Increased expression of interleukin 6 mRNA in cardiac myxomas

Yoshitane Seino, Uichi Ikeda, Kazuyuki Shimada

Abstract
In three patients with cardiac myxoma increased expression of interleukin 6 (IL-6) mRNA was found in the myxoma tissue by polymerase chain reaction (PCR) and in situ hybridisation. These cases suggest that IL-6 is overproduced in the myxoma tissue and secreted into the systemic circulatory system. This might contribute to the systemic inflammatory or autoimmune manifestations seen in cardiac myxoma. This study also showed the usefulness of PCR and in situ hybridisation for the evaluation of mRNA expression in small samples of tissue.

(Cardiac myxomas are benign tumours that can obstruct intracardiac blood flow or cause embolism by fragmentation. The symptoms of cardiac myxoma include weight loss, fever, fatigue, arthralgia, skin flushing and Raynaud’s phenomenon. Laboratory abnormalities include anaemia, an increased erythrocyte sedimentation rate (ESR), and the presence of autoimmune antibodies such as rheumatoid factor and antinuclear antibody. Serum concentrations of interleukin 6 (IL-6) were reported to be increased in patients with cardiac myxoma and IL-6 could cause the inflammatory or immunological features associated with this disorder.\textsuperscript{1-3} We report on three patients with cardiac myxoma in whom the polymerase chain reaction (PCR) and in situ hybridisation showed that expression of IL-6 mRNA was increased in the myxoma tissue.

Case reports
Patient 1—A 72 year old woman was admitted for the evaluation of chest pain. The laboratory examination showed an increased ESR (43 mm/h) and C reactive protein (CRP, 3-1 mg/dl) and detectable antinuclear antibody (table). Cross sectional echocardiography and magnetic resonance imaging (MRI) showed a solid mass in the left atrium, and coronary arteriography showed stenotic lesions in the left anterior descending artery and circumflex artery. The tumour was resected and aortocoronary bypass surgery was performed.

Patient 2—A 63 year old man was admitted with exertional dyspnoea. The concentration of CRP was increased (1-3 mg/dl) and rheumatoid factor was detected (table). Cross sectional echocardiography and MRI showed a mass in the left atrium, which was resected. Patient 3—A 68 year old man was admitted complaining of shortness of breath. There were no immunological abnormalities. Cross sectional echocardiography showed a solid mass in the left atrium. This was excised.

Serum concentrations of IL-6 were measured by a solid phase sandwich immunoassay system (Fuji Revio, Japan) with a monoclonal anti-human-IL-6 antibody. The IL-6 concentration in serum from healthy donors was <3-0 pg/ml. IL-6 was measured in 50 μl serum samples, stored at -80°C.

The resected myxoma from three patients and fragment of aorta punched out during aortocoronary bypass surgery in patient 1 were immediately frozen and stored at -80°C for the analysis of IL-6 mRNA. Total RNA was isolated from 50 mg frozen specimens as previously described.\textsuperscript{1} First, strands of cDNA were synthesised from total RNA by Moloney murine leukaemia virus reverse transcriptase (50 U/ml; BRL, MD) and random primers. Then PCR buffer, which contained 3' and 5' primers for human IL-6 cDNA and Taq polymerase (10 U/ml; Perkin-Elmer/Cetus, CT), was added to the synthesised cDNA and amplification was performed in a DNA thermal cycler (Hoei Science, Japan). The amplification temperatures were 94°C for 20 s for denaturing, 55°C for 20 s for annealing, and 72°C for 30 s for extension. Successive amplification was performed for up to 24 cycles within the exponential phase of the amplification (data not shown).

