Journal of Clinical Immunology, Vol. 25, No. 3, May 2005 (© 2005)
DOI: 10.1007/s10875-005-4088-0

Th1/Th2 Functional Imbalance After Acute Myocardial Infarction: Coronary Arterial Inflammation or Myocardial Inflammation

XIANG CHENG,[1] YU-HUA LIAO,[1,3] HONGXIA GE,[1] BIN LI,[1] JINYING ZHANG,[1] JING YUAN,[1] MIN WANG,[1] YING LIU,[1] ZHANGQIANG GUO,[1] JING CHEN,[1] JIN ZHANG,[1] and LANJING ZHANG[2]

Received: December 8, 2004; accepted: January 27, 2005

Objectives: The study clarified whether the T-helper (Th)1/Th2 imbalance existed only in coronary arterial inflammation or in both coronary arterial inflammation and myocardial inflammation and explored the significance of the imbalance of Th1/Th2 function after acute myocardial infarction (AMI). *Background:* There are two different inflammatory processes in patients with AMI: the coronary arterial inflammation that leads to the pathogenesis of AMI and the myocardial inflammation after AMI that leads to ventricular remodeling, which are positively and negatively regulated by Th1 and Th2 lymphocytes, respectively. *Methods:* Peripheral blood mononuclear cells from 33 AMI patients, 22 unstable angina (UA) patients and splenocytes from 35 AMI Wistar rats were collected. Cytokine-producing Th cells were ambulatorily monitored by 3-color flow cytometry. Interferon (IFN)-γ and interleukin (IL)-4 mRNA in the rat myocardium and chemokine receptors CCR3,CCR5 and CXCR3 mRNA on the surface of rat T-lymphocytes after AMI were measured by RT-PCR. *Results:* IFN-γ-producing T-cells significantly increased in patients with AMI and UA within 24 hours after the onset of symptom. The high ratio of IFN-γ-producing T-cells recovered 1 week after the onset in UA patients, while it could be examined 1 week and even 1 month after the onset in AMI patients. The up-regulation of Th1 cell function is consistent with bad heart function. There was no significant difference on the frequencies of IL-4-producing T-cells between each group. 1 week, 2 weeks and 1 month after AMI, IFN-γ mRNA increased in the myocardium of rats, but there was no significant change on global Th cell functions. *Conclusions:* Th1/Th2 functional imbalance exists in both coronary arterial inflammation and myocardial inflammation processes. The up-regulation of Th1 cell-functions may participate in the immune-mediated ventricular remodeling after AMI.

KEY WORDS: Acute myocardial infarction; T-lymphocyte; helper; inflammation; ventricular remodeling.

INTRODUCTION

Acute myocardial infarction (AMI) is a worldwide problem to human health. In 1986, Jonasson *et al*. (1) reported that T-lymphocytes accumulated in the human atherosclerotic plaque, demonstrated that lymphocytes participated in the atherosclerosis. Recent studies have showed that the pathogenesis of acute coronary syndrome (ACS, including AMI and unstable angina (UA)) is associated with inflammation and at least partly related to immunity or autoimmunity (2, 3). Increased frequencies of lymphocyte and monocyte activation were found in the peripheral blood of patients with UA (4, 5). Liuzzo *et al*. (6) recently reported that interferon-γ [IFN-γ, one characteristic cytokine of T-helper type 1 (Th1)]-producing T-cells were more frequent in the peripheral blood of patients with UA than those of stable angina (SA). It suggests that monocyte activation represents a downstream effect of over-stimulated Th1 lymphocytes.

After AMI, myocardial necrosis releases or exposes normally sequestered antigenic constituents (such as myosin, actin, etc.), that may cause proliferation of antigen-recognizing T-cells. If given the opportunity, these activated T-lymphocytes might target the heart in an autoimmune response matter, assisting the development of ventricular remodeling and congestive heart failure. Maisel *et al*. (7) adoptively transferred splenocytes

[1]Laboratory of Cardiovascular Immunology, Institute of Cardiology, Union Hospital, Tongji Medical College of Huazhong University of Science and Technology, Wuhan 430022, China.
[2]Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, 48109 USA.
[3]To whom correspondence should be addressed at Laboratory of Cardiovascular Immunology, Institute of Cardiology, Union Hospital, Tongji Medical College of Huazhong University of Science and Technology, Wuhan 430022, China; e-mail: liaoyh27@hotmail.com.

of post-MI rats, which were activated by concanavalin A *in vitro*, into syngeneic rats and observed organ specific autoimmune myocarditis in recipient rats. Our previous study indicated that even without *in vitro* activation, transferred splenocytes could also lead to organ specific autoimmune myocarditis in recipient rats and slightly impair the left ventricular systolic function (8). It proves that the autoimmune inflammation plays an important role in post-MI ventricular remodeling and heart failure.

