Glycoprotein 130 ligand oncostatin-M induces expression of vascular endothelial growth factor in human adult cardiac myocytes

Thomas W. Weiss, Walter S. Speidl, Christoph Kaun, Gersina Rega, Christopher Springer, Karin Macfelda, Udo M. Losert, Susan L. Grant, Martin L. Marro, Andrew D. Rhodes, Alexander Fuernkranz, Jan Bialy, Robert Ullrich, Philipp Holzmann, Richard Pacher, Gerald Maurer, Kurt Huber, Johann Wojta

*Department of Internal Medicine II, University of Vienna, Waehringer Guertel 18–20, A-1090 Vienna, Austria
"Department of Biomedical Research, University of Vienna, Vienna, Austria
"The Rotary Bone Marrow Research Laboratories, Royal Melbourne Hospital, Parkville, Victoria, Australia
"Department of Gene Expression and Protein Biochemistry, GlaxoSmithKline R&D, Stevenage, Herts, UK
"Department of Clinical Pathology, University of Vienna, Vienna, Austria
"Department of Cardio Thoracic Surgery, University of Vienna, Vienna, Austria

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Abstract

Objective: In murine and rat cardiac myocytes the gp130 system transduces survival as well as hypertrophic signals and via induction of the expression of the potent angiogenic factor VEGF in these cells also indirectly contributes to cardiac repair processes through the development of new blood vessels. There are, however, species differences in receptor specificity and receptor crossreactivity in the gp130–gp130 ligand system. We asked whether gp130 signaling is also involved in the regulation of VEGF in human cardiac myocytes and if so which gp130 ligands are critical for such an effect.

Methods: Human adult cardiac myocytes (HACMs) were isolated from myocardial tissue and characterised by positive staining for myocardial actin, troponin-I and cardiotin. HACMs were treated with the gp130 ligands CT-1, IL-6, LIF or OSM and VEGF-1 was determined by a specific ELISA in the conditioned media of these cells. RT-PCR and Western blot analysis was used in order to detect gp130, IL-6-receptor, LIF-receptor or OSM-receptor specific protein and mRNA in human adult cardiac myocytes and for detection of VEGF-1 specific mRNA in cardiac myocytes after incubation with OSM. Pieces of myocardial tissue were incubated ex vivo in the presence and absence of OSM and VEGF was determined in supernatants of these cultures and immunohistochemistry was performed on the tissue using specific antibodies for VEGF-1. Immunohistochemistry was also employed to detect VEGF in sections from a healthy human heart and in a heart from a patient suffering from acute myocarditis.

Results: OSM, but not CT-1, IL-6 or LIF increased VEGF-1 production in human adult cardiac myocytes dose-dependently derived from five different donors. This selective stimulation of VEGF by gp130 ligands was also reflected by a specific receptor expression on these cells. We detected high levels of mRNA for gp130 and the OSM receptor in freshly isolated human cardiac myocytes but only low amounts of mRNA for the IL-6 receptor whereas mRNA for the LIF receptor was hardly detectable by RT-PCR. OSM receptor and IL-6 receptor were also detectable by Western blotting whereas LIF receptor was only present as a faint band. OSM also increased the expression of VEGF-1 mRNA in cardiac myocytes. When pieces of human myocardial tissue were incubated with the gp130 ligands in an ex vivo model only OSM resulted in an increase in VEGF-1 in the supernatants of these cultures. Furthermore, VEGF increased in tissue samples treated with OSM in cardiac myocytes as evidenced by immunohistochemistry. In addition, we found increased VEGF-1 expression in myocardial tissue from a patient suffering from acute myocarditis. Conclusion: The gp130–gp130 ligand system is also involved in VEGF regulation in human cardiac myocytes and OSM is the gp130 ligand responsible for this effect in the human system whereas LIF and CT-1 which had been shown to regulate VEGF expression in mouse and rat cardiac myocytes had no effect. Thus we have added OSM, which is produced by activated T lymphocytes and monocytes, to the list of regulatory molecules of VEGF production

*Corresponding author. Tel.: +43-1-40400-2247; fax: +43-1-40400-4216.
E-mail address: johann.wojta@univie.ac.at (J. Wojta).

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in the human heart. Our results lend further support to the notion that besides hypoxia, inflammation via induction of VEGF through autocrine or paracrine pathways plays a key role in (re)vascularisation of the myocardium.