After amplification the reaction mixture was electrophoresed on 1% agarose gel and transferred to nylon membranes (Hybond N+, Amersham, UK). Membranes were hybridised with a 32P-labeled human IL-6 cDNA probe at 60°C for 24 hours. The IL-6 cDNA consisted of a 1.4 kb Eco RI

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restriction fragment. At the end of hybridisation, the membranes were washed twice in 0.2 × SSPE (1 × SSPE contained 150 mM NaCl, 10 mM NaH₂PO₄, 1 mM EDTA-Na₂, pH 7.0) at 60°C, and exposed to Kodak XAR-5 film overnight at −70°C with one intensifying screen. In situ hybridisation was carried out as described. Briefly, the myxoma section was mounted onto olylysine-coated slides and fixed with 4% paraformaldehyde. Before hybridisation, the samples were pretreated with proteinase K (1 µg/ml) for 5 min. Hybridisation was started by adding biotin-labelled sense or anti-sense oligonucleotide probes for human IL-6 (3rd exon) at 37°C for 18 hours. The sections were washed twice with 0.2 × SSC for 15 min (1 × SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0). After treatment with a blocking solution (50 mg/ml bovine serum albumin in 150 mM NaCl and 100 mM Tris-Cl, pH 7-8) for 15 min, slides were incubated with streptavidin-alkaline phosphatase conjugate (BRL, MD) for 5 min at room temperature, then rinsed twice in Tris buffered saline for 15 min at room temperature, and once in the alkaline substrate buffer (150 mM NaCl, 50 mM MgCl₂, 100 mM Tris-Cl, pH 9-5) for 5 min at room temperature. Alkaline phosphatase activity was detected with nitroblue tetrazolium and 4-bromo-5-chloro-3-indolylphosphate solution at 37°C for 30 min.

Serum IL-6 concentrations were measured in patient 1 and patient 2 before and after tumour resection (table) and in both IL-6 concentrations were significantly increased before operation (6.0 and 9.0 pg/ml respectively). Two months after operation they were 4.0 pg/ml in both patients. The immunological abnormalities detected before surgery also disappeared postoperatively.

Figure 1 shows the results of PCR amplification of IL-6 mRNA in the myxoma tissue and the aortic fragment. In patient 1 the aortic fragment expressed little IL-6 mRNA (lane 2), whereas the myxoma tissue showed high levels of IL-6 mRNA expression (lane 3). Increased IL-6 mRNA expression in the myxoma tissue was also seen in patients 2 (lane 4) and 3 (lane 5). In the negative control without RNA samples (lane 1) no IL-6 transcripts were seen.

Figure 2 shows the results of in situ hybridisation of the myxoma tissue in patient 1 when biotin-labelled anti-IL-6 oligonucleotide probes for human IL-6 were used. Transcripts for the IL-6 gene were obtained when the myxoma tissue was hybridised with the anti-sense probe whereas there were no significant signals with the sense probe (data not shown).

Discussion

It is not known why cardiac myxomas sometimes mimic inflammatory or collagen disorders. Hirano et al. showed that cardiac myxomas produce IL-6 constitutively, and Saji et al. and Jourdan et al. reported raised serum concentrations of IL-6 in patients with cardiac myxoma, suggesting the involvement of IL-6 in the inflammatory or immunological features of this disorder.

IL-6, a pleiotrophic cytokine, affects B cell differentiation into plasmocytes, hepatocyte stimulation, induction of CRP release, and hematopoietic stem cell activation. IL-6 is produced by monocytes, B cells, T cells, fibroblasts, keratinocytes, mesangial cells, endothelial cells, vascular smooth muscle cells, and cardiac myocytes. IL-6 has been
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produced in the myxoma tissue and secreted into the systemic circulatory system. This might contribute to the systemic inflammatory or autoimmune manifestations seen in cardiac myxoma.

Our three cases suggest that IL-6 is over-

PCR can be used to amplify DNA and recently has been applied to the quantitative evaluation of mRNA concentrations in small specimens. Feldman et al measured the concentrations of mRNA for atrial natriuretic factor and for β myosin in human endomyocardial biopsy specimens, and Wang et al measured several cytokine mRNAs in human atherosclerotic plaque. In situ hybridisation is another new procedure used to detect mRNA expression in small specimens. We7 and Wilcox et al8 have used this technique to evaluate several types of cytokine mRNA expression in rabbit and human atherosclerotic plaques.

In this study we used PCR and in situ hybridisation to evaluate IL-6 gene transcripts in the myxoma tissue, and we found that IL-6 mRNA expression was increased in the myxoma tissue. In addition, in two patients we measured serum IL-6 concentrations before resection of the tumour, when they were high. After operation they fell and the immunological abnormalities disappeared.

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