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Decreased interferon-gamma (IFN-γ)-producing T cells in patients with acute Kawasaki disease

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SUMMARY

Kawasaki disease (KD) is an acute febrile illness of early childhood, in which the activation of monocytes/macrophages plays a central role in the development of vasculitis during the acute stage of disease. In this study we investigated peripheral blood T cells of 10 patients with KD, focusing on the Th1 and Th2 imbalance, using intracellular cytokine staining and analysis of the cytokine-producing T cells by flow cytometry. We observed a decrease in the numbers of IFN-γ-producing, but not IL-4-producing, $CD3^+$ T cells, during the acute stage. Our results suggest that there is an imbalance of Th1 and Th2 subsets during the acute stage of KD.

Keywords Kawasaki disease Th1/Th2 T cell

INTRODUCTION

Kawasaki disease (KD) is an acute febrile illness of unknown aetiology, which occurs primarily during infancy and early childhood. Histopathological findings in KD indicate panvasculitis with endothelial necrosis, and infiltration of mononuclear cells into small and medium-sized blood vessels. Less than 1% of patients with KD may die due to aneurysms and/or thrombosis caused by coronary arteritis.

Activation of monocytes/macrophages plays a central role in the development of vasculitis during acute KD. At this stage of disease, we have reported an increase in the $CD14^+$ monocyte/macrophage count [1,2], and increased serum levels of tumour necrosis factor-alpha (TNF-α) [1–3], soluble TNF receptor [4], and soluble intercellular adhesion molecule-1 (ICAM-1) [5]. We have also demonstrated activation of monocytes/macrophages by electron microscopy and immunohistochemistry [6].

Infiltration of activated T cells expressing HLA-DR antigen in biopsy skin lesions [7] and coronary vascular lesions at autopsy has been reported [8]. However, it is still uncertain whether peripheral blood T cells are activated in acute KD, as some reports have provided evidence of T cell activation [9,10], whereas others have not [2,11–14]. Leung *et al.* reported that peripheral blood T cell activation with expansion of T cell receptor (TCR) Vβ2 and Vβ8 is evident during the acute stage, which is caused by the production of toxic shock syndrome toxin-1 superantigens by *Staphylococcus aureus* [15–17]. However, others have been unable to provide similar evidence [18–20].

On the basis of the cytokine secretion pattern, two functional classes of murine T helper cells have been identified. T helper 1 (Th1) cells predominantly produce IL-2 and IFN-γ and play a major role in cell-mediated immunity, largely mediated by IFN-γ. Th2 cells produce IL-4, IL-5 and IL-10, and are responsible for antibody-dominated immunity. Th1 and Th2 subsets have been implicated in the regulation of many immune responses [21].

Recently a method for intracellular cytokine staining has been developed, which enables the detection of cytokine-producing cells by single laser flow cytometry [22,23]. We have applied this technique to study the intracellular levels of IFN-γ and IL-4 in circulating $CD3^+$ T cells obtained from KD patients to determine whether peripheral blood T cells are activated during acute KD.

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PATIENTS AND METHODS

Patients and control subjects

We studied peripheral blood obtained from 10 patients with KD who were seen at our hospital between July 1997 and April 1998, and eight healthy children. Informed consent was obtained from the subjects' parents before participation in the study.

Kawasaki disease

The 10 patients with KD comprised four boys and six girls (aged 0·3–4·1 years, mean 1·7 years), who met the diagnostic criteria for KD [24]. All patients received standard treatment with intravenous gammaglobulin (IVGG) 400 mg/kg per day (Venilon, Teijin Co.)

for 5 days, and oral aspirin (30 mg/kg per day). The onset of illness was defined as the day on which fever appeared. Blood samples were obtained on days 3–7 (4·9 ± 1·3 (mean ± s.d.)) prior to treatment (acute stage), and on days 34–52 (42·9 ± 8·3, convalescent stage). In the five out of 10 patients, samples were obtained on days 9–12 (10·2 ± 1·6, subacute stage) after IVGG treatment. Two-dimensional echocardiography was used to detect the presence of coronary artery lesions (CAL). Coronary arteries with diameters of ≥ 4 mm were classified as abnormal, in accordance with the KD Cardiovascular Lesion Diagnostic Criteria of the Research Committee on KD. None of the patients developed CAL.

Control subjects

We also studied eight healthy children (three boys and five girls, aged 0·6–4 years; mean 1·8 years) as a control group. Control samples were tested in parallel with the patients' samples.

Detection of intracellular cytokines by flow cytometry

We detected intracellular cytokines by flow cytometry as reported previously [22,23]. Briefly, whole blood (500 μl) was cultured in tubes for 4 h at 37°C under 7% CO_2 in RPMI 1640 medium. Cells were stimulated with 25 ng/ml phorbol 12-myristate 13-acetate (PMA; Sigma Chemical Co, St Louis, MO) and 1 mg/ml ionomycin (Sigma) in the presence of 10 mg/ml brefeldin A (Sigma).

