ORIGINAL ARTICLE

Altered Immunoglobulin A and M Levels Associated With Changes in BAFF and APRIL After Administration of Intravenous Immunoglobulin to Treat Kawasaki Disease

M Doi,[1] T Takeda,[2,3] Y Sakurai,[2] J Kato,[2] T Hayashi,[2] K Fukuda,[2] T Takase,[1] M Shima[2]

[1]Department of Pediatrics, Yao Municipal Hospital, Yao, Japan
[2]Department of Pediatrics, Nara Medical University School of Medicine, Kashihara, Japan
[3]Clinical laboratory, Uda City Hospital, Uda, Japan

Abstract

Background: Kawasaki disease (KD) is an acute vasculitis of unknown etiology. Immunoregulatory abnormalities have been thought to contribute to its pathogenesis. Although treatment with intravenous immunoglobulin (IVIG) effectively prevents significant cardiac morbidity, the mechanism by which IVIG produces an effect in KD has yet to be fully elucidated.
Objective: To investigate the effects of IVIG on the immune system of patients with KD.
Patients and Methods: Eleven patients with KD (mean [SD] age, 2.2 [1.5] years) were enrolled in this prospective study and treated with high-dose IVIG therapy (2 g/kg in 1 or 2 infusions) during the acute phase of the disease. We examined immunological changes, with special reference to Ig levels and 2 previously unassessed cytokines: B cell–activating factor belonging to the tumor necrosis factor family (BAFF), and a proliferation-inducing ligand (APRIL).
Results: Clinical symptoms disappeared quickly in all cases, with no coronary artery abnormalities. IgA and IgM levels responded more rapidly than previously reported and reached a peak between the 3rd and 10th day after the start of IVIG treatment. The mean (SD) BAFF level was high before IVIG treatment (3234 [1904] pg/mL) and decreased significantly (1085 [257] pg/mL) after IVIG treatment, whereas the mean (SD) APRIL level before IVIG treatment (18.0 [10.0] ng/mL) rose significantly (120.6 [41.2] ng/mL). A significant inverse correlation between BAFF and APRIL was observed in patients with KD.
Conclusions: These results suggest that IVIG may affect the pathogenesis of KD through alteration of BAFF/APRIL.

Key words: A proliferation-inducing ligand (APRIL). B cell–activating factor belonging to the tumor necrosis factor family (BAFF). Immunoglobulin. Kawasaki disease.

Resumen

Antecedentes: La enfermedad de Kawasaki (EK) es una vasculitis aguda de etiología desconocida. Se cree que las anomalías en la inmunorregulación contribuyen a su patogenia. Aunque el tratamiento con inmunoglobulina intravenosa (IGIV) previene de forma eficaz la morbilidad cardíaca significativa, todavía no está claro el mecanismo mediante el cual la IGIV ejerce efecto en la EK.
Objetivo: Investigar los efectos de la IGIV sobre el sistema inmunitario de pacientes con EK.
Pacientes y métodos: En este estudio prospectivo se incluyó a 11 pacientes con EK (edad media [DE]: 2,2 [1,5] años) que fueron tratados con dosis altas de IGIV (2 g/kg en 1 ó 2 perfusiones) durante la fase aguda de la enfermedad. Se examinaron los cambios inmunológicos, con especial atención en los niveles de Ig y 2 citocinas no evaluadas previamente: factor activador de linfocitos B perteneciente a la familia de factores de necrosis tumoral (BAFF) y ligando A inductor de la proliferación (APRIL).
Resultados: En todos los casos los síntomas clínicos desaparecieron rápidamente, sin anomalías de la arteria coronaria. Los niveles de IgA e IgM respondieron más rápidamente respecto a los datos previamente notificados, y alcanzaron un valor máximo entre los días 3 y 10 tras el inicio del tratamiento con IGIV. El nivel medio (DE) de BAFF era elevado antes del tratamiento con IGIV (3.234 [1.904] pg/ml)

y se redujo significativamente (1.085 [257] pg/ml) después del tratamiento con IGIV, mientras que el nivel medio (DE) de APRIL antes del tratamiento con IGIV (18,0 [10,0] ng/ml) aumentó significativamente (120,6 [41,2] ng/ml). En los pacientes con EK se observó una correlación inversa significativa entre BAFF y APRIL.
Conclusiones: Estos resultados indican que la IGIV puede afectar a la patogenia de la EK mediante la alteración de BAFF/APRIL.

