

学位論文内容の要旨
(Summary of dissertation)

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学 位 論 文 題 名
(Title of dissertation)

Studies on the Role of Natural Killer T Cells on Post-Infarct Cardiac Remodeling and Failure in Mice

(マウスの心筋梗塞後心筋リモデリングおよび心不全におけるナチュラルキラーT細胞の役割に関する研究)

(Background and Objectives) Myocardial infarction (MI) leads to the development of heart failure (HF), which is the major cause of death in post-MI patients. The changes in left ventricular (LV) geometry, such as cavity dilatation associated with myocyte hypertrophy and interstitial fibrosis, referred to as remodeling, contribute to the development of depressed cardiac function in HF after MI. Various pathophysiological mechanisms including chronic inflammation have been reported to be involved in the development of LV remodeling and failure after MI. Natural killer T (NKT) cells have been shown to produce inflammatory cytokines and orchestrate tissue inflammation in various tissues including the vascular wall. However, no previous studies have determined the changes of NKT cells and their pathophysiological role in post-MI LV remodeling. The purpose of this study was to examine whether the activation or inhibition of NKT cells might affect the development of remodeling and failure after MI.

(Materials and Methods) MI was created in male C57BL/6J mice by ligating the left coronary artery, and sham operation was also performed. Mice received the intraperitoneal injection of either α -galactosylceramide (α GC), the activator of NKT cells, or phosphate-buffered saline (PBS) on 1 and 4 days after surgery. Four groups of mice, sham+PBS (n=10), sham+ α GC (n=10), MI+PBS (n=31), and MI+ α GC (n=27) were studied. After 28 days, echocardiography and hemodynamic measurements were performed. After collecting blood samples, mice were sacrificed for histological analyses including infarct size, myocyte cross-sectional area, collagen volume fraction, TUNEL staining and MMP zymography. RT-PCR was performed to quantify mRNA expression in the non-infarcted area of LV tissues at 7 and 28 days. To determine the effects of NKT cell deletion, MI was created in male C57BL/6J wild-type (WT; n=39) and NKT cell receptor ($J\alpha 18$; n=42) knockout (KO) and were followed for 28 days.

(Results) Survival rate during 28 days of MI was significantly higher in MI+ α GC than MI+PBS (59% vs 32%, $P<0.05$). LV end-diastolic dimension was smaller (5.0 ± 0.1 vs 5.4 ± 0.1 mm, $p<0.01$), and LV fractional shortening was greater (18.8 ± 0.6 vs 16.5 ± 0.6 %, $p<0.05$) in MI+ α GC than MI+PBS, with no significant changes in infarct size between groups. LV end-diastolic pressure and lung weight/body weight in MI+ α GC were lower than those in MI+PBS. The injection of α GC to sham mice did not affect cardiac function and structure. Histological analysis revealed that α GC attenuated myocyte hypertrophy, interstitial fibrosis with decreased MMP-2 activity, and apoptosis in the non-infarcted area from MI mice. NKT cell receptor ($V\alpha 14J\alpha 18$) gene expression was significantly increased in LV from MI compared with sham at 7 days,

whereas it was not altered at 28 days. The injection of α GC after MI further enhanced NKT cell receptor mRNA expression at 7 and 28 days. In parallel to NKT cell activation, it enhanced LV monocyte chemoattractant protein-1 and tumor necrosis factor- α mRNA at 7 days and also interleukin-10 (IL-10) persistently until 28 days. Anti IL-10 receptor antibody abrogated these protective effects of α GC on MI remodeling. In NKT cell deletion study, survival rate during 28 days of MI was comparable between WT+MI and KO+MI. LV cavity dilatation and dysfunction were significantly exacerbated in KO+MI compared to WT+MI, with no significant changes in infarct size ($56\pm 2\%$ vs $58\pm 1\%$, $P=NS$).

(Discussion) This is the first report to provide direct evidence for increased NKT cells in MI and the inhibitory effects of their activation on the development of post-MI HF. In the acute phase of MI, the infiltration of inflammatory cells is a physiological repair process by removing dead cardiomyocytes and scar formation of infarcted area. However, the chronic inflammatory response in the non-infarcted area causes the further myocardial damage and fibrosis, leading to the progressive LV remodeling and failure. The precise role of various inflammatory cells and chemokines in this disease process has not been fully elucidated. The NKT cells mediate various functions rapidly by producing a mixture of T_H1 and T_H2 cytokines and vast array of chemokines. NKT cells can function as a bridge between the innate and adaptive immune systems, and orchestrate tissue inflammation. In the present study, α GC injection significantly enhanced NKT cell infiltration and could effectively ameliorate post-MI LV remodeling and failure and improved survival. Correspondingly, the inhibition of NKT cells worsened LV remodeling and failure after MI. Therefore, NKT cells play a protective role in LV remodeling and failure after MI. We found that the enhanced expression of IL-10 was involved in the inhibitory effects of NKT cell activation against LV remodeling and failure. The present study demonstrated that both TNF- α and IL-10 were increased in non-infarcted LV from sham and MI animals in association with an increase in NKT cells after the treatment with α GC at 7 days. Interestingly, the enhanced expression of IL-10 gene by α GC persisted only in MI mice, whereas TNF- α gene expression returned to the baseline levels. These changes of IL-10 gene expression completely corresponded to those of NKT cells. Moreover, the inhibitory effects of α GC on LV remodeling were reversed by anti-IL-10 receptor antibody, indicating that IL-10 was involved in the beneficial effects of NKT cell activation against post-MI remodeling and failure. These results are consistent with the previous findings that the therapeutic effects of α GC against T_H1 -like autoimmune diseases included the induction of immunosuppressive cytokine IL-10. IL-10 can inhibit the production of proinflammatory cytokines by macrophages and T_H1 cells, which may regulate extracellular matrix, angiogenesis, and apoptosis. In the present study, the activation of NKT cells by α GC decreased cardiac myocyte hypertrophy and apoptosis, and inhibited interstitial fibrosis possibly through inhibiting the activation of MMP-2 in non-infarcted LV. There are several limitations to be acknowledged. First, we could not directly demonstrate the relation between NKT cells and IL-10 due to the technical difficulties. Second, the underlying mechanisms responsible for the activation of NKT cells after MI remain to be determined. These problems should be explored for future studies.

(Conclusion) NKT cells have a protective effect on LV remodeling and failure after MI via enhanced IL-10 expression. Therapies designed to activate NKT cells may be beneficial against the development of post-MI heart failure.