Recently, it is noticed that the imbalance of Th1/Th2 function is associated with the pathogenesis of autoimmune disease. In order to explore the significance of Th1/Th2 function imbalance on the pathogenesis of AMI and post-MI ventricular remodeling, we investigated the balance between Th1- and Th2-type responses in patients and rats with AMI, because human "natural" AMI includes both coronary arterial inflammation and myocardial inflammation while artificial AMI in non-atherosclerotic rats has only myocardial inflammation.

METHODS

Clinical Population

This study conformed to the approved institutional guidelines. Informed consent was obtained from each patient. We studied 96 patients who underwent diagnostic catheterization (73 men and 23 women, mean age $\pm$ SD 61 ± 10 years). Patients were classified into 4 groups: (1) AMI group (26 men and 7 women, mean age 62 ± 8, inclusion criteria: myocardial infarction confirmed by significant rise of creatine kinase MB and troponin I levels and electrocardiograph abnormalities); (2) UA group (17 men and 5 women, mean age 63 ± 9, inclusion criteria: chest pain at rest with documented transient ST-segment elevation or ST-segment depression of 0.1 mV in at least 2 contiguous electrocardiograph leads); (3) SA group (15 men and 5 women, mean age 60 ± 11, inclusion criteria: typical exertional chest discomfort associated with downsloping or horizontal ST-segment depression >1 mm in an exercise test); (4) and the chest pain syndrome (CPS) group (15 men and 6 women, mean age 58 ± 12, inclusion criteria: chest pain without being accompanied by electrocardiograph changes, coronary artery stenosis, or coronary spasm when acetylcholine was given through intracoronary injection during coronary arteriography (9)).

No patient was treated with anti-inflammatory drugs such as non-steroidal anti-inflammatory drugs, steroids, etc. None had collagen disease, thromboembolism, disseminated intravascular coagulation, advanced liver disease, renal failure, malignant disease, other inflammatory disease (such as septicemia, pneumonia, etc.), valvular heart disease, atrial fibrillation or was using a pacemaker.

Blood Samples

Blood samples were obtained from: (1) group AMI and (2) group UA within 24 h and on days 7 and 30 after the onset of symptom; (3) group SA and (4) group CPS while coronary angiography. 2 mL of blood was collected into evacuated tubes containing 0.2 mL of sodium heparin for cytometric analysis. On days 30 after AMI, group AMI was divided into 2 subgroups according to the New York Heart Association (NYHA) classification system: group AMI1 (NYHA I-II, 15 men and 3 women) and group AMI2 (NYHA III-IV, 11 men and 4 women). At the same time, the heart function of AMI patients was evaluated by echocardiography.

Flow Cytometric Analysis for Human Cytokine Production

One mL of blood was mixed with 1 mL of either nonactivating medium [10% fetal calf serum (FCS)-supplemented RPMI 1640 with 4 μL/mL GolgiStop (BD Pharmingen)] or activating medium [nonactivating medium with 50 ng/mL phorbol myristate acetate (PMA) and 5 μg/mL ionomycin (BD Pharmingen)] and then incubated at 37°C and 5% CO_2 for 5 h. Cells were recovered by centrifugation after being washed with PBS and adjusted to 5×10^5 white blood cells per test, then stained with PE-Cy5-labeled anti-human CD4 monoclonal antibody (BD Pharmingen). The fixation and permeabilization of cells were performed with FACS™ Perm 2 (BD) according to the manufacturer's instructions. Intracellular cytokines were stained with FITC-labeled anti-human IFN-γ and PE-labeled anti-human IL-4 monoclonal antibodies (BD Pharmingen). The IFN-γ- and IL-4-producing CD4+ T-cells were analyzed with FACSCalibur (BD). Nonspecific staining with the isotype-matched control monoclonal antibody was $<1\%$.

Rats

Wistar rats, male, 240 ± 20 g, purchased from animal laboratory of Tongji Medical College, Wuhan, China, randomly divided into AMI group ($n = 35$) and Sham group ($n = 30$). The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication NO. 85-23, revised 1996).

Infarction

The method to produce myocardial infarction used in this study has been described previously (7). Briefly, after anesthesia with intraperitoneal injection of 30 mg/kg sodium pentobarbital, the rats were intubated and ventilated via a rodent respirator. Parasternal thoractomy between the 3rd and 4th ribs was performed. The proximal left anterior descending branch (LAD) of coronary artery was ligated using a terylene suture in MI rats. The same procedure except coronary ligation was undergone in sham-operated rats.

