Research Square Preprint: Please note that this article has not completed peer review.

Functional benefits of corticosteroid and IVIG combination therapy in a coronary artery endothelial cell model of Kawasaki disease

CURRENT STATUS: UNDER REVIEW

Pediatric Rheumatology BMC

Takashi Inoue
National Research Institute for Child Health and Development

Shokei Murakami
National Research Institute for Child Health and Development

Kenji Matsumoto
National Research Institute for Child Health and Development

Akio Matsuda
National Research Institute for Child Health and Development

matsuda-a@ncchd.go.jp*Corresponding Author*
ORCiD: https://orcid.org/0000-0002-7781-0613

DOI:
10.21203/rs.3.rs-16532/v1

SUBJECT AREAS
Rheumatology *Pediatrics*

KEYWORDS
Kawasaki disease, Coronary artery endothelial cells, corticosteroid, IVIG, HMGB1, IL-1α, IL-6, G-CSF

Abstract

Background: Kawasaki disease (KD) is the most common pediatric systemic vasculitides of unknown etiology. Recent clinical studies led to reappraisal of the usefulness of initial combination therapy of intravenous immunoglobulin (IVIG) plus a corticosteroid for patients with severe KD. However, the molecular mechanisms underlying the clinical benefits of early introduction of a corticosteroid to IVIG for severe KD patients remain unclear. We used cultured human coronary artery endothelial cells (HCAECs), which mimic the main lesion sites of KD, in an attempt to elucidate the mechanisms underlying the clinical benefits accruing from adding a corticosteroid to standard IVIG therapy for patients with KD.

Methods: HCAECs were stimulated with TNF-α, IL-1α or IL-1β in the presence and absence of IVIG and/or dexamethasone (DEX). The mRNA and protein concentrations for high-mobility group box-1 (HMGB1), IL-1α, IL-6 and granulocyte-colony stimulating factor (G-CSF) in the culture supernatants were measured by quantitative PCR (qPCR) and ELISA, respectively. Apoptosis was evaluated by the caspase 3/7 activities.

Results: DEX, but not IVIG, significantly inhibited apoptosis caused by inflammatory stimuli, resulting in effective reduction of HMGB1 and IL-1α protein release by HCAECs. As previously reported, DEX or IVIG alone significantly suppressed TNF-α-induced production of IL-6 and G-CSF and mRNA expression, but induction of those cytokines by IL-1s (IL-1α and IL-1β) was resistant to IVIG.

Conclusions: A corticosteroid can effectively inhibit the release of HMGB1 and IL-1α, which may be involved in IVIG resistance in KD. Since IVIG does not have such beneficial anti-cytotoxic effects, adding a corticosteroid to standard IVIG therapy may help prevent the progression of IVIG resistance in KD.

Full-text

Due to technical limitations, full-text HTML conversion of this manuscript could not be completed. However, the manuscript can be downloaded and accessed as a PDF.