Palabras clave: Ligando A inductor de la proliferación (APRIL). Factor activador de linfocitos B perteneciente a la familia de factores de necrosis tumoral (BAFF). Inmunoglobulina. Enfermedad de Kawasaki.

Introduction

Kawasaki disease (KD) is an acute febrile disease of early childhood. It is a self-limiting generalized vasculitis of unknown etiology that was first described in 1967 [1]. High-dose intravenous immunoglobulin (IVIG) therapy (a single 2 g/kg infusion or a dose of 1 g/kg for 2 consecutive days) has been the standard treatment for KD in Japan ever since Newburger et al [2] reported the efficacy and safety of a single high dose of IVIG. The mechanism by which IVIG produces an effect for KD has not been fully elucidated. Changes in immunoglobulin (Ig) levels in KD were systematically investigated over 2 decades [3-5], and recent studies revealed that IVIG may inhibit B-cell proliferation and maturation [6,7]. However, the precise mechanism by which IVIG alters Ig levels also remains unknown.

Enhanced production of inflammatory cytokines such as tumor necrosis factor (TNF) α, interleukin (IL) 1ß, and interferon γ was first reported in 1988 [8]. Nowadays, a direct correlation between disease progression and elevated levels of IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), and vascular endothelial growth factor (VEGF) is thought to be central to the pathogenesis of KD [9-12]. Furthermore, IVIG has been observed to reduce serum levels of inflammatory cytokines such as IL-1ß, TNF-α, IL-6, IL-8, and MCP-1 [13-16].

TNF superfamily ligand members related to B-cell activation, such as B cell–activating factor belonging to the TNF family (BAFF)—also known as BlyS—and a proliferation-inducing ligand (APRIL) have recently attracted attention due to their possible roles in autoimmune diseases. BAFF is involved in the survival and maturation of B cells [17], and APRIL stimulates B- and T-cell proliferation and triggers humoral immune responses. Since IVIG and corticosteroids exert effects, the pathogenesis of KD may be considered similar to that of an autoimmune disease. Therefore, we hypothesized that BAFF, APRIL, or both would participate in the development of KD and in treatment with IVIG. To test our hypothesis, we measured BAFF and APRIL levels before and after administration of IVIG during the acute phase of KD.

Materials and Methods

Patients and Plasma Samples

After obtaining informed consent, we performed a prospective study of 11 patients newly diagnosed with KD between October 2006 and January 2008 according to guidelines set for the disease (5th edition) [18] in Nara Medical University Hospital (Kashahira, Japan) and Yao Municipal Hospital (Yao, Japan). Thirty-three samples were obtained at different time points. All patients were treated with IVIG during the acute phase of the disease. The first day of IVIG treatment was assigned as day 1. We also recruited 15 healthy volunteers.

Blood was drawn into evacuated anticoagulant tubes at a blood to 3.8% [w/v] trisodium citrate solution ratio of 9:1. After centrifugation for 15 minutes at 1500*g*, the platelet-poor plasma was stored at –80°C and thawed at 37°C immediately before performing the assays described. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics committee.

Measurement of Ig Levels and BAFF/APRIL in Plasma

Plasma IgA, IgG, and IgM levels of the 33 samples obtained from 11 patients before and after IVIG treatment were measured using a standard automated immunoturbidimetric assay.

Plasma BAFF and APRIL levels of all the patients were measured using specific enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, Minnesota, USA and Bender MedSystems, Burlingame, California, USA), as previously described [19]. Each sample was tested in duplicate. We validated these assays using citrated plasma samples, as previously described [19].

