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Immunological profile of peripheral blood lymphocytes and monocytes/macrophages in Kawasaki disease

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Summary

Kawasaki disease (KD) is an acute illness of early childhood characterized by prolonged fever, diffuse mucosal inflammation, indurative oedema of the hands and feet, a polymorphous skin rash and nonsuppurative lymphadenopathy. The histopathological findings in KD comprise panvasculitis with endothelial necrosis, and the infiltration of mononuclear cells into small and medium-sized blood vessels. The levels of many proinflammatory cytokines, chemokines and adhesion molecules can be elevated in sera from children with KD at the acute stage. Although many immunological studies on KD involving peripheral blood have been reported, the data obtained remain controversial. This review focuses on the immune response of peripheral blood lymphocytes and monocytes/macrophages during acute KD.

Keywords: Kawasaki disease, peripheral blood, T lymphocytes, monocytes/macrophages, intravenous immnoglobulin

Introduction

Kawasaki disease (KD) is an acute illness of early childhood characterized by prolonged fever, diffuse mucosal inflammation, indurative oedema of the hands and feet, a polymorphous skin rash and nonsuppurative lymphadenopathy [1]. The histopathological findings in KD comprise panvasculitis with endothelial necrosis, and the infiltration of mononuclear cells into small and medium-sized blood vessels [2]. Coronary artery involvement is the most important complication of KD and may cause significant coronary stenosis resulting in ischemic heart disease [3]. Less than 1% of patients with KD may actually die from an aneurysm and/or thrombosis caused by coronary arteritis. Serological testing of patients with this disease reveals nonspecific severe inflammation. The levels of many proinflammatory cytokines, chemokines, and soluble adhesion molecules can be elevated in sera from children with KD at the acute stage [4–9]. In spite of the long history of aetiological investigation in Japan, the cause(s) of KD remains unclear. Recently, two review articles on KD have been reported in 2004 [10,11]. These reviews include the diagnosis, epidemiology, aetiology, pathology, immunopathogenesis and therapy of KD. Although many immunological studies on KD involving peripheral blood have been reported, the data obtained remain controversial. This review focuses on the immune response of peripheral blood lymphocytes and monocytes/macrophages during acute KD, including the effect of intravenous immunoglobulin (IVIG) on peripheral blood monocytes/macrophages.

Numerical changes of peripheral blood lymphocytes and monocytes/macrophages

Table 1 shows the absolute counts of white blood cells, mononuclear cells, monocytes/macrophages and lymphocytes during the acute stage before treatment and during the convalescent stage of KD together with age-matched control subjects [12]. The absolute counts of $CD14^+$ monocytes/macrophages and $CD19^+$ B cells in KD were increased during the acute stage. On the contrary, the absolute counts of $CD4^+$ and $CD8^+$ T cells in KD decreased during the acute stage. These numerical changes of peripheral blood immunocompetent cells are an important finding for investigating the immune response of peripheral blood lymphocytes and monocytes/macrophages during acute KD.

Activation of peripheral blood lymphocytes

The aetiology of KD, particularly the role of staphylococcal and streptococcal superantigens, which have the unique ability to activate a large number of lymphocytes, remains

Table 1. Absolute counts of white blood cells, mononuclear cells, monocytes/macrophages and lymphocytes during the acute stage before treatment and during the convalescent stage of KD and of control subjects.

	Acute stage ($n = 106$)	Convalescent stage ($n = 68$)	Control subjects ($n = 22$)
Mean (SD) day sample taken after onset of fever	5·8 (1·6)	33·9 (19·9)	
White blood cells	15·59 (0·47)†	8·56 (0·29)	8·41 (0·39)
Mononuclear cells	4·58 (0·21)	5·27 (0·20)	4·86 (0·25)
CD14 + monocytes/macrophages	0·52 (0·04)†	0·25 (0·02)	0.18 (0·02)
Lymphocytes	4·06 (0·20)	4·89 (0·19)	4·68 (0·25)
CD4+ T cell	1·81 (0.11)*	2·27 (0·11)	2·33 (0·19)
CD8+ T cell	0·78 (0·04)*	1·14 (0·06)	0·94 (0·06)
CDl9 + B cell	1·23 (0·09)*	1·00 (0·06)	0·94 (0·10)