IFN-γ and IL-4 mRNA Expression

Infarcted hearts at 1 week ($n = 12$), 2 weeks ($n = 11$) and 1 month ($n = 12$) after surgery were sectioned into noninfarcted and infarcted areas by visual inspections. Total RNA was extracted using the Tri-Zol procedure. mRNA was then isolated using an mRNA isolation kit (TaKaRa biotechnology) and was reverse transcribed into first-strand cDNA. The primer sequences to rat IFN-γ and IL-4 were as follows:

rat IFN-γ sense, 5′-GAAAGCCTAGAAAGTCTGAAGAAC-3′
antisense, 5′-GCACCGACTCCTTTTCCGCTTCCT-3′ (387-bp product)
rat IL-4 sense, 5′-ACCTTGCTGTCACCCTGTTCTGC-3′
antisense, 5′-GTTGTGAGCGTGGACTCATTCACG-3′ (352-bp product)

PCR amplification was performed by adding 2 μl aliquots of each cDNA sample to 20.5 μl of reaction mixture. Amplification was done in DNA Thermal Cycler under the following conditions: denaturation at 94°C for 40 s, annealing at 58°C for 40 s, and extension at 72°C for 1 min, with final extension at 72°C for 5 min. Based on the results of preliminary studies, 32 cycles were used for IFN-γ and 40 cycles were used for IL-4. Five microliters of each PCR product were subjected to electrophoresis on 1.5% agarose gel in Tris–acetic acid–EDTA buffer and stained with ethidium bromide. Gels were viewed, and the images were stored digitally by image analysis system (GSD8000, UVP, England). For each cDNA sample, the densitometric units of the amplified cDNA fragments were counted for semiquantitative evaluation by normalization with the GAPDH band.

Splenocytes

Splenocytes were separated according to the protocol described elsewhere (7).

Flow Cytometric Analysis for Rat Cytokine Production

Rat intracellular cytokine production was measured as previously described (10). Briefly, for IL-4-producing T-cells analysis, splenocytes were pre-stimulated with plate bound anti-CD3, soluble anti-CD28, rrIL-2 and rrIL-4 (BD Pharmingen). Then the process was the same as IFN-γ- producing T-cells analysis. Splenocytes were stimulated for 5 h with 50 ng/mL PMA, 5 μg/mL ionomycin (BD Pharmingen) and with or without 4 μL/mL GolgiStop (BD Pharmingen). Then cells were resuspended to a density of 10^6 cells/mL. FITC-labeled anti-rat CD3 monoclonal antibody and RPE-Cy5-labeled anti-rat CD8 monoclonal antibody (Serotec) were used for cell surface staining. After fixed and permeabilised, intracellular cytokines were stained with RPE-labeled anti-rat IFN-γ or IL-4 monoclonal antibodies (BD Pharmingen). The IFN-γ- and IL-4-producing CD3+CD8- T-cells were analyzed with FACSCalibur (BD). Nonspecific staining with the isotype-matched control monoclonal antibody was $<1\%$.

Chemokine Receptors mRNA Expression

Rat T-lymphocytes were enriched by using Rat T-Cell Enrichment Columns (R&D) according to the manufacturer's instructions. 1×10^7 cells were used for RT-PCR. Total RNA and first-strand cDNA syntheses were described above. The primer sequences to rat CCR3, CCR5 and CXCR3 were as follows:

rat CCR3 sense, 5′-GGCATCCAACGAAGAGGAACTCAA-3′
antisense, 5′-ATCTCGCTGTACAAGGCCAGGTAA-3′ (372-bp product)
rat CCR5 sense, 5′-AACCTGGCCATCTCTGACCTG-3′
antisense, 5′-GTAGCAGATGACCATGAC-3′ (432-bp product)
rat CXCR3 sense, 5′-AGGTCAGTGAACGTCAAGTGCTAG-3′
antisense, 5′-GCAAAAAGAGGAGGCTGTAGAGGA-3′ (183-bp product)

PCR amplification and gel analysis were the same as above except that annealing temperature was at 60°C and 32 cycles were used for CCR3, whereas CCR5 and CXCR3 were run for 36 cycles.

Statistical Analysis

All data were given as mean ± SD. The comparisons between >2 groups were made using multivariate

Table I. Clinical Data of Patients with AMI, UA, SA and CPS

Characteristics	Age (year)	Sex (Male/female)	Hypertension	Diabetes mellitus	Total cholesterol (mmol/L)	LDL cholesterol (mmol/L)	HDL cholesterol (mmol/L)	Triglyceride (mmol/L)
AMI ($n = 33$)	62 ± 8	26/7	10	6	4.59 ± 0.81*	2.85 ± 0.65*	0.96 ± 0.22*	1.52 ± 0.57*
UA ($n = 22$)	63 ± 9	17/5	11	5	4.57 ± 0.76*	2.86 ± 0.59*	0.95 ± 0.18*	1.55 ± 0.63*
SA ($n = 20$)	60 ± 11	15/5	7	5	4.65 ± 0.83*	2.89 ± 0.70*	0.98 ± 0.21*	1.54 ± 0.65*
CPS ($n = 21$)	58 ± 12	15/6	8	4	4.47 ± 0.65	2.76 ± 0.52	1.01 ± 0.19	1.47 ± 0.52

Note. Values are expressed as mean ± SD or number.
* $p > 0.05$ vs CPS.

