin rash. It is important to differentiate of these diseases during the acute febrile stage in endemic areas of measles.

Elevated levels of various pro-inflammatory cytokines have been detected in the peripheral blood of patients with Kawasaki disease during the acute stage (2-5, 7-16). During the acute stage of Kawasaki disease, increased production of tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), IL-2, interferon-α (IFN-α), and IL-6 have been detected in the circulation of patients with Kawasaki disease (7-13). TNFα, IL-1β, and IFN-α induce activation of antigens and adhesion molecules, such as endothelial leukocyte adhesion molecule-1 (ELAM-1) and intercellular adhesion molecule-1 (ICAM-1), on endothelial cells, and TNFα and IFN-α cause endothelial injury in vitro studies (21). These four mentioned factors may play a key role in the pathogenesis of both immune activation and endothelial cell damage, and suggested to be as predictable factors of coronary aneurysm in Kawasaki disease (15, 16). Wong et al. (5) reported that RANTES, monocyte chemoattractant protein-1 (MCP-1) and Macrophage Inflammatory Protein-1β (MIP-1β) gene expression levels were significantly elevated in the peripheral blood with Kawasaki disease. Therefore, in Kawasaki disease, the presence of circulating chemokines may lead to an increased immune cell adhesion to activated endothelial cells, resulting in tissue damage.

The chemokines (chemotactic cytokines) are a large family of cytokines composed of small pro-inflammatory peptides that regulate trafficking, activation and sometimes the proliferation of myeloid, lymphoid, melanocytes, and endothelial cells (22). The chemokines presently comprise approximately 60 members. Based on the predicted primary amino acid structure, the superfamily is divided into four groups.
(23-25): the CXC or &alpha; chemokines, the CC or &beta; chemokines, the C or &gamma; chemokines, and the CX-C or &delta; chemokines.

The chemokine Gro-&alpha; is a member of the CXC chemokine and is chemotactic for neutrophils, basophils, monocytes, and lymphocytes, and is responsible for much of the tissue damages associated with chronic infection. Gro-&alpha; is over expressed, contribute to the ongoing inflammatory process and neutrophil infiltration associated with various diseases including psoriasis (26, 27), rheumatoid arthritis (28), and ulcerative colitis (29). And also, it is angiogenic (30), like IL-8, and has a role in wound healing (31, 32) through the stimulation of the proliferation of keratinocytes. It is also speculated, although not yet proven, Gro-&alpha; performs the function of IL-8 in mice (6).

Recent study reports that IL-8 and RANTES have been suggested to be involved in the pathogenesis of Kawasaki disease (5, 33). Moreover, RANTES mRNA was induced by measles virus infection (34). So we focused on RANTES and Gro-&alpha; instead of IL-8.

In this study, the production of Gro-&alpha; was markedly elevated during the acute stage of measles than Kawasaki disease and healthy control. And there were no remarkable changes of plasma level of Gro-&alpha; according to the clinical stages in Kawasaki disease (levels were below 150 pg/mL). This was coincided with the expression patterns of Gro-&alpha; mRNA. The expression of Gro-&alpha; mRNA was increased in every case of measles, but not in Kawasaki disease, even in different clinical stages. Therefore, the production and gene expressions of Gro-&alpha; seem to have no significant correlation on the clinical stages of Kawasaki disease. Otherwise, we assume that Gro-&alpha; might play an important role in the acute febrile stage of measles.

The chemokine RANTES belongs to the CC chemokines and attract mainly T lymphocytes and monocytes, and is involved in a number of inflammatory diseases, including rheumatoid arthritis (35), allergy in airway (36), and multiple sclerosis (37).

The production of RANTES was elevated in the acute stage of Kawasaki disease and measles than healthy control in this study. However, there were no significant changes of plasma RANTES levels according to the clinical stages of Kawasaki disease. The mRNA of RANTES was expressed, but was not consistent according to the clinical stages. We presume that would be the natural course of Kawasaki disease or one of the effects of the IVIG infusion. So we could not find any obvious correlation between the plasma level and gene expression of RANTES in the patients with Kawasaki disease.

And also, there was no obvious relation between clinical characteristics of the disease including the coronary artery dilatation and RANTES. These results were similar to Wong et al. (5), demonstrating that RANTES gene expression levels were significantly elevated, but there was no obvious correlation between the clinical stage of Kawasaki disease and chemokine expression level. Otherwise, they reported that persistence or increased expression of RANTES gene into convalescent stage may contribute to further risk of coronary artery dilatation. In the acute stage of measles, the expression of RANTES mRNA was strong that was similar to Noe et al. (34). The expression patterns of chemokine genes are variable according to the studies (37-40). Further study for the chemokine expressions and roles are still required in Kawasaki disease and measles.

In conclusion, these results suggested that Kawasaki disease differs from measles with regard to Gro-&alpha; production and expression, but not RANTES. Gro-&alpha; might play an important role in the acute febrile stage of measles, but not in Kawasaki disease. Further studies are needed to clarify the efficacy of Gro-&alpha; as a marker for activities of measles.

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