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Keywords: Cell communication; Cytokines; Myocytes; Receptors; Signal transduction

1. Introduction

Glycoprotein (gp) 130 is a common receptor subunit for cytokines of the interleukin-6 (IL-6) family that also includes IL-11, leukemia inhibitory factor (LIF), cardiotrophin-1 (CT-1), ciliary neurotrophic factor and oncostatin M (OSM) [1]. Ligand binding to the receptor leads to activation of the janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway that is critically involved in regulating cell proliferation, differentiation and cell survival as well as in regeneration and remodelling of various tissues [2–5]. In cardiac myocytes gp130 signaling is induced by mechanical stretch and hypoxia and transduces survival as well as hypertrophic signals [6–9]. The essential role of gp130 in maintaining normal cardiac function is underlined by the finding that mice lacking gp130 in the myocardium developed stress induced heart failure and apoptosis in response to pressure overload [10].

Angiogenesis has been shown to be essential for cardiac adaption, remodelling and regeneration [11–13]. Recently it has been demonstrated that vascular endothelial growth factor (VEGF)—a potent angiogenic factor—is upregulated in cardiac myocytes by gp130 activation [14]. In mice with cardiac myocyte specific deletion of the VEGF gene normal cardiac function was significantly impaired [15]. Other studies have demonstrated that VEGF mRNA is constitutively expressed in healthy myocardium whereas increased levels of VEGF mRNA have been detected after transient ischaemic insult [16]. A significant increase of VEGF serum levels was shown after myocardial infarction [17]. These data stress the critical role of VEGF in the maintenance of physiological cardiac function as well as in the regulation of repair processes after injury. This notion is further supported by the fact that VEGF expression in cardiac myocytes is highly regulated by a multitude of stimuli including stretch, hypoxia and inflammatory mediators such as IL-1 and endotoxin [18–22]. Gp130 ligands such as LIF and CT-1 have also been shown to upregulate the expression of VEGF in rat cardiac myocytes in vitro and in mice in vivo [14]. There are, however, significant species-specific differences in receptor selectivity and ligand–receptor crossreactivity in the gp130–gp130 ligand system [23–25]. It was therefore the aim of the present study to investigate possible effects of various gp130 ligands on VEGF expression in human cardiac myocytes and to determine the expression of specific receptors for these ligands on those cells.

2. Methods

2.1. Isolation and cultivation of human adult cardiac myocytes

Primary cultures of human adult cardiac myocytes (HACMs) were prepared from ventricular tissue obtained from donor hearts from patients undergoing heart transplantation by mechanical dispersion of the tissue and separated from fibroblasts by preplating as described recently [26]. This procedure resulted in cell populations of >95% rod-shaped cells with a viability of >90%. HACMs were cultivated in culture flasks that had been coated with fibronectin (Roche, Basel, Switzerland) in M199 containing 10% FCS as well as 100 U/ml penicillin, 100 μg/ml streptomycin, 10 μg/ml transferrin (Sigma, St. Louis, MO, USA) and 10 μg/ml insulin (Sigma) at 37 °C in a humidified atmosphere of 5% CO2–95% air. These cells were either transferred into 24-well plates treated with fibronectin as described above for cytokine experiments or were seeded onto chamber slides (Falcon, Heidelberg, Germany) for immunohistochemical characterisation as described [26]. Fig. 1 shows HACMs stained with a mouse anti-human cardiotin antibody and a goat anti-mouse IgG conjugated with fluorescein isothiocyanate as published recently [26]. Only cultures in which >95% of the cells stained positive for cardiac myocyte markers (troponin I, tropomyosin, cardiotin and myocardial muscle actin) were used in this study. In these cultures contamination with smooth muscle cells, endothelial cells and fibroblasts as judged by staining for smooth muscle actin, vWF and fibroblast specific antigens was <2%.

All human material was obtained and processed according to the recommendations of the hospital’s Ethics Committee and Security Board including informed consent. The recommendations of the hospital’s Ethics Committee and Security Board are in accordance with the Declaration of Helsinki on ethical principles for medical research involving human subjects.