ANOVA of independent groups to determine the overall difference, and a post hoc Bonferroni/Dunn test was used to determine statistical significance between groups. Probability values <0.05 were considered statistically significant.

RESULTS

Basic Clinical Characteristics of Patients with AMI, UA, SA and CPS

Table I shows the clinical data in group AMI, UA, SA and CPS. The basic conditions of the four groups were similar and comparable.

Ambulatory Changes of Intracellular Cytokines in CD4+ T-Lymphocytes in Patients with ACS (Fig. 1)

The frequencies of IFN-γ-producing T-cells (Fig. 2A) were found to be significantly higher in patients with AMI ($22.4 \pm 7.8\%$) and UA ($20.6 \pm 6.4\%$) within 24 h after the onset of symptom than those of SA ($11.3 \pm 3.8\%$) and CPS ($10.7 \pm 3.4\%$) ($p < 0.01$). There was no significant difference on the frequencies of IL-4-producing peripheral T-cells (Fig. 2B) between each group. The high ratio of IFN-γ-producing T-cells lasted shortly in patients with UA and recovered 1 week ($11.7 \pm 4.1\%$) after the onset. In AMI patients, the high ratio of IFN-γ-producing T-cells could be examined 1 week ($17.1 \pm 6.0\%$) and even 1 month ($15.1 \pm 4.8\%$) after the onset.

Assessment of Heart Function in AMI Subgroups

As shown in Table II, compared to group AMI1, there was significant left ventricular (LV) dilatation and systolic dysfunction in group AMI2, as evidenced by increased LV end-diastolic diameter (LVEDd), decreased ejection fraction (EF) and fractional shortening (FS).

Assessment of Frequencies of IFN-γ- and IL-4-Producing T-cells in AMI Subgroups

The frequencies of IFN-γ-producing peripheral CD4+ T-cells and IL-4-producing T-cells in AMI subgroups are shown in Fig. 3. For the former, frequencies were significantly higher in group AMI2 ($16.7 \pm 4.3\%$) than in group AMI1 ($13.3 \pm 3.9\%$). In the case of the latter, the differences were not significant.

Changes of IFN-γ and IL-4 mRNA Expression in the Myocardium of AMI Rats

Compared to Sham group, the expression of IFN-γ mRNA were significantly increased in both infarcted and noninfarcted areas in myocardium of rats with AMI

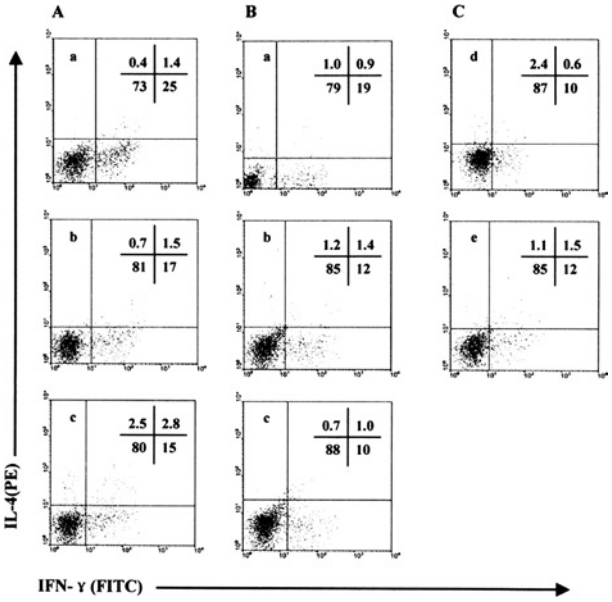

Fig. 1. The representative photographs of 3-color flow cytometric analysis of intracellular cytokines in $CD4^+$ T-cells (gated to CD4). Cytokine-producing T-cells in AMI (A), UA (B) and control (C) group at the different time points ((a), within 24 h; (b), on day 7 and (c), on day 30 after the onset of symptom; (d), group SA and (e), group CPS while coronary angiography).

