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Expression of Fc<gamma>Rs on monocytes among Kawasaki disease patients with coronary artery lesions

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ABSTRACT

Objective: To study expression of Fc gamma receptors (Fc<gamma>Rs) on monocytes in Kawasaki disease (KD) patients with coronary artery lesions (CAL).
Methods: 160 newly diagnosed KD patients and 80 health children were enrolled in this study. All patients were scheduled to receive both aspirin and intravenous immunoglobulin (IVIG). Serial blood samples were obtained before and 3 days after completing IVIG therapy. The first two-dimensional echocardiographic examination was performed for all KD patients within 10 days, and was repeated at 3 weeks. CAL was defined by coronary artery *Z*-scores ≥ 2.5 by echocardiography. Expression of inhibitory and activating Fc<gamma>Rs on $CD14^+$ monocytes (MCs) was assessed by flow cytometry. Cytokine expression in MC was evaluated by PCR.
Results: Of the 160 KD patients enrolled in this study, 36 had coronary artery lesions ($KD\text{-}CAL^+$ group), while 124 did not ($KD\text{-}CAL^-$ group). There was no significant difference in Fc<gamma>RI expression on MCs from KD patients and that of Ctrls. Although Fc<gamma>RIII and Fc<gamma>RIIa levels were significantly higher in KD patients compared with those in Ctrls, there were no significant differences between the $KD\text{-}CAL^+$ and $KD\text{-}CAL^-$ groups. Fc<gamma>RIIb expression in the KD patients was lower than that of Ctrls, meanwhile expression in the $KD\text{-}CAL^+$ group was lower than that in the $KD\text{-}CAL^-$ group. After IVIG therapy, Fc<gamma>RIIb expression increased in $KD\text{-}CAL^+$, but did not reach the normal range. A negative correlation was observed between the levels of IL-6, TNF-α and Fc<gamma>RIIb expression.
Conclusion: Decreased Fc<gamma>RIIb expression on MCs may contribute to the development of coronary artery lesions in patients with KD.

Kawasaki disease (KD) is an acute systemic vasculitis of unknown etiology. The inflammatory process preferentially involves the coronary arteries, potentially resulting in coronary arteritis, aneurismal lesions, arterial thrombotic occlusion and sudden death. The cause of coronary artery lesions (CALs) is unknown, although the incidence can be diminished by IVIG administration. The anti-inflammatory activity of IVIG therapy is generally attributed to interaction with Fc<gamma>Rs [1].

Because of their direct interactions with IVIG, Fc<gamma>Rs are implicated as plausible mediators of CALs in KD. Fc<gamma>Rs can be divided into three classes, Fc<gamma>RI, II and III, which differ in structure, cell distribution and also in affinity [2]. The activating Fc<gamma>Rs (I, IIa and III) are characterized by an immunoreceptor tyrosine-based activation motif (ITAM) that mediates the activation of cells, while the inhibitory Fc<gamma>RIIb contains an inhibitory motif (ITIM) that mediates the inhibition of cells [2]. The balance of inhibitory and activating Fc<gamma>Rs has been shown to determine the behavior of cellular responses [3]. Studies focusing on the changes in Fc<gamma>Rs in KD patients with CALs are rare, although the importance of Fc<gamma>Rs in other immune-related vasculitis conditions such as Behçet's disease has been reported [4]. Here, we evaluated the statuses of distinct Fc<gamma>Rs in KD patients with CALs for the first time.

1. Methods

1.1. Patients and controls

One hundred and sixty (89 male and 71 female; age range 3–58 months; median age, 27 months) newly diagnosed KD patients who met the diagnostic criteria for KD published by the KD Research Committee of Japan were enrolled in this study [5]. Enrollment took place between November 2013 and November 2014 at the Department of Rheumatology, Shenzhen Children's Hospital (China). Eighty similarly aged children (38 males and 42 females; age range: 4–56 months; median age: 26 months) who were physically healthy, without any clinical signs of infection or inflammation, were included as healthy controls (Ctrls). All patients were scheduled to receive both aspirin (30–

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50 mg/kg per day) and IVIG (2 g/kg). Serial blood samples were obtained from all KD patients before and 3–5 days after completing IVIG therapy. Informed consent was obtained from the parents and the study was approved by the Shenzhen Children's Hospital Medical Ethics Committee. In total, 135 KD patients responded favorably to IVIG therapy, while fever persisted in 25 patients for 36 h after IVIG treatment; these patients received a second dose of IVIG. The first two-dimensional echocardiographic examination was performed for all KD patients within 10 days, and was repeated at 3 weeks. CAL was defined by coronary artery *Z*-scores ≥ 2.5 for the left coronary artery (LCA), left anterior descending artery (LAD) or right coronary artery (RCA) measured by echocardiography. *Z*-scores were calculated as follows: $Z(\mathrm{LCA}) = [\mathrm{LCA} - (-0.368 + 4.898 \times -1.761 \times \mathrm{BSA})] / 0.324$, $Z(\mathrm{LAD}) = [\mathrm{LAD} - (-0.383 + 4.226 \times -1.571 \times \mathrm{BSA})] / 0.289$, $Z(\mathrm{RCA}) = [\mathrm{RCA} - (-0.577 + 5.032 \times -2.189 \times \mathrm{BSA})] / 0.332$, where BSA = body surface area. Among the 160 patients enrolled, 36 patients with KD had CALs (KD-CAL$^+$ group), while 124 did not (KD-CAL$^-$ group) (Fig. 1).

