Th1/Th2 Balance Alteration in the Clinical Course of a Patient With Acute Viral Myocarditis

Koichi Fuse, MD; Makoto Kodama, MD; Yoshifusa Aizawa, MD; Masayuki Yamaura, MD*; Yasutaka Tanabe, MD*; Kazuyoshi Takahashi, MD*; Katsuyuki Sakai, MD*; Tsutomu Miida, MD*; Hirotaka Oda, MD*; Norio Higuma, MD*

Cytokines have an important role in the pathogenesis and pathophysiology of myocarditis. In this study, subsets of peripheral helper T lymphocytes (Th) in a patient with acute viral myocarditis were analyzed by 3-color flow cytometry. During the clinical course of myocarditis, the Th1/Th2 ratio of peripheral lymphocytes changed. Th1 was dominant in the acute inflammatory phase during which levels of creatine kinase (CK) increased (day 6), then Th2 levels overtook those of Th1 in the recovery phase during which levels of CK decreased (day 13 and 20). At the time of discharge (day 35), Th1 and Th2 had normalized. Thus, it was speculated that the induction of lymphocytic myocarditis was associated with Th1 dominant status, and recovery was related to Th2 polarity. Th subset imbalances may play an important role in the pathogenesis of acute viral myocarditis and these analyses may be useful for understanding the disease activity of myocarditis. (Jpn Circ J 2001; 65: 1082–1084)

Key Words: Cytokine; Flow cytometry; Helper T lymphocyte (Th); Myocarditis

Viral myocarditis is the most common type of myocarditis, but it has a variable clinical course and the patient's prognosis is unpredictable. Cytokines are being recognized as important factors in the pathogenesis and pathophysiology of myocarditis; elevated levels of circulating cytokines have been reported in patients with acute viral myocarditis, and enhanced expression of cytokine genes has been reported in animal models. Distinct cytokine profiles are clearly associated with the clinical processes of some infectious and immunological diseases and are determined to some extent by 2 functional subsets of T helper (Th) cells, designated as Th1 and Th2. Th1 cells secrete interferon (IFN)-Î, interleukin (IL)-2 and tumor necrosis factor (TNF)-Î and strengthen cell-mediated inflammatory reactions, whereas Th2 cells, which produce IL-4, IL-5, IL-6, IL-10 and IL-13 etc., are mainly responsible for antibody and allergic responses. An imbalance of the Th1 and Th2 subsets has been demonstrated in various diseases, and we analyzed the subsets in a patient with acute viral myocarditis, focusing on the Th1/Th2 balance of peripheral lymphocytes.

Case Report

A 43-year-old man with no previous history of cardiac diseases was admitted to Niigata City General Hospital after suddenly developing chest pain, high fever and dyspnea in June 2000. On examination, blood pressure was 98/68 mmHg and the electrocardiogram revealed a high grade atrioventricular block with a left bundle branch block. Emergency cardiac catheterization was carried out. Mean pulmonary artery wedge pressure was 24 mmHg and the cardiac index was 1.8 L min−1 m−2. The coronary angiography was normal, but left ventricular wall motion was reduced and the ejection fraction was 0.53. Endomyocardial biopsy showed diffuse infiltration of inflammatory cells and myocyte necrosis (Fig 1). Laboratory tests on admission revealed a white blood cell count of 5,200/mm3, C-reactive protein level of 3.9 mg/dl, aspartate aminotransferase of 135 IU/L, alanine aminotransferase of 86 IU/L, lactate dehydrogenase of 796 IU/L, creatine kinase (CK) of 534 IU/L, angiotensin-converting enzyme of 13.9 IU/L, human atrial natriuretic peptide of 93.7 pg/ml and brain natriuretic peptide of 967 pg/ml. On scintigraphy, neither 67Ga citrate (day 14) nor 99mTc-pyrophosphate (day 22) revealed an abnormal uptake and 201Tl (day 22) did not reveal any defects in the heart. In the viral study, coxsackievirus A9 was positive by the measurement of neutralizing antibody titers in a paired-sample (initial antibody titer was <x4, second antibody titer was ×16). He was supported using temporary VVI pacing, mechanical ventilation and intra-aortic balloon pumping. The maximum serum CK level was 3,192 IU/L on the 5th day after the admission and it then decreased. Intra-aortic balloon pumping was ceased on the 7th day and mechanical ventilation on the 13th day (Fig 2). His cardiac function improved, and the atrioventricular block and left bundle branch block also improved. He was treated with noradrenaline, phosphodiesterase inhibitor and diuretic during the acute phase, but was not given steroid hormone, gamma globulin or blood transfusion. On day 6, 13, 20 and 35 after admission, we examined the frequencies of IFN-Î and/or IL-4 producing cells in stimulated peripheral blood lymphocytes by 3-color fluorescence-activated cell sorting (FACS) using the method described by Jung et al. The patient was given a full explanation of the study and agreed to participate. Peripheral blood (4 ml),
which was collected with heparin, was cultured for 4 h in the presence of 40 ng/ml phorbol myristate acetate (Wako, Osaka, Japan), 4 μg/ml ionomycin (Sigma, St Louis, MO, USA), 40 μg/ml brefeldin A (Wako) at 37°C and 5% CO₂. The lymphocyte fraction was obtained using the Ficoll gradient method, and the cells were finally suspended at a concentration of 1×10⁶ cells/100 μl in RPMI-1640 (Flow Laboratories, Irvine, CA, USA) with 10% fetal calf serum. They were triple stained with PC5-labeled anti-CD4, fluorescein isothiocyanate-labeled anti-IFN-γ and phycoerythin-conjugated anti-IL-4 antibodies. The cell preparations were analyzed by EPICS(r) XLII System (Beckman Coulter,
Fullerton, CA, USA) and the percentages of Th1 (IFN-γ single positive cells) and Th2 (IL-4 single positive cells) were counted by FACS. In the acute inflammatory phase (day 6) when the levels of CK were peaking, Th1 was 33.6% (normal control (mean ± SD): 14.5±5.7%, n=12) and Th2 was 5.9% (normal control: 2.4±1.3%). However, in the recovery phase (day 13 and 20) when the levels of CK had decreased, Th1 decreased and Th2 increased. At the time of discharge (day 35), Th1 and Th2 had normalized (Fig 3).