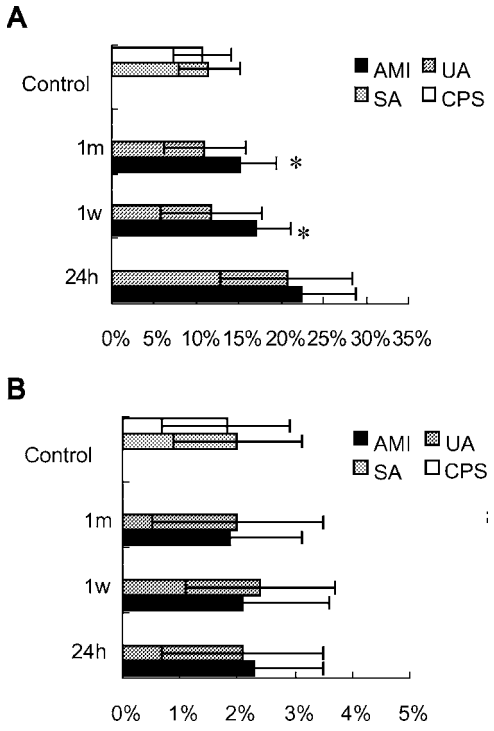

Fig. 2. The ambulatory changes of intracellular cytokines in $CD4^+$ T-cells in patients with ACS. A, IFN-γ- producing T-cells in each group at the different time points (within 24 h, on day 7 and day 30 after the onset of symptom). $^*p < 0.05$ vs UA group at the same time point. B, IL-4- producing T-cells in each group. AMI group: $n = 33$; UA group: $n = 22$; SA group: $n = 20$; CPS group: $n = 21$. Results are expressed as mean $\pm$ SD.

($p < 0.01$). The maximal IFN-γ mRNA expression was examined on 1 week after AMI, and decreased along with the prolongation of the time after AMI. IL-4 mRNA could not be detected at the different time point (Fig. 4).

Changes of Th Lymphocyte Subtypes in Rats with AMI

One week, 2 weeks and 1 month after AMI, there was no significant change on IFN-γ-producing and IL-4-producing T-cells in rats with AMI by flow cytometric analysis (Fig. 5). There was also no significant change on chemokine receptors mRNA expression on the surface of rat T-lymphocytes (Data not shown).

Table II. Assessment of Heart Function in AMI Subgroups

Group	LVEDd (mm)	EF (%)	FS (%)
AMI1 ($n = 18$)	51.3 ± 5.8	52 ± 6	28 ± 5
AMI2 ($n = 15$)	58.6 ± 9.5*	44 ± 9*	17 ± 4*

Note. Values are expressed as mean $\pm$ SD.
$^*p < 0.05$ vs AMI1.

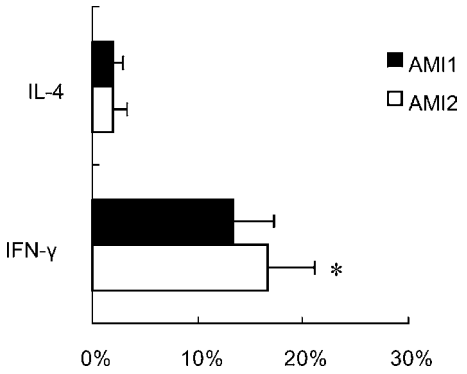

Fig. 3. Assessment of frequencies of IFN-γ- and IL-4-producing T-cells in AMI subgroups. On days 30 after AMI, the frequencies of IFN-γ- and IL-4-producing T-cells in group AMI1 (NYHA I–II, $n = 18$) and group AMI2 (NYHA III–IV, $n = 15$). $^*p < 0.05$ vs AMI1 group. Results are expressed as mean $\pm$ SD.

DISCUSSION

Th1/Th2 Imbalance and Coronary Arterial Inflammation

Recent study demonstrated that almost all the UA patients were accompanied with inflammatory infiltration, suggesting that acute inflammation was not only the reaction to endothelium injury and thrombosis, but also a primary pathological process (11). Liuzzo *et al*. (6) recently reported that interferon-γ-producing T-cells were more frequent in the peripheral blood of patients with UA than those of SA. They suggested that monocyte activation represented a downstream effect of over-stimulated Th1 lymphocytes. Soejima *et al*. (12) demonstrated that in patients with coronary spastic angina (one type of UA), there was a preference toward the Th1-type response. The present study found that within 24 h after the onset, IFN-γ-producing T-cells were more frequent in patient with ACS than those of SA and CPS, indicating that there was a preference toward Th1-type response not only in patients with UA but also in those with AMI. Libby *et al*. (13) found T-lymphocytes in the plaque could modify the proliferation of vascular smooth muscle cells (SMCs) through excreting IFN-γ. IFN-γ could significantly inhibit SMCs to synthesize interstitial collagen and activate monocytes, which could degrade extracellular matrix in vulnerable regions of the plaque's fibrous cap and lead to plaque instability and rupture in patients with UA (6, 14–16). The data we present here show that there is a preference toward the Th1-type response in the patients with ACS and can excrete excessive IFN-γ, suggesting Th1/Th2 functional imbalance may result in the rupture of plaque and lead to the pathogenesis of UA and AMI.