Cultured cells were stained with PerCP-conjugated anti-CD3 MoAb (Becton Dickinson, Mountain View, CA). Erythrocytes were lysed by adding 2 ml of lysing solution (Becton Dickinson) for 10 min. After washing with washing buffer containing PBS with 0·5% bovine serum albumin (BSA) and 0·1% NaN_3, leucocytes were permeabilized using the FACS Permeabilizing Solution (Becton Dickinson) for 10 min. Cells were stained with FITC-conjugated anti-IFN-γ MoAb and PE-conjugated anti-IL-4 MoAb for 30 min. As a last step, the cells were washed with the washing buffer and resuspended in 1% paraformaldehyde.

Stained cells were analysed using a FACScan flow cytometer (Becton Dickinson) equipped with a 15-mW argon ion laser and filter settings for FITC (530 nm), PE (585 nm) and PerCP (677 nm).

Statistical analysis

Statistical analyses were performed using the Mann–Whitney *U*-test and paired Wilcoxon signed rank test for comparison of means.

RESULTS

Figure 1 shows a typical dot plot of IFN-γ and IL-4 staining in $CD3^+$ T cells derived from the peripheral blood of a female KD patient aged 4 years. In this case, there was a decreased percentage of IFN-γ-producing $CD3^+$ T cells at the acute stage in comparison with the convalescent stage. As shown in Fig. 2, the numbers of $CD3^+$ T cells producing IFN-γ were lower in the subjects with acute KD (5·0 ± 3·6% (mean ± s.d.)) than in those at the convalescent stage (14·3 ± 4·8%; $P < 0·01$) and normal subjects (15·9 ± 3·6%; $P < 0·01$). After IVGG treatment during the subacute stage, the percentage of IFN-γ-producing $CD3^+$ T cells was increased (9·8 ± 5·2%, $n = 5$), but was not as high as in the control subjects and the KD patients at the convalescent stage. The percentage of $CD3^+$ T cells producing IL-4 was very low. There were no significant differences in the percentages of $CD3^+$ T cells

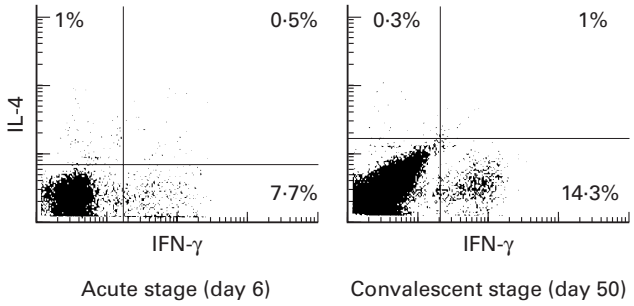

Fig. 1. A typical dot plot of IFN-γ and IL-4 staining in $CD3^+$ T cells derived from peripheral blood of a female Kawasaki disease (KD) patient aged 4 years. The numbers show the percentage of cytokine-producing $CD3^+$ T lymphocytes gated on $CD3^+$ T lymphocytes.

staining for IL-4 among KD patients during the acute, subacute and convalescent stages, and in normal subjects (1·3 ± 1·0%, 1·8 ± 1·4%, 3·1 ± 3·5%, and 1·3 ± 1·1%, respectively), as shown in Fig. 3. One KD patient whose condition was complicated by atopic dermatitis showed an increase of IL-4-producing $CD3^+$ T cells at the convalescent stage.

DISCUSSION

This is the first investigative report of T cell activation in KD focusing on Th1 and Th2 imbalance using flow cytometry for detection of intracellular cytokines. Using this method, several previous studies have demonstrated Th1 and Th2 imbalance in various immunological diseases. These studies revealed a greatly increased proportion of IL-2- and IFN-γ-producing T cells in bronchoalveolar lavage fluid from asthmatic patients [23], reduced production of IFN-γ- and IL-2-producing T cells in atopic patients [25], and a decreased percentage of T cells capable of secreting IFN-γ, but not IL-4, in patients with multiple sclerosis [26]. When T cells are stimulated with PMA and ionomycin, CD4 molecules on the cell surface become difficult to analyse using anti-CD4

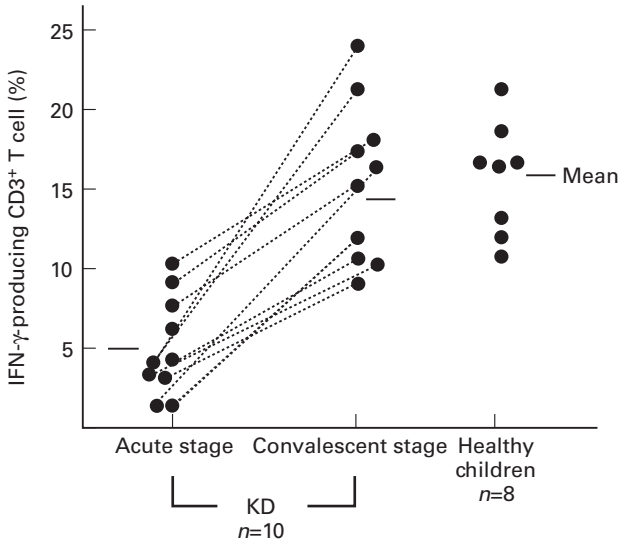

Fig. 2. The percentage of IFN-γ-producing $CD3^+$ T lymphocytes gated on $CD3^+$ T lymphocytes in Kawasaki disease (KD) patients and control subjects.