2.2. Treatment of HACMs with cytokines

HACMs were incubated in M199 containing 0.1% bovine serum albumin (BSA, Sigma) for 24 h prior to treatment with the respective cytokine. Thereafter the medium was replaced with fresh M199 containing 0.1% BSA and recombinant human (rh) CT-1, obtained from Calbiochem (La Jolla, CA, USA), rh IL-6, rh LIF, rh OSM
or recombinant murine (rm) CT-1 all obtained from R&D Systems (Minneapolis, MN, USA), respectively, or rm LIF, obtained from Biotrend (Cologne, Germany) were added at the concentrations indicated. After incubation for 24 h, the culture supernatants were collected following removal of cell debris by centrifugation and stored at −70 °C until used. The total cell number of the respective cultures after trypsinisation was counted with a haemocytometer and viability was determined to be >98% by Trypan Blue exclusion.

2.3. VEGF-1 antigen assays

VEGF-1 antigen in conditioned media was determined by specific enzyme-linked immunosorbent assays (ELISAs) using monoclonal antibodies (R&D Systems).

2.4. Polymerase chain reaction

HACMs were incubated in M199 containing 0.1% bovine serum albumin (BSA, Sigma) for 24 h prior to treatment with the respective cytokine. Thereafter the medium was replaced with fresh M199 containing 0.1% BSA in presence or absence of OSM (100 ng/ml). After incubation for 24 h, supernatant was removed and mRNA was isolated using QuickPrep™ Micro mRNA Purification Kit (Amersham Biosciences, Buckinghamshire, UK). RT-PCR was performed using Titan one step RT-PCR System (Roche) according to the manufacturers instructions. The amplification conditions consisted of an initial incubation at 50 °C for 30 min, followed by incubation at 94 °C for 2 min, 30 cycles of 94 °C for 30 s, the respective annealing temperature for 45 s and 68 °C for 1 min, and a final incubation at 68 °C for 7 min. PCR products were analysed by gel-electrophoresis (3% agarose gel, stained with ethidiumbromide). Primers, as shown in Table 1, were designed using the primer3 software (http://www-genome.wi.mit.edu/genome_software/other/primer3.html).

2.5. Immunoprecipitation and Western blotting

Immunoprecipitation and Western blot analysis were performed as described recently [27]. Briefly, 2×10^6 freshly isolated human cardiac myocytes were lysed and the lysates were immunoprecipitated with polyclonal rabbit anti human IL-6 receptor (IL-6R), rabbit anti human LIFR (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and sheep anti human OSMR antibodies, respectively. Immunoprecipitation was performed with Protein-A sepharose (Sigma) and proteins were electrophoresed on 8% SDS–PAGE gels. Receptors were visualised after transfer onto PVDF membranes using primary antibodies described above and secondary anti-rabbit and anti-sheep antibodies labelled with horseradish peroxidase (Dako, Glostrup, Denmark) and enhanced chemoluminescence reagents (ECL) from Amersham Biosciences.
2.6. Real-time PCR

Stimulation and mRNA preparation of HACMs was performed as described above. Real-time PCR was performed using LightCycler RNA Master SYBR Green I (Roche) according to the manufacturer’s instructions. The amplification conditions consisted of an initial incubation at 61 °C for 20 min, followed by incubation at 95 °C for 30 s, 50 cycles of 95 °C for 1 s, 60 °C for 10 s and 72 °C for 10 s, a melting step from 45 to 95 °C increasing 0.1 °C/s Paraffin embedded pieces of healthy human myocardium and a final cooling to 40 °C. Data were analysed using LightCycler software version 3.5 (Roche).

2.7. Ex vivo immunohistochemical determination of VEGF-1 in human myocardial tissue

Small pieces (approximately 0.2 cm³) of fresh myocardial tissue were incubated in M199 containing 0.1% BSA in the presence or absence of 100 ng/ml IL-6, 10⁻⁵ U/ml LIF or 100 ng/ml OSM for 24 h at 37 °C. Thereafter culture supernatants were collected following removal of cell debris by centrifugation and stored at −70 °C until used for VEGF determination. The tissue was rinsed with PBS and frozen sections were prepared in Tissue Tek™ OCT (Sakura Finetechnical, Tokyo, Japan) in liquid nitrogen. Sections (4 μm) were cut and fixed with cold acetone (4 °C). To inhibit endogenous peroxidase (POX), POX-block was performed with periodic acid. After incubation with normal goat serum (dilution 1:100; Dako) a monoclonal anti VEGF-1 antibody (R&D Systems) at a concentration of 10 μg/ml was added and the sections were incubated for 1 h at room temperature. Thereafter a biotinylated antibody (dilution 1:100; Dako) was added for 1 h. After rinsing with PBS the sections were incubated with a streptavidin–POX complex (dilution 1:100; Dako) 30 min at room temperature. VEGF-1 was visualised by addition of DAB (diaminobenzidine; Dako) for 20 min. Counterstaining was performed with haematoxylin (Merck, Darmstadt, Germany).