All results for cell counts are expressed as $\times 10^9$/l and mean (SEM). *Significant at $P < 0{\cdot}05$ *versus* control subjects. †Significant at $P < 0{\cdot}01$ *versus* control subjects.

controversial. Following superantigen activation, T cells with particular T cell receptor β-chain (TCR Vβ) rapidly proliferate [13]. This is followed by T cell Vβ-restricted deletion mediated by Fas-Fas ligand from the peripheral blood, which is one of the processes of apoptosis [14,15]. Thus, the characteristic immunologocal features mediated by superantigens are the response of T cell Vβ expansion and deletion in the peripheral blood of patients exposed to these toxins. Recently, Brogan *et al.* [16] have reported that Class II MHC-positive endothelial cells operate as competent superantigen-presenting cells for CD4 and CD8 lymphocytes, suggesting that activated T cells are temporarily withdrawn from peripheral circulation during acute KD. In addition, the up-regulatinon of MHC Class II expression on lesion endothelial cells has been reported in a patient with fatal KD [17]. This might be related with slight increase of serum interferon γ (IFN-γ) levels in acute KD patients with coronary artery lesions (CAL) reported by us, since IFN-γ increases MHC Class II exression on endothelial cells [18]. At present, conflicting data have been reported regarding expanded T cell populations with particular TCR Vβ gene segments, suggesting either a superantigen- or a conventional antigen-mediated immune response in KD. Although some studies have demonstrated a significant increase or decrease in the percentage of peripheral blood T cells with any particular TCR Vβ family [19–23], the findings have not been confirmed by other investigators [24–27]. We have reported the lack of increases in the serum levels of soluble Fas and Fas ligand during acute KD [28].

The infiltration of activated T cells expressing HLA-DR antigen in biopsy skin lesions and coronary vascular lesions at autopsy has been reported [17,29]. However, it remains uncertain whether peripheral blood T cells are largely activated in acute KD, as some reports have provided evidence of peripheral blood T cell activation [30,31], whereas other reports suggested that there is a low level of activation of peripheral blood T cells during acute KD [32–34]. Recently, we demonstrated a decrease in the number of IFN-γ-producing, but not IL-4-producing, $CD3^+$ T cells in the peripheral blood obtained from KD patients without CAL, suggesting an imbalance of the peripheral blood T cell function at the acute stage [35]. In addition, it has been reported that plasma levels of IL-4 were significantly higher in the acute KD than control children [36]. Our results suggest that some population of peripheral blood T cells, such as IL-4 producing T cells may be activated, while IFN-γ producing T cells (Th1 and Tc1-type $CD3^+$ T cells) develop hypofunction during acute KD. These results further suggest that great caution should be taken in studies on peripheral blood T cell-mediated responses during acute KD.

Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4, CD152) is a receptor present on T cells that plays a critical role in the down-regulation of antigen-activated immune responses. The expression of CTLA-4 on the T cells depends on cell activation induced by CD28–B7 interaction, which is essential for T cell activation. CTLA-4 is a surface molecule on activated T cells exhibiting sequence homology to CD28 [37]. The essential inhibitory function of CTLA-4 is to maintain the homeostasis of the immune system [38]. We demonstrated that the intracellular T cell expression of CTLA-4 is up-regulated in peripheral blood $CD3^+$, $CD4^+$ and $CD8^+$ T cells in the early part of the acute stage in KD [39]. However, there was a mild increase in intracellular T cells expressing CTLA-4 in KD compared with in Epstein-Barr virus infectious mononucleosis and influenza virus-associated encephalopathy [39,40]. It is an important finding that there is down-regulation of antigen-activated peripheral blood T cell during acute KD, in spite of a mild increase in intracellular CTLA-4 in T cells.

