Expression of CD40 Ligand on CD4\(^+\) T-Cells and Platelets Correlated to the Coronary Artery Lesion and Disease Progress in Kawasaki Disease

Chih-Lu Wang, MD*; Yu-Tsun Wu, MD†; Chieh-An Liu, MD*; Mei-Wei Lin, BS*; Chia-Jung Lee, BS*; Li-Tung Huang, MD*; and Kuender D. Yang, MD, PhD*

ABSTRACT. **Objective.** Kawasaki disease (KD) is an acute febrile vasculitic syndrome in children. CD40 ligand (CD40L) has been implicated in certain types of vasculitis. We proposed that CD40L expression might be correlated with coronary artery lesions in KD.

**Methods.** Blood samples were collected from 43 patients with KD before intravenous immunoglobulin (IVIG) treatment and 3 days afterward. Forty-three age-matched febrile children with various diseases were studied in parallel as controls. CD40L expression on T-cells and platelets were detected by flow cytometry, and soluble CD40L (sCD40L) levels were measured by enzyme-linked immunosorbent assay.

**Results.** We found that CD40L expression on CD4\(^+\) T-cells was significantly higher in patients with KD than in the febrile control (FC) group (28.69 ± 1.17% vs 4.37 ± 0.36%). CD40L expression decreased significantly 3 days after IVIG administration (28.69 ± 1.17% vs 13.53 ± 0.55%). CD40L expression on platelets from patients with KD was also significantly higher than in the FC group (8.20 ± 0.41% vs 1.26 ± 0.12%) and decreased after IVIG therapy. sCD40L levels were also significantly higher in KD patients with those of FC (9.69 ± 0.45 ng/mL vs 2.25 ± 0.19 ng/mL) but were not affected by IVIG treatment 3 days afterward (9.69 ± 0.45 ng/mL vs 9.03 ± 0.32 ng/mL).

More interesting, we found that in KD patients, CD40L expression on CD4\(^+\) T-cells and platelets but not on CD8\(^+\) T-cells or sCD40L was correlated with the occurrence of coronary artery lesions.

**Conclusions.** CD40L might play a role in the immunopathogenesis of KD. IVIG therapy might downregulate CD40L expression, resulting in decrease of CD40L-mediated vascular damage in KD. This implicates that modulation of CD40L expression may benefit to treat KD vasculitis. *Pediatrics* 2003;111:e140–e147. URL: http://www.pediatrics.org/cgi/content/full/111/2/e140; Kawasaki disease, intravenous immunoglobulin, CD40 ligand, soluble CD40L.

**ABBREVIATIONS.** KD, Kawasaki disease; CD40L, CD40 ligand; IgG, immunoglobulin G; sCD40L, soluble CD40L; FC, febrile controls; IVIG, intravenous immunoglobulin; CAL, coronary artery lesions; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PBS, phosphate-buffered saline; PRP, platelet-rich plasma; IL, interleukin; TSST-1, toxic shock syndrome toxin-1.

From the Divisions of *Allergy and Immunology and Cardiology, Chang-Gung Children’s Hospital at Kaohsiung, Chang-Gung University, Kaohsiung, Taiwan.

Received for publication Jul 3, 2002; accepted Oct 2, 2002.

Reprint requests to (K.D.Y.) Office of Vice Superintendents, 123 Ta-Pei Road, Chang Gung Children’s Hospital, Nai-Sung, Kaohsiung 833, Taiwan. E-mail: yangkd@adm.cgmh.org.tw

Des Wang and Wu contributed equally to this study.

PEDIATRICS (ISSN 0031-4005). Copyright © 2003 by the American Academy of Pediatrics.
NS indicates not significant.