Figures

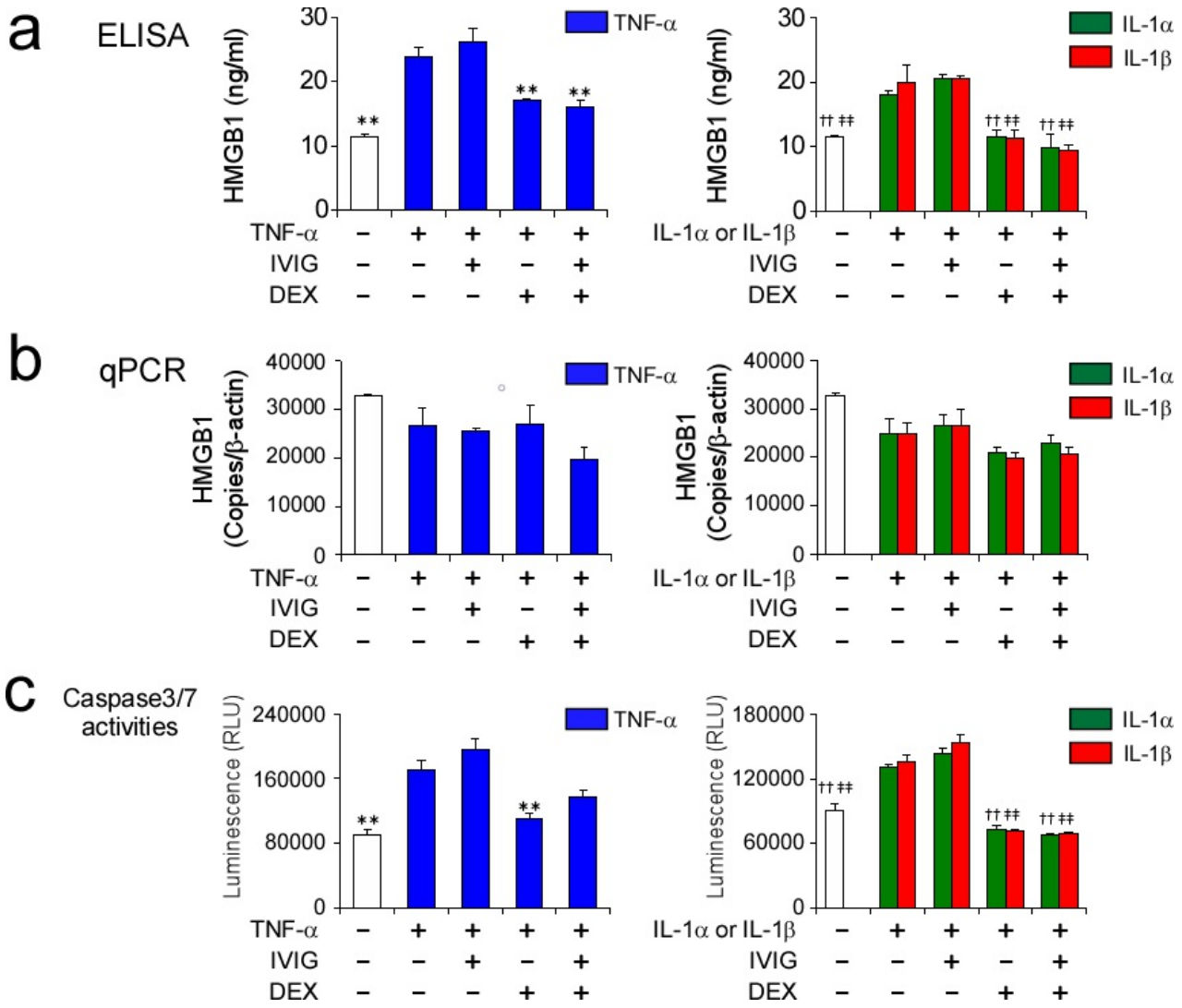

Figure 1

DEX, but not IVIG, inhibits cellular damage to, and HMGB1 protein release by, HCAECs in response to inflammatory stimuli HCAECs were stimulated with 100 ng/ml of TNF-α, or 10 ng/ml of IL-1α or IL-1β for 24 h in the presence and absence of 10 mg/ml IVIG and 1000 nM DEX, alone or in combination. Protein concentrations of HMGB1 in the culture supernatants (a), and 22 HMGB1 mRNA levels (b) and caspase 3/7 activities in HCAECs (c) were measured by ELISA, qPCR and the Caspase-Glo 3/7 Assay System, respectively. Data are shown as the mean ± SD of triplicate samples and are representative of two individual experiments using HCAEC lots from different donors. **P <0.01 compared with 100 ng/ml TNF-α; ††P <0.01 compared with 10 ng/ml IL-1α; and ‡‡P <0.01 compared with 10 ng/ml IL-1β.