A few studies have been reported on the activation of peripheral blood B cells in KD. The findings included polyclonal B cell activation, an increase in the absolute number of B cells and increased expression of CD23 on B cells [12,41–43]. Recently, it was reported that circulating IgA B cells are reduced in acute KD, while IgA plasma cells infiltrate vascular tissue, including coronary arterial walls, in fatal KD [44,45].

Few studies have been reported concerning natural killer (NK) cells in KD. A significant reduction in the absolute number of circulating $CD16^+$ NK cells was observed during the acute phase of KD [46]. It remains unclear whether the alteration in the number of peripheral blood NK cells is primary or secondary to the pathologic condition of acute KD.

Activation of peripheral blood monocytes/macrophages

The immunological features of monocytes/macrophages observed in patients with KD can be summarized as follows:

- infiltration by the cells is notable in affected tissues in autopsy cases and in skin biopsy specimens from KD patients [17,29];
- the numbers of peripheral blood $CD14^+$ monocytes/macrophages and activated $CD14^+CD23^+$ monocytes/macrophages increase during the acute stage of KD [12];
- there are elevated levels of a variety of serum cytokines, such as tumour necrosis factor α (TNF-α, IL-1 and IL-6, which are considered to be produced by monocytes/macrophages during acute KD [4–6,47];
- peripheral blood mononuclear cells from patients with acute KD spontaneously secrete high levels of TNF-α and IL-1 [48,49];
- increases in the number of peripheral blood $CD14^+$ monocytes/macrophages, serum TNF-α level, IL-6 activity in serum and secretion of IL-1 from mononuclear cells are more evident in KD patients with than in ones without CAL [4,6,12,47,49];
- KD patients with a high level of soluble TNF receptor in their serum seem to be susceptible to CAL [50];
- predominant vascular endothelial growth factor expression and enhanced nitric oxide synthase expression in monocytes have been demonstrated in patients with KD [51,52];
- immunocytochemical and immunoelectron microscopic studies have shown that monocytes partly differentiate into macrophages in the peripheral circulation during the acute stage of KD [53,54].

It has been reported that the $CD14^+CD16^+$(FcγRIII) monocyte/macrophage subpopulation plays a more important role in inflammation [55]. We observed an increase in the number of peripheral blood $CD14^+CD16^+$(FcγRIII) monocytes/macrophages in acute KD, which showed positive correlation with the disease severity [56]. Furthermore, we investigated the activation of nuclear factor kappa B (NF-κB) in peripheral blood $CD14^+$ monocytes/macrophages and $CD3^+$ T cells by means of Western blotting and flow cytometric analyses. NF-κB is a pivotal transcription factor for genes that encode the proinflammatory cytokines, chemokines and adhesion molecules that mediate inflammation [57–59]. As shown in Fig. 1, NF-κB activation was more increased in peripheral blood $CD14^+$ monocytes/macrophages than in $CD3^+$ T cells in KD patients during the acute stage [60]. These findings suggest that the activation of peripheral blood monocytes/macrophages plays an important role during acute KD.

Effect of intravenous immunoglobulin on peripheral blood monocytes/macrophages

IVIG therapy has been reported to be effective in reducing the incidence of CAL in patients with KD [61–63]. There have been a few reports on the effect of IVIG on peripheral blood lymphocytes, neutrophils and cytokines in acute KD, including lymphocyte activation and apoptosis, neutrophil apoptosis and cytokine modulation [42,64–66]. The mechanism of IVIG in immune thrombocytopenic purpura (ITP) has been elucidated. In a murine model of ITP, IVIG increases the expression of inhibitory Fc receptor FcγRIIB on splenic macrophages [67]. However, the mode of action of IVIG in monocytes/macrophages during acute KD is not clearly understood.

IVIG therapy for acute KD seems to decrease the absolute number of circulating $CD14^+$ monocytes/macrophages [68]. We revealed that NF-κB activation in peripheral blood $CD14^+$ monocytes/macrophages is significantly decreased after IVIG therapy during acute KD [60]. Recently, we demonstrated that IVIG inhibits NF-κB activation induced by TNF-α, while it remains unclear whether IVIG acts extracellularly and/or intracellularly in U-937 cells, human monocytic leukaemia cell line. Western blotting of cytoplasmic extracts of U-937 cells revealed that IVIG inhibited the degradation of the IκBα protein, which suppresses NF-κB activation [69]. Further examination is necessary to determine whether or not the data *in vitro* reflects those *in vivo*.