*The 43 FC consisted of lobar pneumonia (n = 8), urinary tract infection (n = 6), acute gastroenteritis (n = 8), hand-foot-mouth disease (n = 6), anaphylactoid purpura (n = 5), and simple upper respiratory tract infections (n = 10).
Fig 1. CD40L expression on CD4+ T-cells. A, A representative overlay histogram of CD40L expression on CD4+ T-cells in patients with KD and FC. Fluorescence intensity of the CD40L expression detected by PE-conjugated antibody is shown in the x-axis. Numbers of CD4+ T-cells detected by FITC-conjugated antibody are shown in the y-axis. B, CD40L expression on CD4+ T-cells was significantly higher in patients with KD than in FC (28.69 ± 1.17% vs 4.37 ± 0.36%; P < .001, n = 43) and decreased significantly 3 days after IVIG administration (28.69 ± 1.17% vs 13.53 ± 0.55%; P < .001, n = 43).
Fig 2. CD40L expression on CD8+ T-cells. A, A representative overlay histogram of CD40L expression on CD8+ T-cells in patients with KD and FC. Fluorescence intensity of the CD40L expression detected by PE-conjugated antibody is shown in the x-axis. Numbers of CD8+ T-cells detected by FITC-conjugated antibody are shown in the y-axis. B, CD40L expression on CD8+ T-cells was significantly higher in patients with KD than in FC (19.89 ± 0.97% vs 2.97 ± 0.23%; P < .001, n = 43) and decreased significantly 3 days after IVIG administration (19.89 ± 0.97% vs 8.89 ± 0.55%; P < .001, n = 43).
Patients With KD and FC

Measurement of sCD40L Levels of Serum From Patients With KD and FC

As shown in Fig 4, sCD40L levels were significantly higher in KD patients than in FC (9.69 ± 0.45 ng/mL vs 2.25 ± 0.19 ng/mL; P < .001, n = 43) but were not affected by IVIG treatment 3 days afterward (9.69 ± 0.45 ng/mL vs 9.03 ± 0.32 ng/mL; P = .18, n = 43). As shown in Table 3, there were no differences in sCD40L levels between KD patients with or without CAL before IVIG therapy (9.77 ± 1.05 ng/mL vs 9.65 ± 0.49 ng/mL; P = .924).

**DISCUSSION**

KD is characterized by overactivation of the immune system targeting on vascular endothelium, resulting in systemic vasculitis or even coronary artery aneurysm.

The CD40L–CD40 system is a crucial communication pathway for interaction among bone marrow–derived cells of the immune system. More recently, the interaction of CD40L with its receptor CD40 has been implicated in the modulation of immune and inflammatory responses, which are critical for the activation of tissue structure cells, including endothelial cells, smooth muscle cells, and epithelial cells as well as fibroblast, inducing production of a whole series of proinflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-8, IL-12, and tumor necrosis factor-α, along with induction of adhesion molecule expression on endothelial cells (eg, E-selectin, vascular cell adhesion molecule-1), and RANTES, in macrophages and vascular endothelial cells. Consequently, CD40 signaling has been associated with the pathogenic processes of chronic inflammatory diseases, including autoimmune diseases, neurodegenerative disorders, graft-versus-host disease, cancer, and atherosclerosis. In patients with systemic lupus erythematosus, baseline expression of CD40L and CD40L regulation was recently shown to be abnormal. Most impressive, evidence increasingly shows that CD40L expression on T-lymphocytes, on platelets, or in soluble form plays an important role in the acute coronary syndrome, the most disastrous complication of KD.

Therefore, we explored CD40L expression in KD. To our knowledge, the present study is the first time abnormal CD40L expression in patients with KD has been demonstrated. We found that CD40L expression on CD4+ T-cells was significantly higher in KD than in FC, decreasing significantly 3 days after IVIG administration. Similarly, there was also significantly higher CD40L expression on CD8+ T-cells from patients with KD than in FC. CD40L expression on platelets from patients with KD was significantly higher than on platelets from FC. The increased CD40L expression on platelets in patients with KD

As shown in Table 3, additional analysis disclosed that KD patients in the acute stage with CAL showed significantly higher CD40L expression on CD4+ T-cells than those without CAL (37.26 ± 1.60% vs 25.37 ± 1.00%; P < .001). However, there was no difference in CD40L expression on CD8+ T-cells between KD patients with or without CAL (20.02 ± 1.70% vs 19.84 ± 1.19%; P = .934).