Statistical Analysis

Data are presented as mean (SD). Statistical analysis was performed using the Wilcoxon rank sum test between groups, and significance was set at $P<.05$. All analyses were performed using the ystat 2002 software package (Igaku Tosho Shuppan, Tokyo, Japan).

Results

Patients

The mean (SD) age of the healthy subjects was 3.0 (1.8) years (range, 0.4-6.2) and that of the KD patients was 2.2 (1.5) years (range 0.6-4.9). There were no significant differences in age between the healthy volunteers and the patients. The total IVIG dose was 2.0 g/kg and this was administered in 1 or 2 infusions. The immunoglobulin used was freeze-dried S-sulfonated human immunoglobulin (Kenketsu Venilon-I, Teijin, Tokyo, Japan) or polyethylene glycol-treated human

immunoglobulin (Kenketsu Venoglobulin-IH Yoshitomi, Mitsubishi Tanabe Pharma, Osaka, Japan).

In each case, the clinical symptoms disappeared and levels of inflammation markers such as C-reactive protein (CRP) decreased rapidly. Sequential echocardiographic examinations did not reveal coronary artery aneurysms, stenosis, or occlusion during the course of the disease.

Immunoglobulin

The changes in Ig levels before and after treatment are shown in Figure 1. Mean (SD) IgG level was 617 (179) mg/dL (range, 410-942) before administration of IVIG, increasing significantly to a peak of 2248 (270) mg/dL (range, 1949-2853) after treatment ($P<.005$). Mean (SD) IgA level was 47 (24) mg/dL (range, 19-92) before administration of IVIG, increasing significantly to a peak of 64 (31) mg/dL (range, 25-120) after treatment ($P<.01$). Mean (SD) IgM level was 104 (39) mg/dL (range, 43-193) before administration of IVIG, increasing significantly to a peak of 158 (48) mg/dL (range, 52-248) after treatment ($P<.01$). However, all the levels except that of IgG returned to within the reference range after IVIG treatment.

BAFF/APRIL

The changes in BAFF and APRIL levels during the treatment period are shown in Figure 2. BAFF levels before IVIG treatment (3234 [1904] pg/mL; range, 1504-6956) were higher than in healthy subjects (890 [196] pg/mL; range, 549-1238), although the difference was not statistically significant ($P=.066$). No significant differences in APRIL levels were observed before treatment between patients (18.0 [10.0] ng/mL; range, 7.4-36.1) and healthy volunteers (16.2 [7.5] ng/mL; range, 3.9-26.1).

After treatment, BAFF levels decreased significantly to their lowest value of 1085 (257) pg/mL (range, 640-1625) ($P<.005$), which was comparable to the levels observed in healthy volunteers. In contrast, APRIL levels markedly increased to a peak of 120.6 (41.2) ng/mL (range, 71.6-208.9) after treatment ($P<.005$). Although high compared with the healthy volunteers, the difference was not significant ($P=.066$).

The scatter plot of the plasma BAFF and APRIL levels for all the patient samples (Figure 3) shows a significant inverse correlation between BAFF and APRIL ($P<.001$). No significant correlation was observed between BAFF and APRIL levels in healthy subjects (data not shown).

