Interleukin-6 Family of Cytokines Mediate Angiotensin II-induced Cardiac Hypertrophy in Rodent Cardiomyocytes*

Received for publication, April 12, 2000, and in revised form, June 6, 2000
Published, JBC Papers in Press, June 7, 2000, DOI 10.1074/jbc.M003128200

Motoaki Sano‡, Keiichi Fukuda‡§, Hiroaki Kodama‡, Jing Pan‡, Mikiyoshi Saito¶, Junichi Matsuzaki‡, Toshiyuki Takahashi‡, Shinji Makino‡, Takahiro Kato‡, and Satoshi Ogawa‡

From the ¶Cardiopulmonary Division, Department of Internal Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582 and €Chugai Pharmaceutical Co. Ltd., 1-135 Komakado, Gotenba, Shizuoka 412-8513, Japan

This study was designed to investigate whether angiotensin II induces the interleukin (IL)-6 family of cytokines in cardiac fibroblasts and, if so, whether these cytokines can augment cardiac hypertrophy. Angiotensin II increased IL-6, leukemia inhibitory factor (LIF) and cardiotrophin-1 mRNA by 6.5-, 10.2-, and 2.0-fold, respectively, but did not affect IL-11, ciliary neurotrophic factor, or oncostatin M in cardiac fibroblasts. Enzyme-linked immunosorbent assay revealed that angiotensin II-stimulated conditioned medium from cardiac fibroblasts contained 9.3 ng/ml IL-6 at 24 h, which was 24-fold higher than the control. It phosphorylated gp130 and STAT3 in cardiomyocytes, which was reduced with RX435 (anti-gp130 blocking antibody). It increased [3H]phenylalanine uptake and cell area by 44% and 86% in cardiomyocytes compared with mock medium. RX435 suppressed these increases by 26% and 38%, while TAK044 (endothelin-A/B-R blocker) suppressed them by 52% and 52%, respectively. Antisense oligonucleotides against LIF and cardiotrophin-1 blocked their up-regulation, and attenuated the conditioned medium-induced increase in [3H]phenylalanine uptake by 21% and 13%, respectively. The combination of antisense oligonucleotides to LIF and cardiotrophin-1 decreased their uptake by 33%. These results indicated that angiotensin II induced IL-6, LIF, and cardiotrophin-1 in cardiac fibroblasts, and that these cytokines, particularly LIF and cardiotrophin-1, activated gp130-linked signaling and contributed to angiotensin II-induced cardiomyocyte hypertrophy.

Both the circulating and local renin-angiotensin system play an important role in the progression of cardiac remodeling (1–3). Angiotensin II (AngII) causes cardiomyocyte hypertrophy and cardiac fibroblast proliferation concomitant with deposition of extracellular matrix, both actions being mediated by the AT-1 receptors (4). Cardiac fibroblasts have been clearly shown to express AT-1 receptors (5–7), but the presence of AT-1 receptors on cardiomyocytes remains controversial. AT-1 receptor mRNA levels were found to be markedly higher in cardiac fibroblasts than cardiomyocytes from neonatal rats (8). Binding experiments using 125I-AngII revealed that the specific binding observed on cardiomyocytes was <10% of the specific binding obtained in a comparable number of fibroblasts (8). Although there is a disparity in distribution of AT-1 receptor between cardiac fibroblasts and cardiomyocytes, ACE inhibitors or AT-1 receptor antagonists may not only prevent cardiac fibrosis but also cardiomyocyte hypertrophy. These discrepancies may be explained by the potential involvement of a paracrine mechanism between cardiomyocytes and cardiac fibroblasts in the hypertrophic effect of AngII. AngII did not stimulate hypertrophy in pure cardiomyocyte cultures, but did induce an increase in cell size and augmentation of atrial matriuretic peptide and B-type natriuretic peptide secretion in co-cultured cardiomyocytes and cardiac fibroblasts (9).