2.8. Immunohistochemical determination of VEGF-1 in healthy human myocardial tissue and in human myocardial tissue with acute inflammation

Paraffin embedded pieces of healthy human myocardium obtained from a donor heart obtained from an accident victim that was unsuitable for transplantation and paraffin embedded pieces of myocardium obtained by biopsy from a patient suffering from acute myocarditis were subjected to immunohistochemical staining for VEGF-1 as described above. This material was obtained and processed according to the recommendations of the hospital’s Ethics Committee and Security Board including informed consent.

2.9. Statistical analysis

Data were compared statistically by ANOVA. Values of \( P<0.05 \) were considered significant.

### Table 1

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Annealing temperature (°C)</th>
<th>Fwd-Primer (corresponding position)</th>
<th>Rev-Primer (corresponding position)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>gp130</td>
<td>60</td>
<td>5’-gag gtt tga gta gga tgg tgg-3’ (681–702)</td>
<td>5’-gct gca tct gat ttg cca ac-3’ (1490–1510)</td>
<td>829</td>
</tr>
<tr>
<td>IL-6R</td>
<td>60</td>
<td>5’-cat tgc cat tgt tct gag gtt-3’ (1580–1601)</td>
<td>5’-agt agt ctg tat tgc tga tgt-3’ (1830–1851)</td>
<td>271</td>
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<tr>
<td>LIFR</td>
<td>50</td>
<td>5’-gaa aac tgt aaa gca tta ca-3’ (2782–2802)</td>
<td>5’-aga gtc tgg aga cac taa-3’ (3285–3303)</td>
<td>521</td>
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<tr>
<td>OsMR</td>
<td>60</td>
<td>5’-gac act gcc ttg ggg tgg tc-3’ (1151–1171)</td>
<td>5’-cat ctc cag gtt ggg gtt tc-3’ (2027–2047)</td>
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<tr>
<td>VEGFα</td>
<td>55</td>
<td>5’-gga cat ctt cca gga gta-3’ (232–250)</td>
<td>5’-tgc aac gcc agt ctg tgt-3’ (555–573)</td>
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<td>GAPDH</td>
<td>60</td>
<td>5’-aca gtc cat gcc atc act gcc-3’ (604–625)</td>
<td>5’-gcc tgc ttc acc acc ttc ttg-3’ (869–890)</td>
<td>286</td>
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</tbody>
</table>

Primers were designed using the PRIMER3 software (http://www-genome.wi.mit.edu/genome_software/other/primer3.html).

*VEGF isoforms 165 and 189 are detected by these primers.
Table 2
Effect of gp130 ligands CT-1, IL-6, LIF and OSM on VEGF-1 production in human adult cardiac myocytes (HACMs) isolated from five different donors

<table>
<thead>
<tr>
<th></th>
<th>Donor 1</th>
<th>Donor 2</th>
<th>Donor 3</th>
<th>Donor 4</th>
<th>Donor 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>368.3±48.2</td>
<td>210.0±40.2</td>
<td>482.5±65.9</td>
<td>262.3±15.8</td>
<td>367.5±63.6</td>
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<tr>
<td>CT-1 (100 ng/ml)</td>
<td>327.5±48.7</td>
<td>N.d.</td>
<td>N.d.</td>
<td>N.d.</td>
<td>405.5±56.8</td>
</tr>
<tr>
<td>IL-6 (100 ng/ml)</td>
<td>399.9±46.6</td>
<td>222.0±20.6</td>
<td>482.5±14.7</td>
<td>301.5±17.4</td>
<td>363.1±60.7</td>
</tr>
<tr>
<td>LIF (10^4 U/ml)</td>
<td>356.3±23.5</td>
<td>169.2±52.1</td>
<td>419.5±12.9</td>
<td>223.6±30.6</td>
<td>412.6±63.2</td>
</tr>
<tr>
<td>OSM (100 ng/ml)</td>
<td>1271.3±163.4</td>
<td>1320.5±95.6</td>
<td>2750.3±112.9</td>
<td>724.7±82.5</td>
<td>967.9±25.4</td>
</tr>
</tbody>
</table>