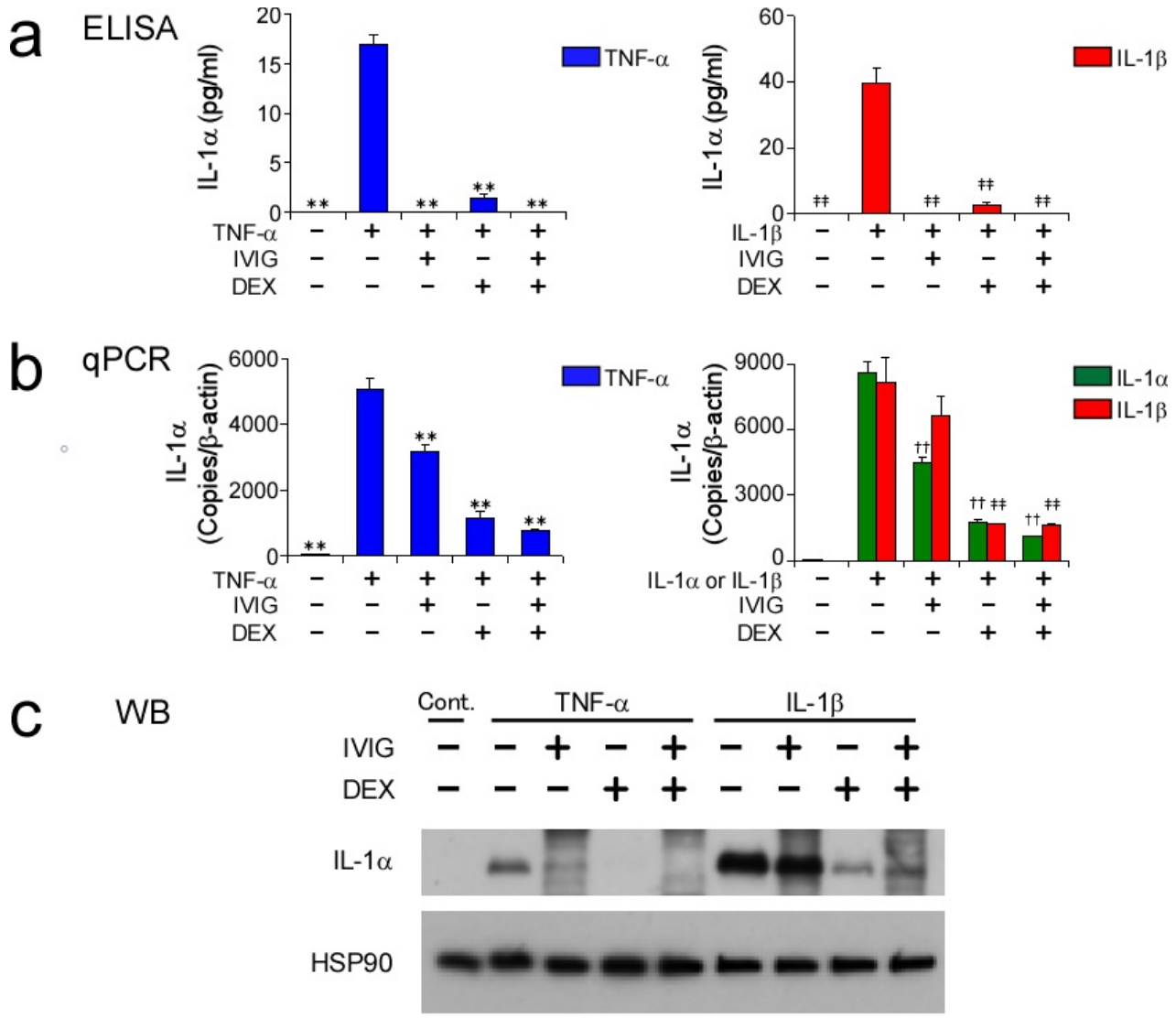

Figure 2

DEX inhibits expression and release of IL-1α by HCAECs in response to inflammatory stimuli

HCAECs were stimulated with 100 ng/ml of TNF-α, or 10 ng/ml of IL-1α or IL-1β for 48 h in the presence and absence of 10 mg/ml IVIG and 1000 nM DEX, alone or in combination. Protein concentrations of IL-1α in HCAEC culture supernatants (a) and mRNA levels of IL-1α in HCAECs (b) were measured by ELISA and qPCR, respectively. Whole-cell lysates of HCAECs were subjected to Western blot analysis of the expression of IL-1α and heat shock protein 90 (HSP90; as a loading control) (c). Data shown in a and b are the mean ± SD of triplicate samples. All data are representative of two individual experiments using HCAEC lots from different donors. P <0.01 compared with 100 ng/ml TNF-α; ††P <0.01 compared with 10 ng/ml IL-1α; and ‡‡P <0.01 compared with 10 ng/m l IL-1β.