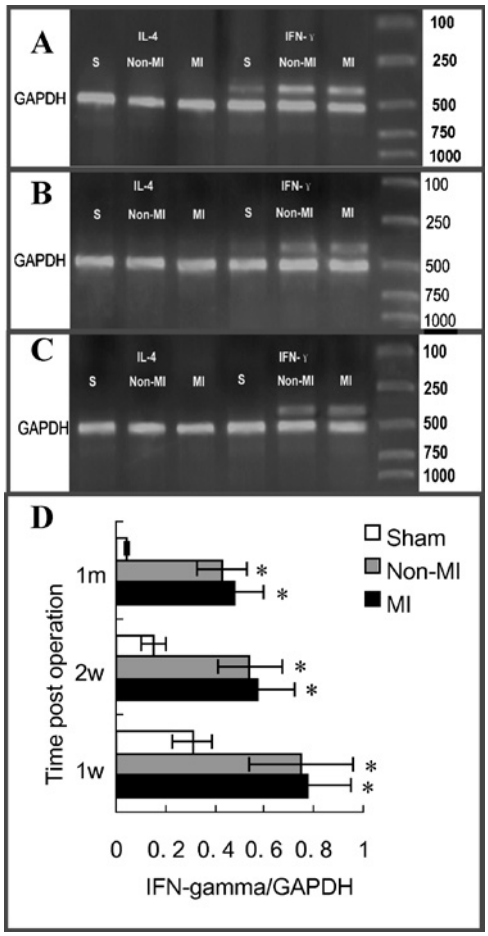

Fig. 4. IFN-γ and IL-4 mRNA expression in the myocardium of AMI and Sham rats. A, 1 week (AMI group: $n = 12$; Sham group: $n = 10$), B, 2 weeks (AMI group: $n = 11$; Sham group: $n = 10$) and C, 1 month (AMI group: $n = 12$; Sham group: $n = 10$) after operation, the representative RT-PCR photographs: IFN-γ and IL-4 mRNA expression in MI, Non-MI area of post-MI rats and Sham (S) rat. D, IFN-γ mRNA expression at the different time points after operation. $^{*}p < 0.01$ vs Sham rats at the same time point. Results are expressed as mean ± SD.

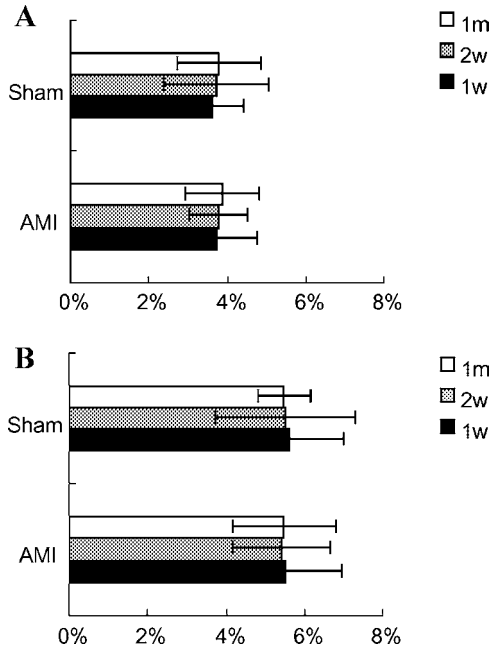

Fig. 5. Changes of Intracellular Cytokines in $CD3^{+}$ $CD8^{-}$ Lymphocytes of Rats post-MI. A, IFN-γ-producing T-cells in AMI and Sham rats at the different time points [1 week (AMI group: $n = 12$; Sham group: $n = 10$), 2 weeks (AMI group: $n = 11$; Sham group: $n = 10$) and 1 month (AMI group: $n = 12$; Sham group: $n = 10$) after operation]. B, IL-4-producing T-cells in AMI and Sham rats at the different time points, after pre-stimulated with plate bound anti-CD3, soluble anti-CD28, rrIL-2 and rrIL-4. Results are expressed as mean ± SD.

Th1/Th2 Imbalance and Myocardial Inflammation after AMI

AMI is much more severe cardiac disorder than UA, that is characterized by acute myocardial necrosis. There are two different inflammatory processes in patients with AMI: the coronary arterial inflammation that leads to the pathogenesis of AMI and the myocardial inflammation that leads to ventricular remodeling post-MI**.** Through follow-up, we noticed that significantly higher ratio of Th1 lymphocytes in AMI patients compared with UA after 1 week and 4 weeks. The outcomes suggest that the imbalance of Th1/Th2 function in patients with UA is only a short term result of acute coronary arterial inflammation, whereas the preference toward the Th1-type response is not only the result of coronary arterial inflammation, but secondary to the inflammation because of the myocardial necrosis post-MI.