Confluent monolayers of HACMs, isolated from five different donors (donors 1, 2, 4 and 5: explanted hearts from patients suffering from ischemic cardiomyopathy, donor 3: healthy donor heart, unsuitable for transplantation), were incubated for 24 h in the absence or presence of the respective cytokine at concentrations indicated. Conditioned media of such treated cells were collected and VEGF-1 antigen was determined as described in Methods. Values are given in pg/10^4 cells/24 h and represent mean values±S.D. of three independent determinations. N.d., not determined.

discrepancy we incubated HACMs with murine CT-1 (100 ng/ml) and murine LIF (104 U/ml). However, in contrast to human OSM and similar to human LIF and CT-1 used at concentrations described above, these murine gp130 ligands had no effect on VEGF production in human cardiac myocytes (control: 461.5±51.3 pg/10^4 cells/24 h; human OSM: 1711.4±299.8 pg/10^4 cells/24 h; human CT-1: 423.6±47.9 pg/10^4 cells/24 h; human LIF: 456.7±33.4 pg/10^4 cells/24 h; murine CT-1: 419.6±42.6 pg/10^4 cells/24 h; murine LIF: 375.6±31.8 pg/10^4 cells/24 h).

In order to address the different effects of these gp130 ligands on VEGF expression in HACM, RT-PCR using specific primers for gp130, IL-6R, LIFR and OSMR was performed. As shown in Fig. 2A freshly isolated HACM express significant amounts of mRNA specific for gp130 and OSMR, but low levels of IL-6R specific mRNA and barely detectable levels of LIFR specific mRNA. Fig. 2B shows replicates of RNA samples shown in Fig. 2A subjected to RT-PCR using specific primers for GAPDH. Corresponding to the results of RT-PCR presented in Fig. 2A Western blot analysis shown in Fig. 2C revealed detectable amounts of IL-6R and OSMR on the protein level as evidenced by bands at the expected molecular masses of approximately 80 000 and 180 000 Da, respectively, whereas no clear band was visible after immunoprecipitation with anti LIFR antibodies at the expected molecular mass of 190 000 Da. No bands were visible when immunoprecipitation was performed with preimmune rabbit or sheep antibodies, respectively (data not shown).

When HACMs were treated with increasing concentrations of OSM (0.1 pg/ml to 100 ng/ml) for 24 h a dose dependent increase in VEGF-1 production by these cells was observed with maximum effects reached at concentrations between 10 and 100 ng/ml OSM (Fig. 3). In order to test a specific interaction of OSM with its receptor, HACMs were pretreated with the aptamer ADR58 (1 μM), which interferes with OSM binding to its receptor as described by Rhodes et al. [28], with a OSM-neutralising polyclonal goat antibody (AB-295-NA, 1 μg/ml; R&D Systems), or with a nonimmune polyclonal goat antibody (1 μg/ml; Dako) for 1 h at 37 °C and incubated for 4 or 8 h in the presence or absence of 1 ng/ml OSM. VEGF-mRNA was quantitated after 4 h in these cells by real-time PCR. The increase in VEGF specific mRNA after treatment of HACM with OSM was reduced by 67% in the presence of aptamer ADR58 and by 98% in the presence of the neutralising anti-OSM antibody. As can be seen from Fig. 4 after 8 h the neutralising antibody also...
abolished the effect of OSM on VEGF protein production whereas the nonimmune goat antibody had no effect.