Discussion

We examined the systemic imbalance of the Th subsets in a case of acute viral myocarditis in whom the Th1/Th2 ratio in peripheral lymphocytes changed in accordance with the clinical course; that is, Th1 was dominant in the acute inflammatory phase, during which serum levels of CK increased (day 6). Subsequently, the proportion of Th2 increased and the levels of CK decreased during the recovery phase (day 13 and 20). Thus, systemic Th1/Th2 polarity seems to reflect the clinical phase of myocarditis and so analyses of systemic Th subsets using flow cytometry may be useful for understanding the disease activity.

Cytokines play important roles in the pathogenesis of myocarditis and in order to understand the pathogenesis of a disease, the role of individual cytokines has to be understood. However, every cytokine has pleiotropic actions, and many cytokines share similar biologic effects. Even if one proinflammatory cytokine is activated, counter-acting cytokines may overcome its effects. Therefore, recognition of such a cytokine environment as the Th1/Th2 balance is valuable in understanding the nature of the disease.

Can the Th1/Th2 balance of peripheral lymphocytes represent the immune-inflammatory condition of the heart? We could not analyze locally infiltrating lymphocytes or myocardial expression of cytokines in this case. The expression of cytokine mRNA in the heart has been reported from other laboratories using a murine model of viral myocarditis in which the mRNA of Th1-related cytokines, such as IFN-γ and TNF-α, was expressed throughout the early phase of infection and the mRNA of Th2-related cytokines, such as IL-4 and IL-10, was expressed only on day 5 or day 7, when massive cell infiltration appeared in the heart? Huber et al reported that a Th2 dominant immune response was associated with enhanced recovery from murine viral myocarditis induced by coxsackievirus. The Th1/Th2 balance alteration in viral myocarditis may be associated with the elimination of the virus, as a similar pattern has been demonstrated in experimental autoimmune myocarditis (EAM) of rats. In rat EAM, the peripheral Th1/Th2 balance by flow cytometry reflects the expression pattern of cytokine mRNA in the heart (unpublished data).

The present case suggests that the induction of viral myocarditis is associated with systemic Th1 dominant status and that recovery is related to systemic Th2 polarity. The Th subset imbalance seems to play an important role in the pathogenesis of human myocarditis and further studies of more patients and analysis of other subtypes of myocarditis should be investigated to elucidate the precise mechanism of the Th1/Th2 balance. Analysis of the systemic Th1/Th2 balance using flow cytometry may be useful for understanding the disease activity of myocarditis.

Acknowledgments

We thank Dr Sadao Aoki for his technical assistance with the flow cytometric analyses.

References