As we know, the human "natural" AMI versus artificial AMI in non-atherosclerotic rats are completely different situations. Because the construction of AMI rat model through *in vivo* ligation of coronary artery could only lead to myocardial inflammation but not coronary arterial inflammation, we selected the AMI rat model as the experimental object to observe whether the imbalance of Th1/Th2 function occurred in the myocardial inflammation post-MI. This study showed that 1 week, 2 weeks and 1 month after AMI, IFN-γ mRNA increased in both infarcted and noninfarcted areas in the rat myocardium, but there was no significant change on cytokine-producing Th cells and chemokine receptors CCR3, CCR5 and CXCR3 mRNA expression on the surface of rat T-lymphocytes. Th1/Th2 function can be evaluated by measuring of chemokine receptors CCR3 (17, 18), CCR5 (19) and CXCR3 (17, 20) on the surface of T-lymphocytes or by intracellular cytokines analysis. Our study demonstrated that there was no Th1/Th2 functional imbalance of

the global immune system in post-MI rats, but the preference toward Th1 type cytokine in local myocardium (including MI area and non-MI area) lasted at least 1 month after AMI. We consider that the difference of Th1/Th2 function of the global immune system between AMI patients and AMI rats is perhaps concerned with the species difference.

Th1/Th2 Imbalance and Ventricular Remodeling after AMI

From the subgroup assessment of AMI patients on days 30 after AMI, we observed the higher frequencies of IFN-γ-producing T-cells in patients with heart function class NYHA III-IV than that of class NYHA I-II. The results showed that up-regulation of Th1 cell function is consistent with bad heart function, which suggests that imbalance of Th1/Th2 cell functions participated in ventricular remodelling after AMI.

As the characteristic cytokine of Th1 lymphocyte, IFN-γ is very important in the whole process of inflammation (21). This study showed that the preference toward Th1 type cytokine in local myocardium (including MI area and non-MI area) lasted long period after AMI. Because IFN-γ could cause cardiac myocytes necrosis through the induction of local nitric oxide production (22–25), we suggests that local Th1/Th2 functional imbalance participate in the myocardial injury and ventricular remodeling after AMI.

Varda-Bloom *et al*. (26) previously showed that in MI area and non-MI area of post-MI rats, focal myocarditis, perivasculitis and lymphocytes infiltration could be observed, indicating the occurrence of the cell mediated autoimmune response in post-MI rats. They co-cultured lymphocytes from post-MI rats and cardiomyocytes from normal neonatal rats, found that cytotoxic T-lymphocytes were activated following MI and could recognize and kill normal cardiomyocytes *in vitro*. Maisel *et al*. (7) and our previous study (8) showed that adoptively transferred splenocytes of post-MI rats could induce organ specific autoimmune myocarditis in recipient rats, proving the autoimmune inflammation plays an important role in post-MI ventricular remodeling. Previous study demonstrated that up-regulation of Th1 cell-functions could promote the development of organ specific autoimmune disease such as experimental autoimmune encephalomyelitis (27) while up-regulation of Th2 cell-functions could promote the development of systemic autoimmune disease such as systemic lupus erythematosus (28). We suggest that up-regulation of Th1 cell-functions may participate in the immune-mediated myocardial injury and ventricular remodeling and lead to heart failure post-MI.

Hence we demonstrate myocardial inflammation caused by Th1 is associated with post-MI injury and heart failure. Further studies or clinical trials should be focused on the immunotherapy aiming at decrease inflammation caused by Th1 lymphocytes.

ACKNOWLEDGMENTS

This study was supported by grants from National Natural Science Foundation of China (No. 30370574).

REFERENCES

1. Jonasson L, Holm J, Skalli O, Bondjers G, Hansson GK: Regional accumulations of T-cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque. Arteriosclerosis 6:131–138, 1986
2. Entman ML, Ballantyne CM: Inflammation in acute coronary syndromes. Circulation 88:800–803, 1993
3. Buja LM, Willerson JT: Role of inflammation in coronary plaque disruption. Circulation 89:503–505, 1994
4. Semeri GG, Abbate R, Gori AM, Attanasio M, Martini F, Giusti B, Dabizzi P, Poggesi L, Modesti PA, Trotta F: Transient intermittent lymphocyte activation is responsible for the instability of angina. Circulation 86:790–797, 1992
5. Neri Serneri GG, Prisco D, Martini F, Gori AM, Brunelli T, Poggesi L, Rostagno C, Gensini GF, Abbate R: Acute T-cell activation is detectable in unstable angina. Circulation 95:1806–1812, 1997
6. Liuzzo G, Vallejo AN, Kopecky SL, Frye RL, Holmes DR, Goronzy JJ, Weyand CM: Molecular fingerprint of interferon-gamma signaling in unstable angina. Circulation 103:1509–1514, 2001
7. Maisel A, Cesario D, Baird S, Rehman J, Haghighi P, Carter S: Experimental autoimmune myocarditis produced by adoptive transfer of splenocytes after myocardial infarction. Circ Res 82:458–463, 1998
8. Liao YH, Tao R, Cheng X: Autoimmune mechanism in ventricular remodeling after acute myocardial infarction of rats. J HK Coll Cardiol 10:220, 2002
9. Yasue H, Horio Y, Nakamura N, Fujii H, Imoto N, Sonoda R, Kugiyama K, Obata K, Morikami Y, Kimura T: Induction of coronary artery spasm by acetycholine in patients with variant angina: possible role of the parasympathetic nervous system in the pathogenesis of coronary artery spasm. Circulation 74:955–963, 1986
10. Caraher EM, Parenteau M, Gruber H, Scott FW: Flow cytometric analysis of intracellular IFN-gamma, IL-4 and IL-10 in CD3(+)4(+) T-cells from rat spleen. J Immunol Methods 244:29–40, 2000
11. Arbustini E, De Servi S, Brsmucci E, Porcu E, Costante AM, Grasso M, Diegoli M, Fasani R, Morbini P, Angoli L: Comparison of coronary lesion obtained by directional coronary atherctomy in unstable angina, stable angina, and restenosis after either atherectomy or angioplasty. Am J Cardiol 75:675–682, 1995
12. Soejima H, Irie A, Miyamoto S, Kajiwara I, Kojima S, Hokamaki J, Sakamoto T, Tanaka T, Yoshimura M, Nishimura Y, Ogawa H: Preference toward a T-helper type 1 response in patients with coronary spastic angina. Circulation 107:2196–2120, 2003
13. Libby P, Hansson GK: Involvement of the immune system in human atherogenesis: Current knowledge and unanswered questions. Lab Invest 64:5–15, 1991