AngII has been reported to stimulate autocrine/paracrine release of other growth factors such as platelet-derived growth factor, TGF-β1, insulin-like growth factor-1, and endothelin-1 in various cell types (10–12) These growth factors may in part indirectly mediate the physiological effects of AngII. In the case of cardiac fibroblasts, AngII has been shown to stimulate TGF-β1 and ET-1 production (13–15). TGF-β1 has a major influence on extracellular matrix production, particularly collagen and fibronectin. Simultaneous treatment of cardiac fibroblasts with AngII and neutralizing antibody to TGF-β1 has been shown to reduce type I/III collagen mRNA expression. These data suggested that AngII-induced collagen synthesis might be mediated in part by autocrine/paracrine stimulation by TGF-β1.

In rat adrenal zona glomerulosa cells and mouse mesangial cells, AngII was shown to increase IL-6 release, and the growth promoting effects of AngII on mesangial cells is at least partially mediated by IL-6 released from mesangial cells (16, 17). The IL-6 family cytokines includes leukemia inhibitory factor (LIF), cardiotrophin-1 (CT-1), ciliary neurotrophic factor (CNTF), oncostatin M (OSM), and IL-11. This family shares a common co-receptor gp130, and has many similar physiological effects. In the cardiovascular system, all the cytokines of this family have been reported to have a hypertrophic effect on unulated cardiac fibroblasts; UCM, conditioned medium from unstimulated cardiac fibroblasts; MCM, mock conditioned medium; MOPS, 4-morpholinepropanesulfonic acid; ACE, angiotensin-converting enzyme.
cardiomyocytes, although with different potencies (18, 19). Among these, CT-1 and LIF have a potent hypertrophic effect on cardiomyocytes. Based on these findings, we hypothesized that AngII induced secretion of IL-6 family cytokines from cardiac fibroblasts, which in turn contributes to cardiac hypertrophy by a paracrine mechanism. Here we report that cross-talk between cardiomyocytes and cardiac fibroblasts via the IL-6 family cytokines, mainly LIF and CT-1, plays a critical role in AngII-induced cardiomyocyte hypertrophy.

MATERIALS AND METHODS

Cell Culture—Primary cultures of cardiomyocytes were prepared from the ventricles of neonatal Wistar rats or ICR mice as described (20). Cardiomyocytes and cardiac nonmyocytes were separately prepared by differential adhesiveness. Attached cells (mostly cardiac fibroblasts) were subcultured two times to deplete residual cardiomyocytes, and the third passage cells were used. The murine cells were used in RT-PCR for OSM and IL-11, and in some experiments, since anti-gp130 antibody RX435 specifically blocks murine gp130.

Preparation of Fibroblast Conditioned Medium—Cardiac fibroblasts were grown to confluence, and incubated in the serum-free medium for 24 h. The medium was replaced with fresh serum-free medium, and then conditioned medium was cultured either with or without AngII (10^{-7} M). Conditioned medium from AngII-stimulated and unstimulated cardiac fibroblasts (AngII-CM, UCM) was collected. Mock conditioned medium (MCM) was generated by adding the serum-free medium to dishes without cells.

Immunoprecipitation and Western Blot Analysis—Polyclonal anti-STAT3 (signal transducer and activator of transcription-5) and anti-gp130 antibodies were purchased from Santa Cruz Biotechnology. Monoclonal antibody to phosphotyrosine (4G10) was purchased from Upstate Biotechnology Inc. (Lake Placid, NY). Cardiomyocytes, stimulated, or AngII, or conditioned medium, were lysed at 5 min. Immunoprecipitation-Western blot analysis was performed as described (20). The membranes were incubated with anti-phosphotyrosine antibody for 2 h at 23 °C, and incubated with peroxidase-conjugated goat anti-mouse IgG. Signals were visualized by ECL (Amersham Pharmacia Biotech).

