T-cell activation outside the peripheral circulation during acute Kawasaki disease

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要約：
To clarify the activation of peripheral blood T-cells in KDpatients, we investigated whether expression of LFA-1 and/or ICAM-1 on peripheral blood T-cells increases during the acute stage, in comparison with those of convalescent stage. There was a decrease in the percentage of CD3+ T-cells in the bright LFA-1α and LFA-1β population and a concomitant increase in the dim population of LFA-1α and LFA-1β during the acute stage, in comparison to those of the convalescent stage. In our view the present data, in conjunction with previous reports on T-cell function during acute KD, suggest that there is the obvious anergy of peripheral blood T-cells during acute KD, and that sequestration of activated T-cells may be a feature of this disease.

見出し語：Adhesion molecules, T-cell activation

INTRODUCTION

We have reported that skin lesions in biopsy specimens of Kawasaki disease(KD) were infiltrated by HLA-DR+ T-cells. This suggested the activation of T-cells in skin lesions in KD. However, peripheral blood T-cell counts were observed to decrease and the expression of HLA-DR on peripheral blood CD4+ or CD8+ T-cells was not increased during acute KD. In addition, KD patients had low levels of soluble CD4 and CD8 in serum during the acute stage, compared to measles and infectious mononucleosis, although these levels were slightly increased during the acute stage of KD. It has been reported that these soluble antigens of T-cells are released from activated CD4+ or CD8+ T-cells. Furthermore, KD patients had decreased delayed hypersensitivity skin testing to purified protein derivative, phytohemagglutinin and *Candida albicans* during the acute stage. These previous results suggest that there is only a low level of activation of peripheral blood T-cells during acute KD, if any.

Several adhesion molecules have been identified that are thought to play a central role in the interaction of the antigen presenting cells with the T-cells. Intercellular adhesion molecule-1(ICAM-1) serves as the major ligand for lymphocyte

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function-associated antigen-1(LFA-1). Complete T-cell activation, demonstrated by interleukin-2(IL-2) production and DNA synthesis, has been shown to occur after simultaneous cross-linking of LFA-1 and CD3. To clarify the activation of peripheral blood T-cells in KD patients, we investigated whether expression of LFA-1 and/or ICAM-1 on peripheral blood T-cells increases during the acute stage, in comparison with those of convalescent stage.

MATERIALS and METHODS

Kawasaki disease(KD)

The subjects of the study were 10 patients with acute KD. The day of onset of fever was recognized as the first day of illness. Blood samples were taken for examination of expression of adhesion molecules on peripheral blood T-cells on the fourth to the eighth day(mean, 5.9±1.4) of illness preceding treatment. All KD patients were also examined for expression of adhesion molecules on peripheral blood T-cells on the 20th to the 57th day(mean,32.2±11.9) of illness during the convalescent stage. The control subjects were 7 healthy children,aged 8-37 months(mean, 16 months).

Analysis of expression of cell adhesion molecules

Expression of cellular adhesion molecules was measured using flow cytometry as previously reported by us. Fluorescein isothiocyanate- or phycoerythrin-conjugated monoclonal antibodies used in two colour flow cytometric analyses were as follows: CD3/Leu-4, CD11a/LFA-1α, CD18/LFA-1β and CD54/Leu-54/ICAM-1(Becton Dickinson Monoclonal Center, San Jose, CA). Analysis of stained mononuclear cells was performed on a Becton-Dickinson FACScan flow cytometer(Becton Dickinson ICS, Mountain View, CA) by gating on the lymphocyte populations using forward and side light scatter. From the ratio of peripheral blood lymphocytes to the leukocyte counts, the absolute counts of CD3+ T-cell were calculated. In all experiments, the autofluorescence (background fluorescence) for the negative population of cells was set using a mouse isotype(IgG_1, IgG_{2a} or IgG_{2b}) control monoclonal antibody(Becton Dickinson). Since each individual has a different width of autofluorescence, the horizontal or vertical axis was further divided into three areas, a fluorescence-negative population, a population with intermediate (dim) fluorescence intensity and a brightly stained population, according to the width of autofluorescence. The percentages of LFA-1α, LFA-1β or ICAM-1 positive T-cells in each area were calculated. To reveal any quantitative differences in KD patients between the acute and convalescent stages, statistical analysis was made by paired t tests.