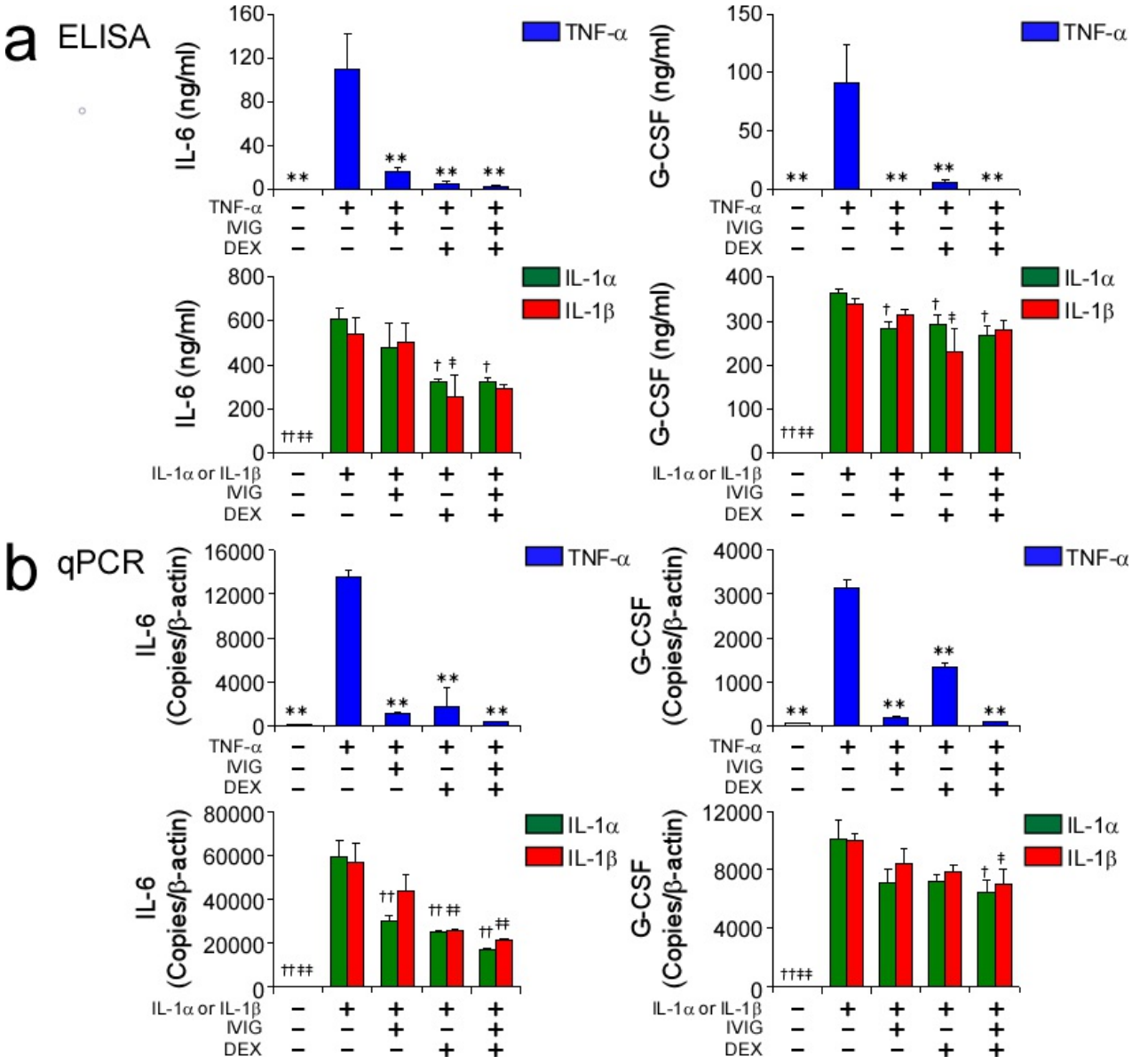

Figure 3

Effects of DEX and IVIG on inflammatory cytokine induced expression of IL 6 and G CSF in HCAECs 23 HCAECs were stimulated with 100 ng/ml of TNF-α, or 10 ng/ml of IL-1α or IL-1β for 48 h in the presence and absence of 10 mg/ml IVIG and 1000 nM DEX, alone or in combination. Protein concentrations of IL-6 and G-CSF in the culture supernatants (a) and mRNA levels of IL-6 and G-CSF (b) in HCAECsin HCAECs were measured by ELISA and qPCR, respectively. Data are shown as the mean ± SD of triplicate samples andData are shown as the mean ± SD of triplicate samples and are are representative of two individual experiments using HCAEC lots fromrepresentative of two individual experiments using

HCAEC lots from twotwo different different donors. **donors. **PP <0.01 compared with 100 ng/ml <0.01 compared with 100 ng/ml TNF-α; †P <0.05 and ††P <0.01 compared with 10 ng/ml compared with 10 ng/ml IL-1α; and ‡P <0.05 and ‡‡P < 0.01 compared with 10 ng/ml compared with 10 ng/ml IL-1β.