RNA Extraction and Northern Blot Analysis—Total RNA was extracted using TRIzol Reagent (Life Technologies, Inc.), and poly(A)+ RNA was isolated. Rat IL-6, LIF, and CT-1 cDNA were obtained by RT-PCR from the heart RNA and cloned into the pCR II plasmid (21). Monoclonal antibody to phosphotyrosine (4G10) was purchased from Upstate Biotechnology Inc. (Lake Placid, NY). Cardiomyocytes, stimulated, or AngII, or conditioned medium, were lysed at 5 min. Immunoprecipitation-Western blot analysis was performed as described (20). The membranes were incubated with anti-phosphotyrosine antibody for 2 h at 23 °C, and incubated with peroxidase-conjugated goat anti-mouse IgG. Signals were visualized by ECL (Amersham Pharmacia Biotech).

RESULTS

AngII Increased IL-6, LIF, and CT-1 mRNA Expression in Cardiac Fibroblasts—To demonstrate whether AngII induces the expression of IL-6 family cytokines in cardiac fibroblasts, we performed poly(A)+ RNA Northern blot analysis for IL-6, LIF, and CT-1 (Fig. 1), and examined quantitative RT-PCR analysis to detect mRNA expression of CNTF, OSM, and IL-11. AngII augmented IL-6, LIF, and CT-1 mRNA expression by 6.5-, 10.2-, and 2.0-fold, respectively, at 30 min. The expressions of IL-6 and LIF mRNA were gradually decreased, but were still elevated at 2 h. Although basal expression of CT-1 mRNA was higher than those of IL-6 and LIF, its increase by AngII was lower than those of IL-6 and LIF, and it returned to the control level at 1 h. CNTF expression was unaffected (data not shown). IL-11 and OSM mRNA could be detected by RT-PCR, but levels were too low to quantify (data not shown). Next, we preincubated the cells with either CV11974 or PD123319 (AT2 receptor blocker), stimulated with AngII, and detected the expression of these cytokines at 30 min. Up-regulation of IL-6, CT-1, and LIF was mediated not by AT2 receptor but by AT1 receptor (data not shown).

AngII Augmented IL-6 Production in Cardiac Fibroblasts—To confirm that AngII induced the production of these cytokines in cardiac fibroblasts, we also measured the protein levels of these cytokines. ELISA showed that cardiac fibroblasts secreted basal levels of IL-6 protein in the unstimulated condition. As shown in Fig. 2, AngII significantly increased IL-6 secretion in a time-dependent manner. IL-6 content in the UCM and AngII-CM at 2 h were 0.04 ± 0.02 and 0.07 ± 0.03 pg/ml. IL-6 concentration in the conditioned media was increased with AngII by 24.0-fold (p < 0.01) at 24 h, and was 9.3 ± 2.9 ng/ml.

AngII-stimulated Cardiac Fibroblast-conditioned Medium Phosphorylated gp130 in Cardiomyocytes—Ligand binding is
known to induce tyrosine phosphorylation of co-receptor gp130. To determine whether the secreted cytokines can transduce signals in cardiomyocytes, we stimulated cardiomyocytes with conditioned media for 5 min and detected the phosphorylation of gp130. AngII-CM significantly increased the tyrosine phosphorylation of gp130, and its phosphorylation increased in a conditioning time-dependent manner, while UCM did not (Fig. 3). We also confirmed that AngII itself did not phosphorylate gp130 at this time (data not shown), indicating AngII does not directly stimulate gp130.

**Phosphorylation of STAT3 by AngII-stimulated Fibroblast-conditioned Medium Was Mediated by gp130 Receptor**—The signaling pathways downstream of gp130 are reported to consist of two distinct pathways, the Janus kinase/signal transducer(s) and activator(s) of transcription (JAK/STAT) pathway and the mitogen-activated protein kinase pathway (20, 25–27). The signaling through STAT3 was considered to be specific to this cytokine family. To determine whether the conditioned media can induce the tyrosine phosphorylation of STAT3, we stimulated murine cardiomyocytes for 5 min with conditioned media. Only 2 h of AngII-stimulated conditioned media was sufficient to phosphorylate STAT3 in cardiomyocytes, and phosphorylation of STAT3 increased in a conditioning time-dependent manner (Fig. 4A). However, unstimulated media conditioned for up to 12 h could not stimulate STAT3 phosphorylation.