RESULTS

Table 1 shows expressions of LFA-1α, LFA-1β and ICAM-1 by CD3+ T-cells of KD patients and control subjects. There was a decrease in the percentage of CD3+ T-cells in the bright LFA-1α and LFA-1β population and a concomitant increase in the dim population of LFA-1α and LFA-1β during the acute stage, in comparison to those of the convalescent stage($p<0.01$, respectively). However, we observed no significant differences in ICAM-1 expression during the acute stage compared to that of the convalescent stage.

DISCUSSION

In our view the present data, in conjunction with previous reports on T-cell function during acute KD, suggest that there is the obvious anergy of peripheral blood T-cells during acute KD, and that sequestration of activated T-cells may be a feature

of this disease. This has several implications regarding the problem of immunosuppession during acute KD and may reflect a situation beneficial to the patient rather than the opposite. The anergy of peripheral T-cells during acute KD is compatible with a state of T-cell activation outside the peripheral circulation, as suggested by the mild elevation of soluble CD4 and CD8 levels in serum and the infiltration of activated T-cells in skin biopsy specimens of acute KD. It may be plausible that these activated T-cells are derived from circulating T-cells that have been sequestered following the recognition of antigen.

Since 1983 we have analyzed peripheral blood mononuclear cell subsets including macrophages/monocytes in patients with KD by fluorescence-activated cell sorter. Our results showed that peripheral blood CD14+ macrophages/monocytes have been activation during acute KD in terms of numerical changes of immunocompetent cells, expression of activated antigens on their cell surfaces, and monokine production. Furthermore, it has been reported that the increased numbers of CD14+ macrophages/monocytes, serum tumor necrosis factor-α levels, IL-6 activities in serum, and secretion of IL-1 from peripheral blood mononuclear cells are more evident in KD patients with CAL than in patients without CAL. These reports suggest that CD14+ macrophages/monocytes in peripheral circulation play an important role in the exacerbation of vascular damage in KD.

Our results suggest that great caution should be exerted in the planning of studies on peripheral blood aimed at evaluating T-cell-mediated responses during acute KD, in particular when the studies on causal agent(s) of KD are conducted on peripheral blood T-cells.

Table1 Expressions of LFA-1α, LFA-1β and ICAM-1 on CD3+ T-cell of KD patients during the acute and convalescent stages, and of control subjects

		KD, n=10		Control
		Acute	Convalescent	subjects, n=7
Leukocyte	/mm^3	14,067±3,223**	8,389±1,554	8,200±2,408
Lymphocyte	/mm^3	3,648±1,523*	5,419±1,493	4,932±1,411
CD3+ T-cell	/mm^3	2,332±989**	4,367±1,252	4,016±1,142
Adhesion molecule	Fluorescence intensity %			
LFA-1α	Negative	0.1±0.2	0.7±2.1	0.0±0.0
	Dim	82.9±16.8**	64.0±28.1	64.2±15.7
	Bright	17.0±16.9**	35.2±28.7	35.8±15.6
LFA-1β	Negative	0.1±0.2	0.1±0.3	0.0±0.1
	Dim	76.0±21.6**	59.5±28.9	58.7±17.8
	Bright	23.8±21.7**	40.3±29.0	41.2±17.8
ICAM-1	Negative	73.3±8.9	78.0±8.3	74.2±11.8
	Dim	26.3±8.7	21.1±7.7	24.4±10.8
	Bright	0.4±0.3	0.9±0.9	1.5±1.2

** Significant at $p<0.01$ vs. convalescent stage.
* Significant at $p<0.05$ vs. convalescent stage.

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