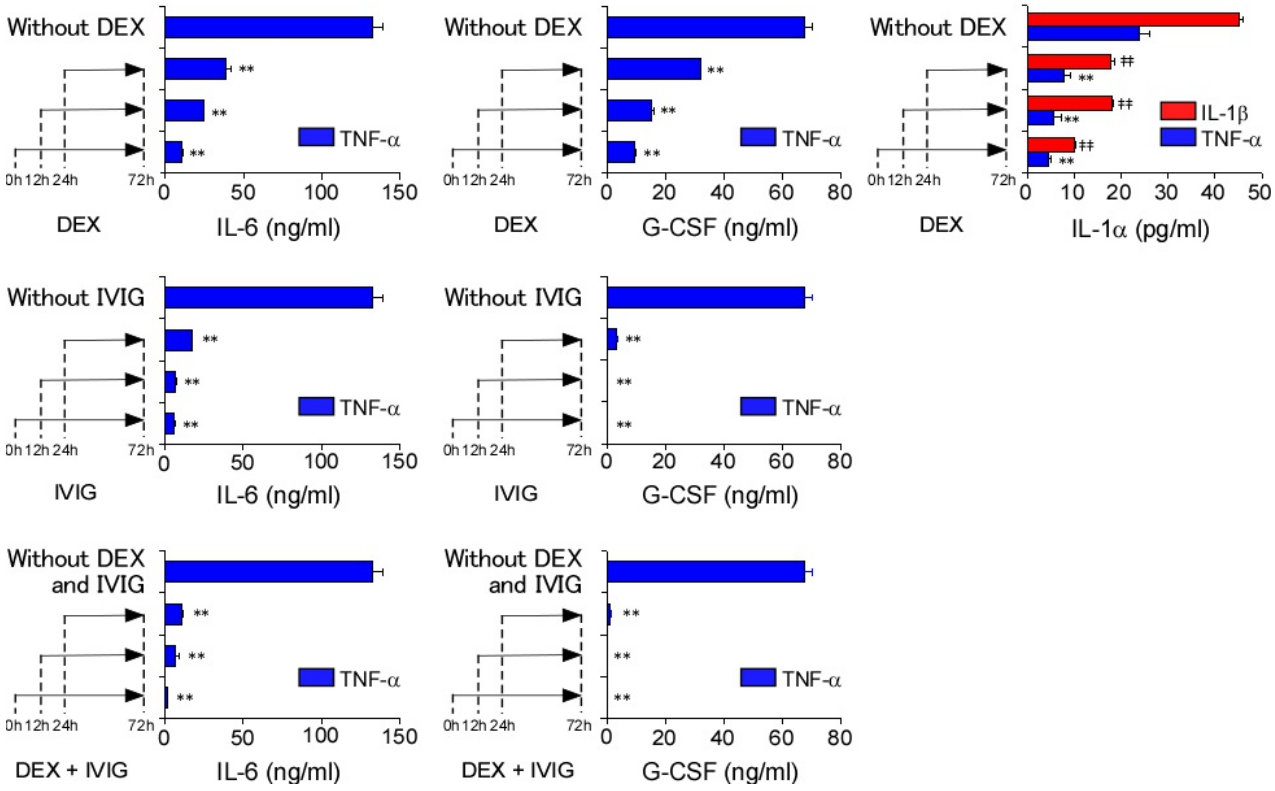

Figure 4

Inhibitory kinetics of IVIG and DEX on cytokine-induced production/release of IL-6, G-CSF and IL-1α by HCAECs HCAECs were stimulated with 100 ng/ml of TNF-α or 10 ng/ml of IL 1β alone (without drugs) for 72 h and then treated with 1000 nM of DEX and/or 10 mg/ml of IVIG at 0, 12 and 24 h after stimulation. The cell supernatants were collected at 72 h after cytokine stimulation. The protein concentrations of IL-6, G-CSF and IL-1α in the culture supernatants were measured by ELISA. Data are shown as the mean ± SD of triplicate samples and are representative of two individual experiments using HCAEC lots from two different donors. ** P <0.01 compared with 100 ng/ml TNF-α; and ‡‡P <0.01 compared with 10 ng/ml IL-1β.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

Additional file 2.pdf
Additional file 1.pdf