To confirm that this phosphorylation was not caused by AngII itself, we preincubated the cardiomyocytes with CV11974 for 1 h, and then stimulated the conditioned medium. Pre-
treatment with CV11974 did not inhibit the phosphorylation, indicating that it was not caused by AngII (Fig. 4B).

To further investigate the cause of this phosphorylation, we preincubated the cells with RX435 for 1 h, and stimulated the cells. Interestingly, RX435 strongly inhibited the phosphorylation of STAT3 (Fig. 4C). These results were reproducible in four separate experiments. Densitometric analysis revealed that the pretreatment of RX435 inhibited the phosphorylation of gp130 and STAT3, and that most of these factors were IL-6 family cytokines.

Contribution of Paracrine-secreted IL-6 Family Cytokines to Increase in Cardiomyocyte Protein Synthesis—Given the observation that paracrine-secreted IL-6 family cytokines activated gp130-related signaling in cardiomyocytes, we considered that the hypertrophic effects of AngII on cardiomyocytes were partially mediated by these cytokines. We stimulated the cells with the 24-h conditioned medium, and measured [3H]Phe uptake. Then, we assessed the role of these cytokines in the conditioned medium-induced increase in [3H]Phe uptake by using RX435.

Murine cardiomyocytes were stimulated with MCM, UCM, or AngII-CM in the presence of CV11974. The MCM with AngII did not significantly increase the [3H]Phe uptake compared with the MCM alone, suggesting that “carried-over” AngII in the conditioned medium was completely blocked by CV11974. The UCM and AngII-CM increased [3H]Phe uptake by 31% and 44% more than MCM, respectively (Fig. 5A). These results indicated that fibroblast-conditioned medium contained paracrine hypertrophic growth factors, and that AngII augmented their secretion.

To determine the contribution of these cytokines, cardiomyocytes were pretreated with RX435. To compare the actions of these cytokines with paracrine-secreted endothelin-1, the effect of TAK044 was also examined. RX435 and TAK044 suppressed the AngII-CM-induced increase in [3H]Phe uptake by 26.2% and 51.7%, respectively (Fig. 5B). The combination of TAK044 and RX435 suppressed this increase by 64.6%. The inhibitory effect of RX435 in the absence of CV11974 was equivalent to that in the presence of CV11974. These results suggested that the contribution of the IL-6 family cytokines to the conditioned medium-induced increase in [3H]Phe uptake by using RX435 was approximately 50% of endothelin-1.

Effect of Paracrine-secreted IL-6 Family Cytokines on Cardiomyocyte Size—To investigate whether these paracrine factors can increase cardiomyocyte size, cardiomyocytes were pretreated with TAK044 or RX435 and stimulated with the MCM or AngII-CM in the presence of CV11974 for 60 min. Cells were fixed and stained with anti-myosin antibody, and cell area was measured. *, p < 0.01; **, p < 0.05.
and stimulated with the conditioned medium in the presence of CV11974. RX435 inhibited the conditioned medium-induced increase in cell area by 38%, whereas TAK044 inhibited it by 52% (Fig. 6). These findings revealed that this cytokine family plays a pivotal role in AngII-induced cardiac hypertrophy, although their contribution was weaker than that of ET-1.