**Detection of CD40L Expression on Platelets From Patients With KD and FC**

CD40L expression on platelets from patients and FC was also studied by flow cytometric analysis. As shown in Fig 3, ex vivo expression of CD40L on platelets was significantly higher in KD than in FC (8.20 ± 0.41% vs 1.26 ± 0.22%; P < .001, n = 43) and decreased significantly after IVIG treatment 3 days afterward (8.20 ± 0.41% vs 3.88 ± 0.22%; P < .001, n = 43). After in vitro stimulation by thrombin (0.5 U/mL) for 15 minutes, there was no difference between the KD group and FC group (Table 2).

As shown in Table 3, patients with CAL also expressed significantly higher CD40L on platelets in the acute stage than those without CAL (9.48 ± 0.67% vs 7.70 ± 0.49%; P = .043).

**PMA indicates phorbol ester.**

CD4+ T-cells, CD8+ T-cells and platelets obtained from KD patients before and after IVIG therapy were subject to analysis of CD40L expression.

* P values calculated from analysis of variance.

**Table 2.** Overexpression of CD40L on T-Cells and Platelets From Patients With KD

<table>
<thead>
<tr>
<th>CD4+ T-Cells (%)</th>
<th>CD8+ T-Cells (%)</th>
<th>Platelets (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resting</td>
<td>PMA + A23187</td>
</tr>
<tr>
<td>Pre-IVIG</td>
<td>28.69 ± 1.17</td>
<td>75.31 ± 7.20</td>
</tr>
<tr>
<td>Post-IVIG</td>
<td>13.53 ± 0.55</td>
<td>54.14 ± 3.28</td>
</tr>
<tr>
<td>FC</td>
<td>4.37 ± 0.36</td>
<td>42.83 ± 4.43</td>
</tr>
</tbody>
</table>

*P values* <.001 <.01 <.001 <.01 <.001 >.05

**Table 3.** CD40L Expression in Patients With KD With and Without CAL*

<table>
<thead>
<tr>
<th>CD4+ T-Cells (%)</th>
<th>CD8+ T-Cells (%)</th>
<th>Platelets (%)</th>
<th>sCD40L (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resting</td>
<td>PMA + A23187</td>
<td>Resting</td>
</tr>
<tr>
<td>With CAL (n = 12)</td>
<td>37.26 ± 1.60</td>
<td>20.02 ± 1.70</td>
<td>9.48 ± 0.67</td>
</tr>
<tr>
<td>Without CAL (n = 31)</td>
<td>25.37 ± 1.00</td>
<td>19.84 ± 1.19</td>
<td>7.70 ± 0.49</td>
</tr>
</tbody>
</table>

P values calculated from analysis of variance.

* CAL was defined by internal diameter of coronary artery >3 mm.
decreased dramatically 3 days after IVIG treatment. In contrast, sCD40L levels were not affected by IVIG treatment 3 days afterward. It is interesting that CD40L expression on CD4+ T-cells and platelets but not on CD8+ T-cells is significantly correlated with the occurrence of coronary artery dilation, whereas sCD40L is not. This suggests that CD40L may play an important role in the immunopathogenesis of CAL in KD.

In patients with KD, overexpression of CD40L may
activate CD40-positive target cells, either hematopoietic or structure cells, with the potential to activate further the immune system and elicit inflammatory reactions, resulting in vascular endothelial damage. Endothelial cells are able to express cell surface CD40 that, when ligated, signals augmented expression of vascular cell adhesion molecules and cytokines and secretion of tissue factors. Kotowicz et al showed that ligation of CD40L with CD40 on human endothelial cells can lead to endothelial cell activation, induced expression of vascular cell adhesion molecule-1, intracellular adhesion molecule-1, and E-selectin with recruitment and activation of T-cells and neutrophils at sites of inflammation. Mach et al demonstrated that activated human T-cells mediate contact-dependent expression of matrix metalloproteinases in vascular endothelial cells through CD40/CD40L signaling. They concluded that ligation of CD40 on endothelial cells can mediate aspects of vascular remodeling and neovessel formation during chronic immune reactions or even atherogenesis. We believe that the increase of CD40L expression on T-cells and platelets associated with elevated shedding of sCD40L in patients with KD may not only trigger immune activation but also attribute to pathogenic process of vascular inflammation.