It is known that gp130–gp130 ligand interaction activates JAK–STAT, mitogen activated protein kinase (MAPK) and the phosphatidylinositol 3 kinase (PI3 K)–Akt pathways [29,30]. In order to investigate which of these pathways was involved in OSM induced VEGF expression we preincubated HACMs for 30 min with AG-490, a specific JAK-inhibitor, with LY294002, a specific PI3K inhibitor, with PD98059, a specific MEK inhibitor and with SB202190, a specific inhibitor of p38–Akt. Thereafter medium was removed and fresh M199 with or without OSM at a concentration of 100 ng/ml was added and the cells were incubated for 24 h. As can be seen from Fig. 5 treatment of HACM with AG-490 at a concentration of 2 μM abolished the effect of OSM on VEGF production. In contrast neither LY294002 nor PD98059, used at a concentration of 100 μM, nor SB202190, used at a concentration of 20 μM, affected the OSM-induced expression of VEGF in these cells. SB202190 could not be used at 100 μM because of cytotoxicity as determined by Trypan Blue exclusion (control: 401.5±51.3 pg/10⁴ cells/24 h; OSM: 1711.4±299.8 pg/10⁴ cells/24 h; LY294002+OSM: 1542.0±92.5 pg/10⁴ cells/24 h; PD98059+OSM: 1499.9±178.2 pg/10⁴ cells/24 h; SB202190+OSM: 1526.3±366.4 pg/10⁴ cells/24 h). These data indicate that OSM upregulates VEGF production in HACMs via activation of the JAK–STAT pathway.

The effect of OSM on VEGF expression was also evident on the level of specific mRNA expression as demonstrated by real-time PCR using specific primers for VEGF-1. As can be seen from Fig. 6, OSM increased VEGF-1 specific mRNA levels in HACMs approximately four-fold over control.

When pieces of fresh myocardial tissue were incubated with OSM, an increase in VEGF in the supernatants of these cultures was seen, whereas IL-6 and LIF had no effect (Fig. 7). Immunohistochemistry with anti VEGF-1 antibodies revealed an increase in VEGF-1 in cardiac myocytes treated with OSM as evidenced by the more
Fig. 5. JAK inhibitor AG-490 abolishes the VEGF-inducing effect of OSM in HACMs. Confluent monolayers of HACMs isolated from a single donor were preincubated with the specific JAK-inhibitor AG-490 (2 μM) or without any addition for 30 min. Thereafter the medium was removed and fresh M199 with or without OSM (100 ng/ml) was added. Conditioned media of such treated cells were collected after 24 h and VEGF-1 was determined as described in Methods. Values are given in pg/10^4 cells/24 h and represent mean values±S.D. of three independent determinations. Experiments were performed three times with HACM isolated from three different donors. A representative experiment is shown.

intense and extensive staining (Fig. 7). When healthy myocardial tissue and myocardial tissue from a patient suffering from acute myocarditis was subjected to immunohistochemistry analysis for VEGF-1, a more intense staining for this protein was evident in the diseased heart (Fig. 8).

4. Discussion

The essential role of the gp130–gp130 ligand system for maintaining normal cardiac function is emphasised by studies demonstrating that in cardiac myocytes gp130 signalling transduces survival as well as hypertrophic signals [8,9]. This notion is further supported by findings that mice lacking gp130 in the myocardium developed stress-induced heart failure and apoptosis in response to pressure overload and that targeted disruption of the gp130 gene leads to impaired development of the myocardium [10,31]. In addition to this direct role on cardiac function, the gp130–gp130 ligand system has been linked to angiogenesis in the heart. It was shown that vascular endothelial growth factor (VEGF)—a potent angiogenic factor—is upregulated in cardiac myocytes by gp130 activation [14]. In that respect it should be pointed out that in mice with cardiac myocyte specific deletion of the VEGF gene normal cardiac function was significantly impaired [15]. Thus—since angiogenesis is known to be essential for cardiac development, adaption, remodelling and regeneration—these findings provide evidence for an indirect role for gp130 in regulating these processes via the induction of VEGF [11–13].

These studies, however, were performed in vivo in mice or using rat cardiac myocytes in vitro. Given the significant species-specific differences in receptor selectivity and ligand–receptor cross reactivity in the gp130–gp130 ligand system (e.g. in the mouse LIF signals exclusively through gp130–LIF receptor heterodimers and OSM signals exclusively through gp130–OSM receptor heterodimers whereas in humans OSM can signal through gp130–LIF receptor and gp130–OSM receptor heterodimeric and LIF uses exclusively gp130–LIF receptor heterodimers) we have in this paper addressed the question whether gp130 signalling is also involved in regulating VEGF expression in human adult cardiac myocytes [23–25].

