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.

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 autoimmunity 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 2 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 anti-ribonucleoprotein (RNP) antibody. Cardiac catheterisation showed normal coronary arteries and a left atrial mass (figure). Imaging studies showed a solid mass in the left atrium and resection was performed. The mass was resected at the left atrial appendage, and the mass was divided into two parts. One part was resected and stained with haematoxylin and eosin after fixation with formalin. The other part was resected and stained with haematoxylin and eosin after fixation with formalin.

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

<table>
<thead>
<tr>
<th>Patient 1</th>
<th>Patient 2</th>
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<tbody>
<tr>
<td><strong>ESR (mm/h)</strong></td>
<td>45</td>
</tr>
<tr>
<td><strong>CRP (mg/dl)</strong></td>
<td>3.1</td>
</tr>
<tr>
<td><strong>RF</strong></td>
<td>(−)</td>
</tr>
<tr>
<td><strong>ANA</strong></td>
<td>(+)</td>
</tr>
<tr>
<td><strong>IL-6 (pg/ml)</strong></td>
<td>6.0</td>
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</tbody>
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ESR, erythrocyte sedimentation rate, CRP, C reactive protein, RF, rheumatoid factor, ANA, antinuclear antibody.

*IL-6 concentration in serum.

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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-indolyolphosphate 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 and 3 (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 patients 1 and 2 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. 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 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, and cardiac myocytes. IL-6 has been
implicated in rheumatoid arthritis, mesangial proliferative glomerulonephritis, multiple myeloma, Castleman's disease, psoriasis, atherosclerosis, 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.\(^5\) measured the concentrations of mRNA for atrial natriuretic factor and for \(\beta\) myosin in human endomyocardial biopsy specimens, and Wang et al.\(^6\) measured several cytokine mRNAs in human atherosclerotic plaque. In situ hybridisation is another new procedure used to detect mRNA expression in small specimens. We\(^7\) and Wilcox et al.\(^8\) 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.