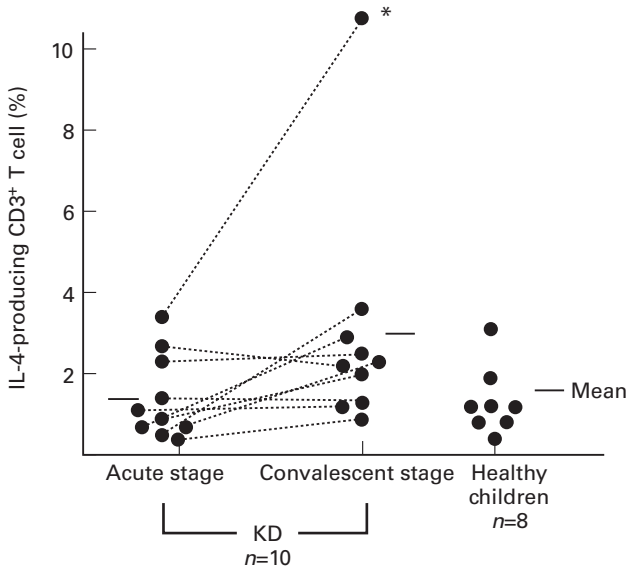

Fig. 3. The percentage of IL-4-producing $CD3^+$ T lymphocytes gated on $CD3^+$ T lymphocytes in Kawasaki disease (KD) patients and control subjects. *This case complicated by atopic dermatitis.

MoAb. Therefore we investigated only $CD3^+$ T cells in the present study.

We found that the number of IL-4-producing $CD3^+$ T cells was much lower than that of IFN-γ-producing $CD3^+$ T cells in both patients with KD and control subjects, as reported previously. There was a decrease of IFN-γ-producing $CD3^+$ T cells, but not IL-4-producing $CD3^+$ T cells, during the acute stage of KD. These findings suggest that there is an imbalance of Th1 and Th2 in the peripheral blood during acute KD. We have already reported that only 30% of KD patients show slightly increased serum levels of IFN-γ [3]. We have also demonstrated that peripheral blood $CD4^+$ and $CD8^+$ T cell counts are decreased [2], and that the cells are not activated, at least in terms of cell surface activation markers such as HLA-DR [12] and LFA-1 [13], and the serum level of soluble CD2 [14].

The decrease of IFN-γ-producing $CD3^+$ T cells may reflect the hypofunction of a proportion of peripheral blood T cells during acute KD. It is not unlikely that this hypofunction of peripheral blood T cells is responsible for the unresponsiveness observed during acute KD. It is not completely clear how T cells acquire their functional unresponsiveness, although it has been reported that a lack of CD28 costimulation through the CD80 and CD86 molecules on antigen-specific cells might play a causative role [27]. It has also been reported that Th1 helper T cells are completely inhibited by prostaglandin E_2 (PGE_2), whereas Th2 cells are largely unaffected [28]. We have already demonstrated that plasma PGE_2 levels are markedly increased during the acute stage of KD [29]. Since we found that IFN-γ-producing $CD3^+$ T cells were decreased, whereas the number of IL-4-producing $CD3^+$ T cells was unchanged, the increased production of PGE_2 by activated monocytes/macrophages might be responsible for the decreased activation of Th1-type T cells.

Another possible explanation for the decrease of IFN-γ-producing $CD3^+$ T cells during acute KD is the infiltration of activated T cells expressing the HLA-DR antigen, which has been observed in biopsied skin [7] and coronary vascular lesions at autopsy [8]. It is possible that IFN-γ-producing $CD3^+$ T cells are temporarily withdrawn from the peripheral circulation during acute KD, and that sequestration of IFN-γ-producing $CD3^+$ T cells may be a feature of this disease. A decrease of IFN-γ-producing $CD3^+$ T cells may therefore simply reflect a shift of Th1-type T cells into the vascular tissue compartment.

With regard to Th2-type T cells, we demonstrated that the numbers of IL-4-producing $CD3^+$ T cells were not decreased during acute KD. One patient who had large numbers of IL-4-producing $CD3^+$ T cells at the convalescent stage developed atopic dermatitis. Th2-type T cells are involved in the production of IgE and the development of allergic diseases, and are also responsible for antibody-dominated immunity [21]. It has been reported that the serum levels of IL-4 and IL-10 are increased [30] and that polyclonal B cell activation is evident during acute KD [9]. In addition, we have also reported that KD patients have a background of allergy [31]. Taken together, these findings suggest that Th2-type T cells might be activated during acute KD.

In conclusion, we have demonstrated a decrease in the number of Th1-type $CD3^+$ T cells in the peripheral blood of patients with acute KD. Our results suggest the importance of studying the function of peripheral blood T cells in terms of Th1-type and Th2-type in KD at the acute stage.

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