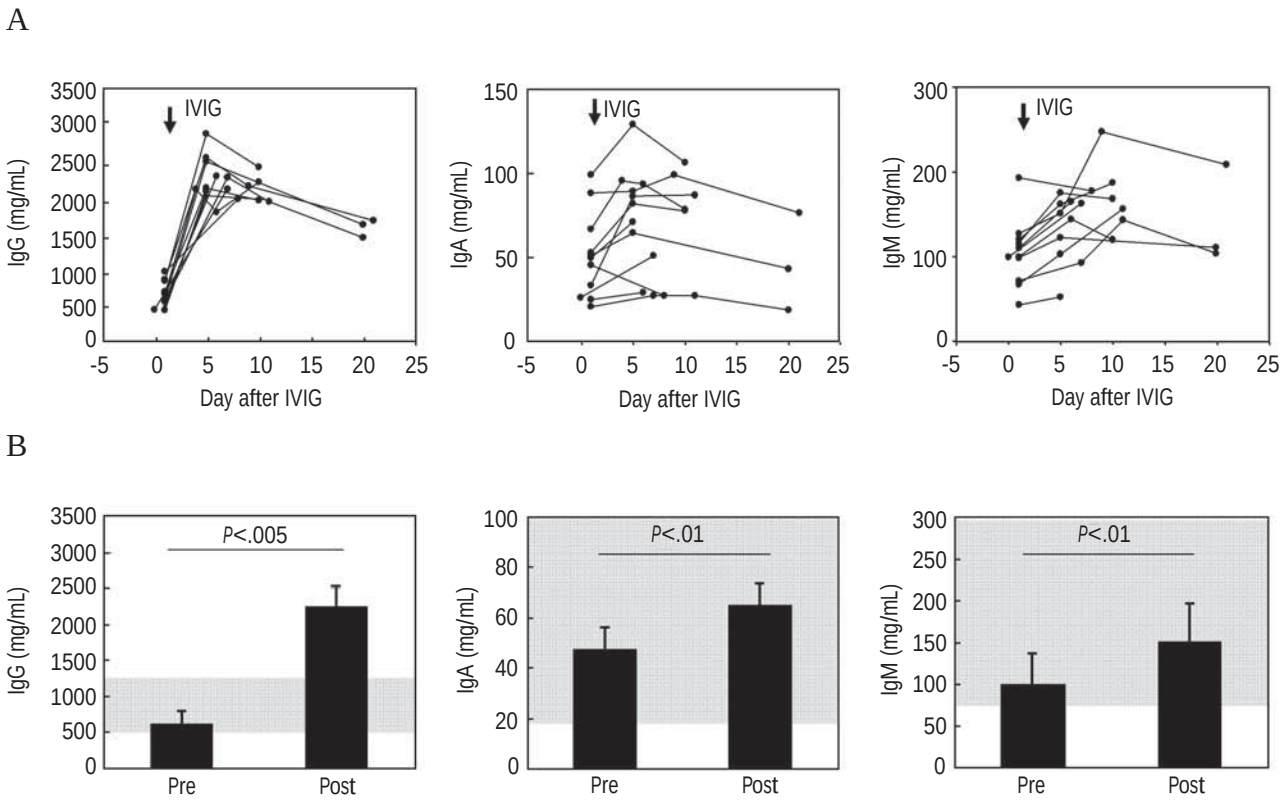

Figure 1. A, Changes in Ig levels during IVIG treatment. Levels of IgG (left), IgA (middle), and IgM (right) are shown. The first day of IVIG treatment was set as day 1. B, Ig levels before IVIG treatment and the highest values after treatment. The shaded region shows the values within normal limits for age. Pre, Ig levels in KD patients before IVIG treatment; Post, those in KD patients after IVIG treatment. Ig indicates immunoglobulin; IVIG, intravenous immunoglobulin.

