

Increased expression of interleukin 6 mRNA in cardiac myxomas

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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.

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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.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.⁴ 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.⁵ 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

Laboratory findings before and after tumour resection in two patients with cardiac myxoma.

	Patient 1		Patient 2	
	Before	After	Before	After
ESR (mm/h)	43	47	17	14
CRP (mg/dl)	3.1	0.5	1.3	0.1
RF	(-)	(-)	(+)	(±)
ANA	(+)	(-)	(-)	(-)
IL-6 (pg/ml)*	6.0	4.0	9.0	4.0

ESR, erythrocyte sedimentation rate, CRP, C reactive protein, RF, rheumatoid factor, ANA, antinuclear antibody. *In serum from healthy donors the concentration of IL-6 was <3.0 pg/ml.

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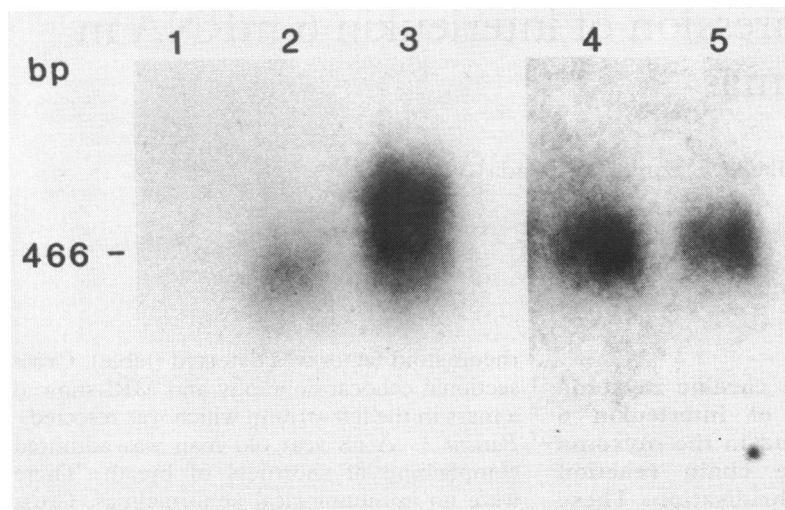
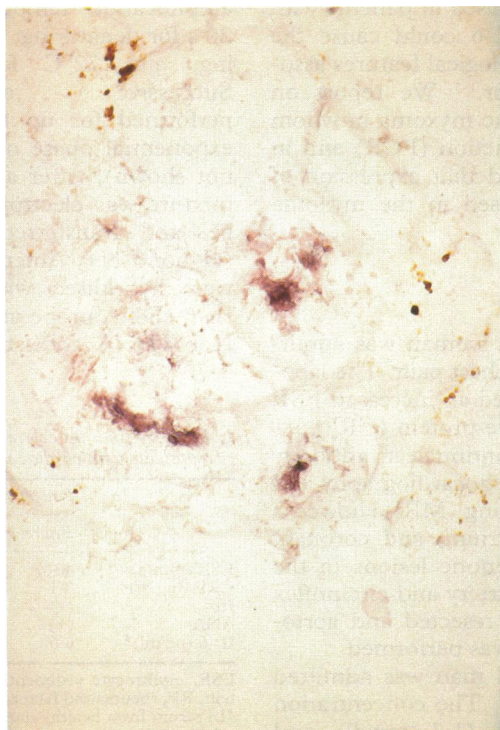


Figure 1 Identification of IL-6 mRNA transcripts by the polymerase chain reaction (PCR). Total RNA was extracted from the myxoma tissue in three patients and the aortic fragment in patient 1. The cDNA synthesised by reverse transcription of RNA was amplified in a DNA thermal cycler, separated by electrophoresis, and hybridised with 32 P-labelled human IL-6 cDNA probe. Lane 1; no RNA sample (negative control); lane 2, the aortic fragment from patient 1; lanes 3, 4, and 5, myxoma tissue from patients 1, 2, and 3, respectively. The size of amplification products was 466 base pairs.

restriction fragment. At the end of hybridisation, the membranes were washed twice in $0.2 \times$ SSPE ($1 \times$ SSPE contained 150 mM NaCl, 10 mM NaH_2PO_4 , 1 mM EDTA- Na_2 , pH 7.0) at 60°C , and exposed to Kodak XAR-5 film overnight at -70°C with one intensifying screen.⁶

In situ hybridisation was carried out as described.⁷ Briefly, the myxoma section was mounted onto olylysine-coated slides and fixed with 4% paraformaldehyde. Before hybridisation, the samples were pretreated

Figure 2 In situ hybridisation of IL-6 gene transcripts in the myxoma tissue of patient 1. Serial sections of the myxoma tissue were fixed with 4% paraformaldehyde and hybridised with biotin labelled human IL-6 oligonucleotide sense and anti-sense probes. The antisense probe showed the distribution (violet stain) of IL-6 mRNA in the myxoma tissue. (original magnification, $\times 32$).



with proteinase K ($1 \mu\text{g}/\text{ml}$) 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 $0.2 \times$ SSC for 15 min ($1 \times$ 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 MgCl_2 , 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-sense 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*¹ showed that cardiac myxomas produce IL-6 constitutively, and Saji *et al*² and Jourdan *et al*³ 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 plasmacytes, hepatocyte stimulation, induction of CRP release, and haematopoietic stem cell activation. IL-6 is produced by monocytes, B cells, T cells, fibroblasts, keratinocytes, mesangial cells, endothelial cells, vascular smooth muscle cells,⁶ and cardiac myocytes.⁴ IL-6 has been

implicated in rheumatoid arthritis, mesangial proliferative glomerulonephritis, multiple myeloma, Castleman's disease, psoriasis, atherosclerosis,⁷ and acute myocardial infarction.⁴

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*⁸ measured the concentrations of mRNA for atrial natriuretic factor and for β myosin in human endomyocardial biopsy specimens, and Wang *et al*⁹ 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 al*¹⁰ 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.

Our three cases suggest that IL-6 is over-

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.

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