In the past 30 years, identification of any infectious agent that may cause KD has been difficult. However, certain intracellular pathogens and superantigens from bacteria have been implicated in the immunopathogenesis of KD. By using a 3-phase screening procedure, Takahashi and colleagues recently identified a novel lymphotropic virus from peripheral blood mononuclear cells in patients with KD. This novel virus has an extensive but low-level homology (25%–33% identities and 28%–45% positives) with the African swine fever virus, which replicates in monocyte-macrophage lineage cells. Whether this lymphotropic virus contributes to the CD40L expression of CD4+ T-cells and platelets deserves additional study. In contrast to viral pathogenesis, expansion of T-cells expressing TCRBV2 and TCRBV6 chains has been reported to be stimulated by streptococcal and staphylococcal superantigens, such as toxic shock syndrome toxin-1 (TSST-1), which likely contributes to immunopathogenesis of KD in a number of ways. Jabara et al showed that TSST-1 promotes CD40L expression on T-cells. CD40L was preferentially expressed in the Vβ2 subset of T-cells expanded by TSST-1. Kum et al also revealed that ligation of Vβ2-TCR by TSST-1 induced a rapid surface expression of CD40L on CD4+ T-cells, leading to sequential T-cell proliferation and monocyte activation.

KD now has become the leading cause of acquired heart disease among children. There is evidence of persistent abnormal vascular wall morphology and vascular dysfunction in patients with previous KD at the site of regressed coronary aneurysms, including premature atherosclerosis. The arterial lesions continue to undergo active remodeling processes several years after the onset of the disease, even into adulthood. Takahashi et al revealed evidence of atherosclerotic lesions by pathologic examination from autopsy patients older than 15 years of age with previous KD. Multiple lines of evidence also support the view of atherosclerosis as a chronic inflammatory disease and implicate components of immune activation in atherogenesis. Recent work has documented overexpression of CD40L in experimental and human atherosclerotic lesions. Notably, interruption of CD40/CD40L interaction not only di-
diminished the formation and progression of atheroma but also fostered change in lesion biology and structure.\(^{57,38}\) On the basis of the findings, we propose that patients with KD, especially those patients associated with CAL, should be counseled to avoid potential risk factors for atherosclerosis, with long-term follow-up into adulthood. Furthermore, advanced understanding of the basic mechanisms of the relationship between CD40L interaction and long-term coronary arterial remodeling, as well as atherogenesis, may lead us to more effective and innovative treatments for patients with KD.

**CONCLUSION**

CD40L might play an important role in the immunopathogenesis of KD. IVIG treatment decreased cell-surface CD40L expression on CD4\(^+\) T-cells, CD8\(^+\) T-cells, and platelets but not on sCD40L, suggesting that downregulation of CD40L expression rather than shedding of sCD40L is involved in the decrease of CD40L-mediated vascular damage in KD. The precise mechanisms deserve additional investigation. On the basis of the results of this study, therapeutic modalities that are able to downregulate CD40L–CD40 interactions may represent a new therapeutic approach for KD vasculitis.

**ACKNOWLEDGMENTS**

This work was supported, in part, by grant NSC 89-2314-B-182A-142 from National Science Council, Taiwan. We thank the nursing staff in the Chang Gung Children's Hospital at Kaohsiung for helpful collection of blood samples. This manuscript was reviewed and edited by James F. Steed, MS.

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*Pediatrics* 2003;111:e140

DOI: 10.1542/peds.111.2.e140

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