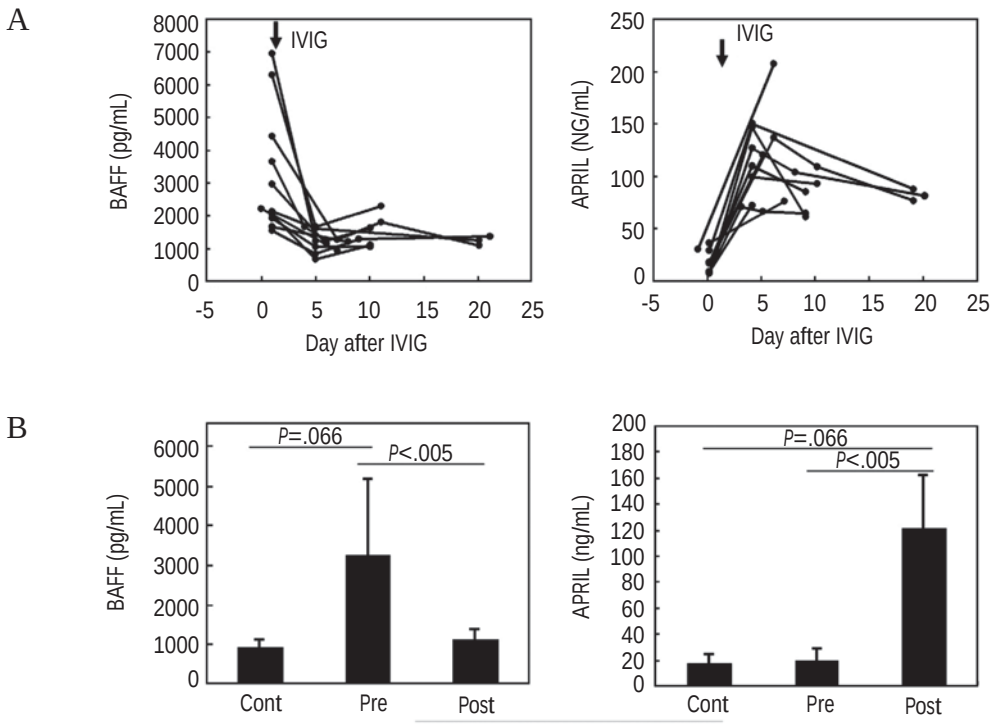

Figure 2. A, Changes in levels of BAFF and APRIL during IVIG treatment. BAFF levels were elevated before IVIG treatment and showed a downward trend after IVIG treatment (left). Conversely, APRIL levels were not elevated before IVIG treatment, but showed an upward trend just after IVIG treatment (right). B, BAFF and APRIL levels before IVIG treatment and the lowest values for BAFF after treatment or the highest values for APRIL. Cont, Ig levels in healthy subjects; Pre, Ig levels in patients with Kawasaki disease before IVIG treatment; Post, those in patients with Kawasaki disease after IVIG treatment. APRIL indicates a proliferation-inducing ligand; BAFF, B-cell–activating factor belonging to the tumor necrosis factor family; Ig, immunoglobulin; IVIG, intravenous immunoglobulin.

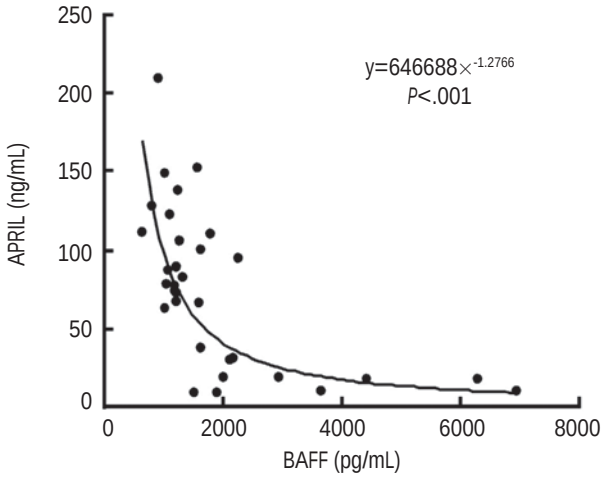

Figure 3. Relationship between APRIL and BAFF levels in patients with Kawasaki disease. APRIL levels were plotted against BAFF levels. A significant inverse correlation was observed between BAFF and APRIL ($P<.001$). APRIL indicates a proliferation-inducing ligand; BAFF, B-cell–activating factor belonging to the tumor necrosis factor family.

Discussion

Immunoregulatory abnormalities have been thought to contribute to the pathogenesis of KD [20,21]. More than a quarter century ago, immunoregulatory abnormalities were known to cause polyclonal B-cell activation in acute KD [21]. As a result of this activation, IgA, IgM, and IgG reached a peak at different points after onset (10-20 days, around day 20, and after day 20, respectively) [5-8]. A recent study revealed that IgA and IgM levels rose immediately after administration of high-dose IVIG, although the differences were not statistically significant [22].