Effect of Antisense Oligonucleotides on AngII-induced Up-regulation of Cytokine mRNA Expression in Cardiac Fibroblasts—To block CT-1 and/or LIF expression, we prepared three kinds of AS oligonucleotide against CT-1 and two kinds of AS oligonucleotides against LIF, respectively, and tested their inhibitory effect on mRNA expression (Fig. 7, A and B). Cardiac fibroblasts were pretreated with AS oligonucleotides for 6 h, followed by stimulation with AngII for 1 h. Sense oligonucleotide was used as a control. RT-PCR revealed that two of three AS oligonucleotides to CT-1 (AS-1-CT-1 and AS-2-CT-1) markedly inhibited AngII-induced up-regulation of CT-1 mRNA expression, and that both AS-1-LIF and AS-2-LIF strongly inhibited that of LIF.

Effect of Antisense Oligonucleotides on AngII-stimulated Medium-induced Increase in [3H]Phe Uptake in Cardiomyocytes—To demonstrate whether CT-1 and/or LIF secreted from AngII-stimulated cardiac fibroblasts were involved in the conditioned medium-induced cardiac hypertrophy, CT-1 or LIF production was inhibited by preincubation with specific AS oligonucleotides, and [3H]Phe uptake was measured in cardiomyocytes incubated in AngII-CM containing AS or sense oligonucleotides.

At first, we preincubated cardiac fibroblasts with the AS or sense oligonucleotides, stimulated the cells with AngII for 48 h, and collected the conditioned media. Then, we stimulated the cardiomyocytes with these conditioned media, and measured [3H]Phe uptake (Fig. 7C). AngII-CM containing sense oligonucleotides increased [3H]Phe uptake by 76% compared with the control. Pretreatment with AS-1-CT-1 or AS-2-CT-2, which completely perturbed AngII-induced CT-1 mRNA expression, decreased uptake by 14.2 ± 3.4% and 11.1 ± 5.9%, respectively. However, pretreatment with AS-3, which did not alter CT-1 mRNA expression, did not affect the [3H]Phe uptake. Pretreatment with AS-1-LIF and AS-2-LIF decreased uptake by 23.4 ± 10.8% and 18.7 ± 3.7%, respectively. Moreover, the combination of AS-1-CT-1 and AS-1-LIF decreased uptake by 33.1 ± 4.2%. These findings suggested that IL-6, LIF and CT-1 can all stimulate gp130-mediated [3H]Phe uptake to a various extent.

DISCUSSION

The heart is a syncytium of myocytes and the surrounding support cells, collectively referred to as nonmyocytes, most of which are fibroblasts. Although cardiomyocytes are the most predominant cellular constituent of the heart by mass, cardiac fibroblasts are twice as plentiful as cardiomyocytes. Previous studies revealed that cardiomyocyte hypertrophy was stimulated in cultures by increasing numbers of cardiac fibroblasts, and that this effect of cardiac fibroblasts on cardiac hypertrophy was more pronounced than that of cardiomyocytes.
IL-6 Family Mediates AngII-induced Cardiac Hypertrophy

phy could be reproduced by fibroblast-conditioned medium (28). These findings suggested that cardiac fibroblasts modulate cardiomyocyte growth by production of one or more growth factors (29–31).

A number of clinical and experimental studies revealed that ACE inhibitors and AT-1 receptor antagonists were very effective for preventing cardiac hypertrophy and cardiac remodeling (32, 33). Since cardiomyocytes only express less than 10% of the number of AT-1 receptors on the cardiac fibroblast, it was difficult to explain why cardiomyocytes respond effectively to ACE inhibitors and AT-1R antagonists in this setting. Recent studies have demonstrated that the interaction between cardiomyocytes and cardiac fibroblasts plays an important role during the process of AngII-induced cardiomyocyte hypertrophy (8). Harada et al. (9) reported that AngII did not stimulate hypertrophy in pure cardiomyocyte culture, but that it induced an increase in cell size and augmentation of atrial natriuretic peptide and B-type natriuretic peptide secretion in cardiomyocyte-cardiac fibroblast co-culture. This effect of AngII was inhibited by 55% by ET-A receptor antagonist and AngII-stimulation augmented ET-1 secretion in cardiac fibroblasts, but not in cardiomyocytes. Their findings suggested that the hypertrophic effect of AngII was mediated at least partially via an increased secretion of ET-1 from cardiac fibroblasts. The possibility remains that other unknown paracrine factors from cardiac fibroblasts might play a role in this phenomenon.

