Gene expression profiles of cardiomyocytes in rat autoimmune myocarditis by DNA microarray and increase of regenerating gene family

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Cardiomyocytes with myocarditis compared with the normal state are thought to change the expressions of various genes greatly, some of which may be new biomarkers or new biologic medicinal products. However, until now, little comprehensive analysis has been made of gene-expression changes in cardiomyocytes with myocarditis. In this study, we performed a DNA microarray analysis by using cardiomyocytes from rat experimental autoimmune myocarditis (EAM). On day 0, rats were immunized with porcine cardiac myosin and cardiomyocytes were isolated and purified from EAM hearts and normal hearts by a method that is hardly thought to change gene expressions in cardiomyocytes. RNA from normal cardiomyocytes and cardiomyocytes of EAM on day 18 was analyzed for 7711 gene expressions by DNA microarray. Some gene expressions showed over 10-fold changes. In particular, the regenerated gene (Reg)2/pancreatitis-associated protein (PAP)1 messenger RNA (mRNA) level most markedly increased in the genes, which were clearly expressed in cardiomyocytes rather than in noncardiomyocytes, and it was approximately 2000-fold greater in cardiomyocytes under active myocarditis than normal by real-time reverse transcription polymerase chain reaction analysis. Moreover, we demonstrated that Reg2/PAP1 proteins determined by Western blot analysis and immunohistochemistry and other Reg/PAP family gene expressions were remarkably increased in EAM hearts; in addition, interleukin (IL)-6 expression was significantly related to Reg2/PAP1. It seemed that these data were useful as a reference database of gene-expression changes in cardiomyocytes with myocarditis. The Reg/PAP family, which was found to show dramatically increasing gene expressions by DNA microarray analysis, was suspected to play an important role in myocarditis. (Translational Research 2008;152:119–127)

Abbreviations: aRNA = antisense RNA; BSA = bovine serum albumin; EAM = experimental autoimmune myocarditis; IL = interleukin; mRNA = messenger RNA; NCNI = noncardiomyocytic, noninflammatory; PAP = pancreatitis-associated protein; PE = phycoerythrin; PSP = pancreatitis stone peptide; Reg = regenerating gene; RT-PCR = reverse transcription polymerase chain reaction; TBS = Tris-buffered saline

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AT A GLANCE COMMENTARY

Background

Cardiomyocytes with myocarditis are assumed to change the expressions of various genes greatly; however, until now, little comprehensive analysis has been made of gene-expression changes in cardiomyocytes with myocarditis.

Translational Significance

We demonstrate that regenerated gene (Reg)/pancreatitis-associated protein (PAP) family messenger RNA (mRNA) levels dramatically increase by DNA microarray and real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis of gene-expression changes in cardiomyocytes with myocarditis. Reg2/PAP1 are clearly expressed in cardiomyocytes rather than in noncardiomyocytes. Interleukin (IL)-6 expression is significantly related to Reg2/PAP1. These results strongly suggest that the Reg/PAP family plays an important role in myocarditis.

Fulminant myocarditis often causes lethal heart failure with severe cardiac dysfunction, but if it can pass from the acute phase to the recovery phase, then cardiac dysfunction spontaneously recovers in most cases. The reversibility means that various factors of myocarditis temporarily inhibit contraction-relaxation; however, most cardiomyocytes in myocarditis are alive. Cytokines, which are other chemical and mechanical factors in myocarditis hearts, are thought to change gene expressions dramatically in such viable cardiomyocytes. Tumor necrosis factor-α, interleukin (IL)-6, and IL-2 have been considered critical factors in the pathogenesis of cardiac contractile dysfunction in a concentration-dependent, reversible manner.[1–3] Moreover, the changes of gene expressions in cardiomyocytes are assumed to affect cardiac remodeling profoundly. However, no comprehensive study has been conducted on the changes of gene expression not in myocarditis hearts that contain inflammatory cells but in purified cardiomyocytes under myocarditis. In this study, we purified cardiomyocytes under myocaridits and normal cardiomyocytes by a method that is thought to have little influence on gene expressions,[4] and we investigated the expressions of 7711 genes by DNA microarray. We assumed that these data might be useful as a reference database for new biomarkers and new drugs to diagnose and treat myocarditis. Here, as a result of gene-expression profiling analysis, we found that the regenerated gene (Reg) 2/pancreatitis-associated protein (PAP) 1 messenger RNA (mRNA) level most markedly increased in the genes, which were clearly expressed not in noncardiomyocytes but in cardiomyocytes. Subsequently, we also investigated the gene expression of the Reg/PAP family by real-time reverse transcription-polymerase chain reaction (RT-PCR).

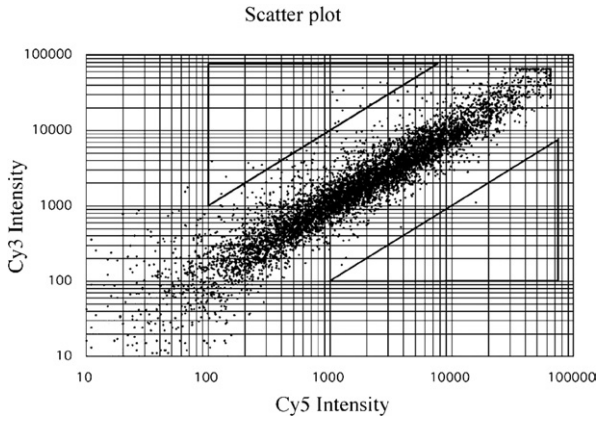

Fig 1. Scatter plot of 7711 gene expression by DNA microarray. Cy3, EAM heart; Cy5, normal heart.

MATERIAL AND METHODS

Animals. Lewis rats were obtained from Charles River, Japan (Atsugi, Kanagawa, Japan) and were maintained in our animal facilities until they reached 8 weeks of age. Throughout the studies, all animals were treated in accordance with the guidelines for animal experiments of our institute and the guide for the care and use of laboratory animals published by the U.S. National Institutes of Health.

Induction of experimental autoimmune myocarditis (EAM). Whole cardiac myosin was prepared from the ventricular muscle of porcine hearts as previously described. It was dissolved in phosphate buffering solution at a concentration of 10 mg/mL and emulsified with an equal volume of complete Freund's adjuvant supplemented with 10 mg/mL of *Mycobacterium tuberculosis* H37RA (Difco, Detroit, Mich). On day 0, the rats received a single immunization at 2 subcutaneous sites with 0.2 mL of emulsion for each rat.[5] As control, rats were immunized with only complete Freund's adjuvant on day 0.

Isolation and purification of cells. Cardiomyocytes and noncardiomyocytes in normal and EAM hearts were isolated after collagenase perfusion treatment for 20 min using Langendorff apparatus and purified serially through a 38-μm stainless-steel sieve twice and a 20-μm stainless-steel sieve twice as reported previously. Cells larger than 38 μm and smaller than 20 μm were considered as cardiomyocytes and noncardiomyo-