In the present study, significant elevation of IgA, IgM, and IgG levels was evident after administration, although—except for IgG—they remained within normal limits. IgA, IgM, and IgG levels reached a peak between days 3 and 10 after the start of treatment. Even though it takes more than 5 days to make a definitive diagnosis, elevation of Ig levels occurred earlier than previously reported [5-8].

One might think that elevation of IgA and IgM levels is the result of treatment, because IVIG contains both IgA and IgM in small amounts. However, a calculation based on the

concentration of IgA and IgM in IVIG preparations (maximum IgA/IgG ratio of 0.060% and IgM/IgG ratio of 0.054%: recent data on file, Teijin, 2008) shows that the increase in IgA and IgM levels due to IVIG contamination was trivial compared to the real IgA and IgM levels: IgA and IgM contained in IVIG contributed to an increase in IgA and IgM levels to a maximum of 6.1% and 2.1%, respectively. Furthermore, since the half-life of IgA and IgM (5-6 days) is shorter than that of IgG (around 20 days), the contribution of contaminating IgA and IgM in IVIG to the increase in IgA and IgM levels would actually be smaller. IVIG would modify Ig levels, and this modification could be involved in the mechanism of action of IVIG.

BAFF level before treatment was significantly higher in patients, although no differences were observed in the APRIL level between patients and controls. BAFF plays a critical role in B-cell maturation and survival, and failure of the BAFF system results in a breakdown of peripheral B-cell tolerance and autoreactive antibody production. In mice, constitutive BAFF overexpression results in the survival of autoreactive B cells through a survival signal, thus leading to a breakdown in peripheral tolerance and the onset of autoimmune disorders [17,23,24]. In humans, elevated BAFF levels are closely related to hypergammaglobulinemia and several B-cell–mediated autoimmune diseases [25,26]. An elevated BAFF level before IVIG treatment may cause pathogenic IgG production associated with KD. APRIL, on the other hand, stimulates B- and T-cell proliferation, triggers humoral immune responses, activates nuclear factor (NF)–κB, and induces cell death. However, the physiological significance of APRIL has not been fully elucidated.

BAFF/APRIL ligand–receptor interaction comprises a complex network, because BAFF binds to TNF-related receptors such as B-cell maturation antigen (BCMA), transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI), and BAFF receptor (BAFFR), whereas APRIL binds to TACI and BCMA and to heparan sulfate proteoglycans [27]. TACI and BAFFR mediate isotype switching in B cells [28]. Recent studies have revealed that BAFF is involved in B-cell antibody production [29] and that APRIL promotes IgA class switching [30].

Rowley et al [31] reported IgA plasma cell infiltration into vessel lesions in the early, acute, and subacute stages of KD. The same group also identified an oligoclonal IgA response [32,33] and suggest that entry of the etiologic agent of KD induces an unusual antigen-driven immune response [32-34]. However, whether and how IgA plays a role in the pathogenesis of KD has not been elucidated. Future challenges include explanation of the mechanism of rapid increase in IgA levels after IVIG treatment in patients with KD.

Taken together, elevated BAFF levels in acute-phase KD may cause pathogenic IgG production similar to that observed in autoimmune disorders. Falling BAFF levels and rising APRIL levels after high-dose IVIG might contribute to the suppression of IgG production and enhanced IgA production. IVIG might improve the pathogenesis of KD through modification of BAFF/APRIL production. Although elevated BAFF/APRIL levels have been reported in many conditions, to our knowledge, this is the first report of an inverse relationship between BAFF levels and APRIL levels after IVIG treatment.

Although the effectiveness of high-dose IVIG for acute KD has been established, refractory KD has occasionally been reported. Further large-scale studies including intractable cases will provide essential information on the role of the BAFF/APRIL system in high-dose IVIG treatment for patients with KD. Our findings add to knowledge of the pathogenesis of KD and the mechanism of action IVIG.

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Yoshihiko Sakurai, MD, PhD

Department of Pediatrics
Nara Medical University School of Medicine
Kashihara, Nara 634-8522, Japan
E-mail: ysakurai@naramed-u.ac.jp