We previously observed an increase in the number of $CD14^+CD16^+$(FcγRIII) monocytes/macrophages in acute KD and a decrease after IVIG therapy, as shown in Table 2 [56]. *In vitro* study of activation of Fc receptor FcγRIII by flow cytometry demonstrated that IVIG decreased the expression of FcγRIII in U-937 cells and peripheral blood $CD14^+$ monocytes/macrophages, and that this phenomenon is transient [69]. On the other hand, IVIG did not affect FcγRIIB expression on the membranes of U-937 cells or peripheral blood $CD14^+$ monocytes/macrophages. More recently, we observed that $CD14^+CD32B^+$(FcγRIIB) monocytes/macrophages were not increased during subacute KD after IVIG therapy [70]. Regarding FcγR expression in peripheral blood monocytes/macrophages during acute KD, the main effect of IVIG therapy may be based on a decrease in $CD14^+CD16^+$ (FcγRIII) monocytes/macrophages, and not an increase in $CD14^+CD32B^+$ (FcγRIIB) monocytes/macrophages.

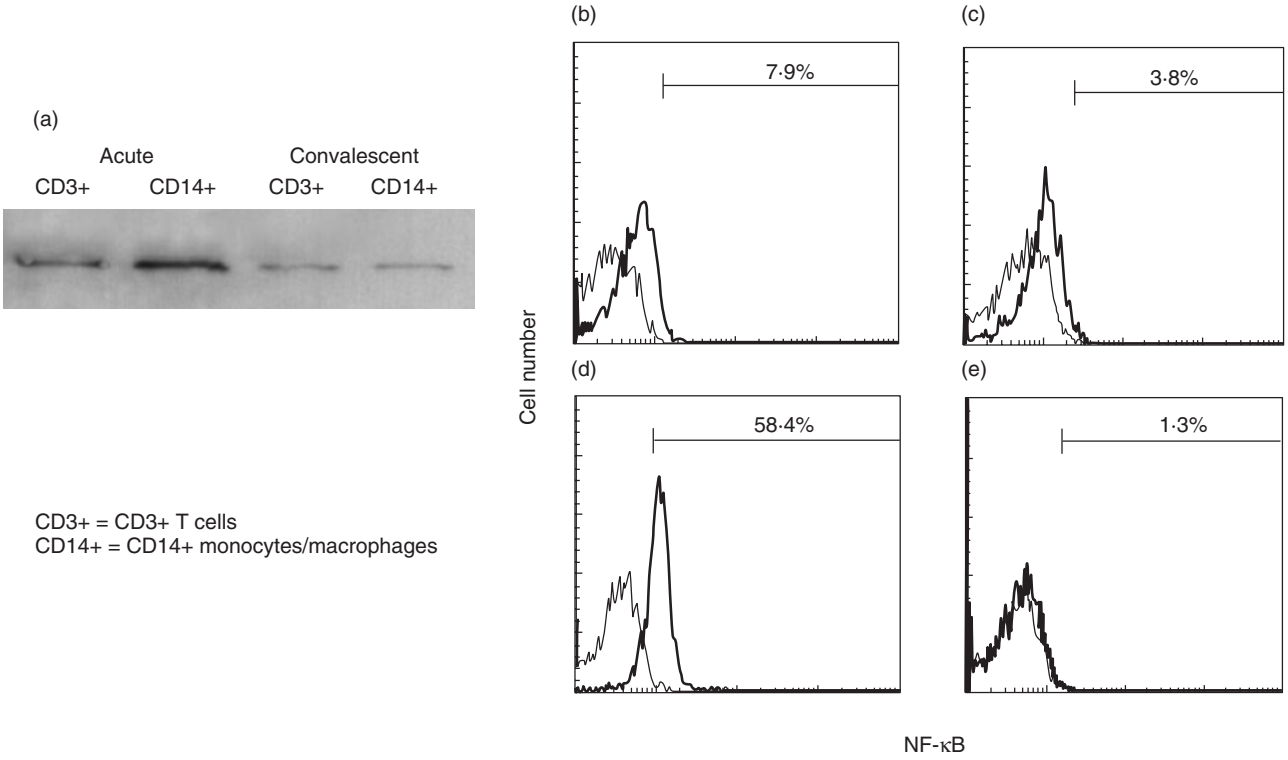

Fig. 1. NF-κB activation in peripheral blood CD3+ T cells and CD14 + monocytes/macrophages of a 2-month-old boy with KD. (a) Nuclear extracts were harvested from CD14+ monocytes/macrophages or CD3+ T cells. The nuclear extracts were used as the sample for Western blotting because activated NF-κB existed in the nucleus. Rabbit polyclonal antibodies against NF-κB-p65 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used as the primary antibodies.Western blot analysis demonstrated that intranuclear amount of NF-κB was increased in CD14 + monocytes/macrophages and CD3+ T cells at the acute stage compared with that at the convalescent stage. (b–e) Whole blood was labelled with phycoerythrin-conjugated with anti-CD14 moclonal antibodies and peridinin chlorophyll protein-conjugated anti-CD3 monoclonal antibodies and then permeabilized in 4% paraformaldehyde in phosphate-buffered saline, pH 7·2, containing 0·1% saponin and 10 mm HEPES. The cells were then labelled with a mouse anti-NF-κB (nuclear-localized signal) antibody (IgG3; Boehringer Mannheim, Mannheim, Germany). The mouse anti-NF-κB (nuclear-localized signal) antibody recognizes an epitope