1.2. Blood samples

Venous blood samples (5 ml) were taken from patients with KD and Ctrls using heparin as an anti-coagulant. Blood samples were analyzed immediately without mitogen stimulation or in vitro culture. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by Ficoll density gradient centrifugation (500 × *g*, 20 min). CD14$^+$ MC cells were immediately isolated from peripheral blood using microbeads (Dynal, USA) according to the manufacturer's instructions. Whole blood (2 ml) was prepared for flow cytometric (FCM) analysis. Purified cells were identified as >97% CD14+ by FCM.

1.3. Flow cytometric analysis

The antibodies CD14-FITC (MC), CD64-PE (Fc<gamma>RI), CD16-PE (Fc<gamma>RIII) and mouse IgG1-PE were obtained from Becton Dickinson. Because of the high degree of sequence similarity within the extracellular regions of human CD32B (Fc<gamma>RIIb) and CD32A (Fc<gamma>RIIa), none of the commercially available fluorescently labeled antibodies can distinguish CD32B from CD32A. Consequently, we used an indirect immunofluorescent staining method based on the usage of two polyclonal antibodies specific for CD32A and CD32B (Santa Cruz, USA), respectively, which were detected by the secondary PE-conjugated antibody (Santa Cruz, USA). PE-conjugated donkey anti-rabbit IgG (Santa Cruz, USA) was used as an isotype control. After gating on the MC populations, the data were obtained using FACS Canto II and FACS Diva Ver 6.1.3 software. The mean fluorescence intensity (MFI) of each type of Fc<gamma>R was estimated.

1.4. LightCycler real-time polymerase chain reaction (PCR)

Expressions of proinflammatory cytokine (IL-6, TNF-α), Fc<gamma>RIIa and Fc<gamma>RIIb mRNA in MCs were quantitated by real-time PCR, using Quantitect™ SYBR green PCR Kit (Qiagen, Germany) and a LightCycler 480 Instrument (Roche Molecular Biochemicals, Switzerland). The final results were calculated as ratios of the transcript levels of the target genes relative to the amount of β-actin by Relative Quantification Software V1.0 (Roche Molecular Biochemicals, Switzerland). The following primers were used: IL-6 forward, ACTCACCTCT TCAGAACGAATTG, and reverse, CCATCTTTGGAAGGTTCAGGTTG; TNF-α forward, CCTCTCTCTAATCAGCCCTCTG, and reverse, GAGGACCT GGGAGTAGATGAG; IL-1β forward, TTCGACACATGGGATAACGAGG, and reverse, TTTTTGCTGTGAGTCCCGGAG; Fc<gamma>RIIa forward, GACGAAGGGATGCTGCAGTTC, and reverse, TGAGGCACAGAAGGTGCAG TC; Fc<gamma>RIIb forward, GACAAAGTTGGGGCTGAGAAC, and reverse, CCAATGCAAGACAATGGAGAC; β-actin forward, GGCATTCACGA GACCACCTAC, and reverse, CGACATGACGTTGTTGGCATAC.

2. Statistical analysis

All statistical analyses were performed using SPSS for Windows version 19.0 (SPSS, USA). Data are presented as mean ± standard deviation (SD). Statistical analyses were performed using the Wilcoxon matched-

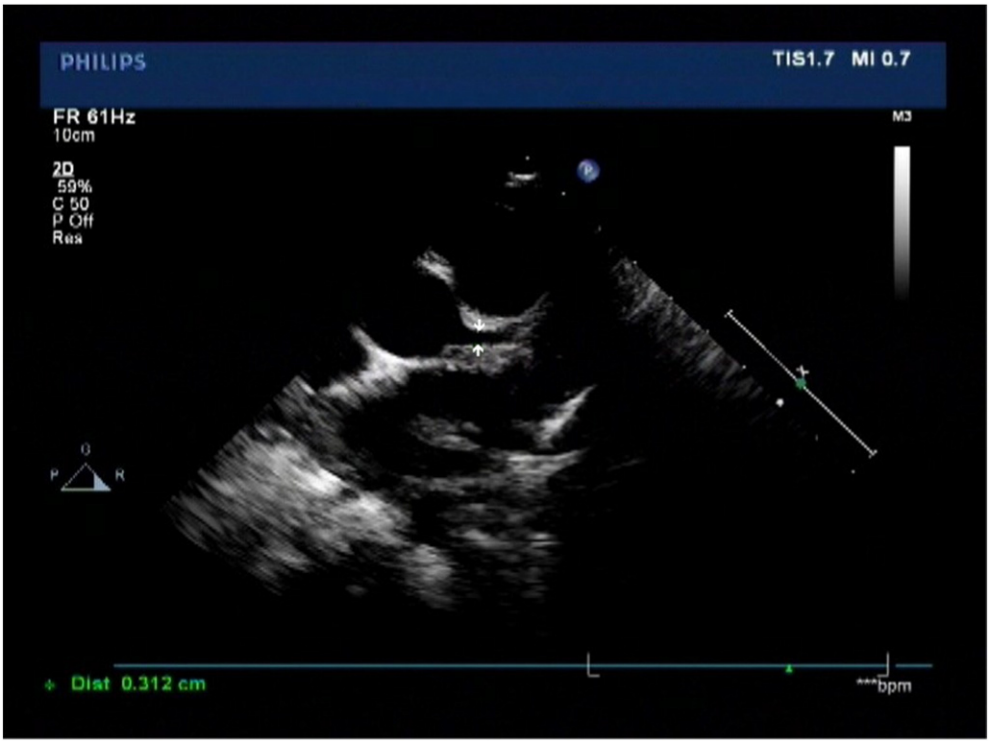

Fig. 1. An echocardiographic image for CAL in KD. Arrow: enlarged coronary artery.