Using human adult cardiac myocytes isolated from five different hearts we were—at least to our knowledge—for the first time able to demonstrate that human OSM increased the production of VEGF-1 in these cells significantly. In contrast neither human CT-1, nor human IL-6 nor human LIF, three other gp130 ligands, showed an
all receptors tested. The highest levels, however, were found for gp130 and the OSM receptor, with low levels for the IL-6 receptor whereas mRNA specific for the LIF receptor was hardly detectable. Correspondingly using immunoprecipitation with specific antibodies followed by Western blot analysis we could show that freshly isolated human adult cardiac myocytes express detectable levels of IL-6 receptor and OSM receptor whereas no clear band was detectable at the expected molecular mass of the LIF receptor. It should be emphasised that CT-1 as LIF signals through the LIF receptor but not through the OSM receptor [23,32,33]. Thus the selective induction of VEGF by OSM in human cardiac myocytes could be brought about by the differential receptor expression in these cells. The fact that the aptamer ADR58, which had been shown to block the interaction of human OSM with its receptor as well as a neutralising anti-OSM antibody abolished the stimulating effect of OSM on VEGF production supports the view that this effect is brought about by a specific interaction of OSM with its receptor [28]. The effect of OSM on VEGF expression, which was concentration dependent with maximum stimulation achieved between 10 and 100 ng/ml of OSM, was mediated via activation of the JAK–STAT pathway because it was blocked by A-490. Ligand binding to gp130 is also known to activate MAPK and the PI3K–akt pathways [29,30]. However, specific inhibitors of these pathways did not affect the OSM induced production of VEGF by human cardiac myocytes. In correlation with data obtained on the level of protein, OSM also induced VEGF-1 specific mRNA as demonstrated by RT-PCR. Note that the brown staining for VEGF-1 is predominantly located within myocardial tissue and in tissue treated with IL-6 or LIF. Examples of VEGF-1 positive cardiac myocytes are highlighted by black arrows. An isotype-matched control is shown in the insert to (A). Experiments were performed three times with tissue obtained from three different donors. A representative experiment is shown.

Fig. 7. Effect of OSM on VEGF-1 expression in human myocardial tissue: Upper panel: pieces of fresh myocardial tissue were incubated in the absence (control) or presence of 100 ng/ml IL-6, 10^4 U/ml LIF or 100 ng/ml OSM for 24 h at 37 °C and VEGF-1 was determined in the supernatants as described in Methods. Values are given in pg/mg tissue/24 h and represent mean values±S.D. of three independent determinations. Experiments were performed twice with tissue obtained from two different donors. A representative experiment is shown. *, P<0.01. Next page: pieces of fresh myocardial tissue were incubated in the absence (A) or presence of 100 ng/ml IL-6 (B), 10^4 U/ml LIF (C) or 100 ng/ml OSM (D) for 24 h at 37 °C and VEGF-1 was visualised as described in Methods. Note that the brown staining for VEGF-1 is predominantly located within cardiac myocytes after OSM treatment and is hardly visible in untreated tissue and in tissue treated with IL-6 or LIF. Examples of VEGF-1 positive cardiac myocytes are highlighted by black arrows. An isotype-matched control is shown in the insert to (A). Experiments were performed three times with tissue obtained from three different donors. A representative experiment is shown.

Effect on VEGF in human cardiac myocytes. It should be emphasised that in rat cardiac myocytes and in murine hearts CT-1 and LIF upregulated the expression of VEGF whereas IL-6 also had no effect [14]. We could also show here that murine CT-1 and murine LIF had no effect on VEGF expression in human cardiac myocytes. As indicated above, no data are yet available on the effect of OSM on VEGF in cardiac myocytes either in the rat or in the human system. In order to further investigate this selective stimulation of VEGF expression in human cardiac myocytes we performed quantitative RT-PCR using specific primers for human gp130 and the human receptors for IL-6, LIF and OSM. Employing this method we were able to demonstrate that freshly isolated primary cultures of human adult cardiac myocytes express specific mRNA for VEGF.
Fig. 7. (continued)
Fig. 8. VEGF-1 expression in healthy human myocardial tissue and in human myocardial tissue obtained from a patient suffering from acute myocarditis: VEGF-1 was visualised as described in Methods. As compared to the healthy myocardium (A) more intense and extensive VEGF-1 staining in cardiac myocytes is visible throughout the tissue obtained from the patient suffering from acute myocarditis (B). Examples of VEGF-1 positive cardiac myocytes are highlighted by black arrows.

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