overlapping the nuclear location signal of NF-κB-p65 and therefore selectively recognizes the activated form of NF-κB. The cells were then labelled with a FITC-conjugated rat antimouse IgG3 monoclonal antibody (Pharmingen, San Diego, CA, USA). Immunofluorescence was analysed with a FACScan flow cytometer equipped with CellQuest software (Becton-Dickinson Biosciences, San Jose, CA, USA). The percentages of cells with intranuclear NF-κB in CD14+ monocytes/macrophages and CD3+ T cells by flow cytometric analysis are indicated. (b) CD3+ T cells at the acute stage; (c) CD3+ T cells at the convalescent stage; (d) CD14 + monocytes/macrophages at the acute stage; (e) CD14 + monocytes/macrophages at the convalescent stage.

Table 2. CD14+ CD16+ (FcγRIII) monocytes/macrophages in the patients with KD during the acute stage and the convalescent stage, and in control subjects.

	KD ($n = 28$)			
	Acute			
	Before IVGG	After IVGG	Convalescent	Control subjects ($n = 20$)
Mononuclear cells (cells/μl)	5271 ± 2705	5779 ± 2354	5374 ± 2274	5585 ± 1783
$CD14^+CD16^+$monocytes/macrophages (%)	3·6 ± 3·5*	0·6 ± 0·6	0·5 ± 0·3	0·7 ± 0·3
$CD14^+CD16^+$monocytes/macrophages (cells/μl)	155 ± 132*	35 ± 32	25 ± 18	35 ± 18
Percentage of $CD14^+CD16^+$ monocytes/macrophages among $CD14^+$ monocytes/macrophages (%)	21·6 ± 12·5*	6·6 ± 6·8	6·7 ± 3·7	10·1 ± 4·3

Values are expressed as mean ± s.d. * Significant at $P < 0{\cdot}01$ *versus* convalescent stage and control subjects.

Concluding remarks

Many conflicting data regarding peripheral blood T cell activation during acute KD have been reported. We speculate that these conflicting data might be due to the bipolarity of the peripheral blood T cell function observed in patients with acute KD. There is now ample evidence of a central role of peripheral blood monocytes/macrophages during acute KD, including the observation of an anti-inflammatory action of IVIG on monocytes/macrophages.

Informed consent for participation was obtained from the subjects' parents in our studies. Our studies were approved by the Institutional Review Board of Yamaguchi University Hospital.

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