14. Libby P: Molecular bases of the acute coronary syndromes. Circulation 91:2844–2850, 1995
15. Liuzzo G, Kopecky SL, Frye RL, O'Fallon WM, Maseri A, Goronzy JJ, Weyand CM: Perturbation of the T-cell repertoire in patients with unstable angina. Circulation 100:2135–2139, 1999
16. Liuzzo G, Goronzy JJ, Yang H, Kopecky SL, Holmes DR, Frye RL, Weyand CM: Monoclonal T-cell proliferation and plaque instability in acute coronary syndromes. Circulation 101:2883–2888, 2000
17. Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, Sozzani S, Allavena P, Gray PA, Mantovani A, Sinigaglia F: Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med 187:129–134, 1998
18. Sallusto F, Mackay CR, Lanzavecchia A: Selective expression of the eotaxin receptor CCR3 by human T helper 2 cells. Science 277:2005–2007, 1997
19. Loetscher P, Uguccioni M, Bordoli L, Baggiolini M, Moser B, Chizzolini C, Dayer JM: CCR5 is characteristic of Th1 lymphocytes. Nature 391:344–345, 1998
20. Sallusto F, Lenig D, Machay CR, Lanzavecchia A: Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. J Exp Med 187:875–883, 1998
21. Williams JG, Jurkovich GJ, Maier RV: Interferon-gamma: A key immunoregulatory lymphokine. J Surg Res 54:79–93, 1993
22. Pinsky DJ, Cai B, Yang X, Rodriguez C, Sciacca RR, Cannon PJ: The lethal effects of cytokine-induced nitric oxide on cardiac myocytes are blocked by nitric oxide synthase antagonism or transforming growth factor beta. J Clin Invest 95:677–685, 1995
23. Bick RJ, Wood DE, Poindexter B, McMillin JB, Karoly A, Wang D, Bunting R, McCann T, Law GJ, Buja LM: Cytokines increase neonatal cardiac myocyte calcium concentrations: The involvement of nitric oxide and cyclic nucleotides. J Interferon Cytokine Res 19:645–653, 1999
24. Sun X, Delbridge LM, Dusting GJ: Cardiodepressant effects of interferon-gamma and endotoxin reversed by inhibition of NO synthase 2 in rat myocardium. J Mol Cell Cardiol 30:989–997, 1998
25. Keira N, Tatsumi T, Matoba S, Shiraishi J, Yamanaka S, Akashi K, Kobara M, Asayama J, Fushiki S, Fliss H, Nakagawa M: Lethal effect of cytokine-induced nitric oxide and peroxynitrite on cultured rat cardiac myocytes. J Mol Cell Cardiol 34:583–596, 2002
26. Varda-Bloom N, Leor J, Ohad DG, Hasin Y, Amar M, Fixler R, Battler A, Eldar M, Hasin D: Cytotoxic T-lymphocytes are activated following myocardial infarction and can recognize and kill normal myocytes in vitro. J Mol Cell Cardiol 32:2141–2149, 2000
27. Kennedy MK, Torrance DS, Picha KS, Mohler KM: Analysis of cytokine mRNA expression in the central nervous system of mice with experimental autoimmune encephalomyelitis reveals that IL-10 mRNA expression correlates with recovery. J Immunol 149:2496–2505, 1992
28. Hagiwara E, Gourley MF, Lee S, Klinman DK: Disease severity in patients with systemic lupus erythematosus correlates with an increased ratio of interleukin-10-interferon gamma-secreting cells in the peripheral blood. Arthritis Rheum 39:379–385, 1996