In this study, we showed that AngII induced IL-6, LIF, and CT-1 mRNA expression, and confirmed that it augmented IL-6 protein secretion in cardiac fibroblasts. AngII-CM phosphorylated gp130 and STAT3 and increased [3H]Phe uptake and cell area in cardiomyocytes. Pretreatment with RX435 partially inhibited these phosphorylation events and the increase in [3H]Phe uptake and cell area. Preincubation with CT-1 and LIF AS oligonucleotide blocked the up-regulation of CT-1 and LIF mRNA in AngII-stimulated cardiac fibroblasts and attenuated the AngII-CM-induced increase in [3H]Phe uptake. We showed that the IL-6 family of cytokines were another essential autocrine/paracrine factor that mediated AngII-induced cardiac hypertrophy, and that their contribution was as high as that of endothelin-1.

A previous report using smooth muscle cells revealed that AngII could significantly increase the expression of IL-6 mRNA and protein in a dose-dependent manner (10 –10 to 10 mol/liter) (34). We stimulated the cardiac fibroblasts with AngII at a concentration of 10 mol/liter and obtained compelling evidence for AngII-mediated cytokine production. The AngII concentration we used, however, was higher than the accepted in vivo concentration. Sadoshima et al. (2) reported that mechanical stretch causes secretion of AngII from cardiomyocytes to a concentration of 452 ± 56 pmol/liter after 10 min stretch in the stretch-conditioned media, and that these levels were sufficiently high to trigger the hypertrophic response. Yamazaki et al. (36) reported that conditioned media after cardiomyocyte stretch contained approximately 5 × 10 mol/liter AngII. However, since these studies used conditioned media, the autocrine/paracrine-secreted AngII may have been diluted, and the concentration of AngII in vivo might be much higher than these values.

Although poly(A)+ RNA and ELISA revealed that AngII strongly augmented IL-6 production from cardiac fibroblasts, we supposed that IL-6 might not be a critical paracrine hypertrophic factor for cardiomyocytes. Previous studies had reported that IL-6 is not significantly expressed in neonatal cardiomyocytes (37), while gp130 and LIF receptor-β were abundantly expressed. Although we could not directly show the release data of LIF and CT-1 due to the lack of a radioimmunoassay or ELISA for these murine cytokines, the present paper demonstrated that AS oligonucleotides that specifically blocked CT-1 and LIF expression significantly attenuated AngII-CM-induced [3H]Phe uptake. The inhibitory effect of the combination of AS oligonucleotides to LIF and CT-1 on [3H]Phe uptake corresponded to that of RX435. These findings indicated that both LIF and CT-1 were secreted from AngII-stimulated cardiac fibroblasts, and were critically involved in the conditioned medium-induced cardiac hypertrophy. Very recently, Kuwahara et al. (35) revealed that CT-1 secreted from cardiac non-myocytes is significantly involved in hypertrophic changes of cardiac myocytes in co-culture, and suggested that CT-1 is an important local regulator in the process of cardiac hypertrophy. The present study demonstrated that, although the basal expression of CT-1 mRNA was higher than that of LIF, AngII-induced increase in mRNA expression of LIF was much higher than that of CT-1. These findings could easily explain the results that the inhibitory effect of the AS oligonucleotides to LIF on [3H]Phe uptake was bigger than those of AS oligonucleotides to CT-1. Based on these findings, we speculated that both CT-1 and LIF might be critical paracrine hypertrophic growth factors released from cardiac fibroblasts in response to AngII, and that the role of LIF in this paracrine process was as strong or even stronger than that of CT-1.

In conclusion, to our knowledge this is the first report to show that AngII-induced cardiomyocyte hypertrophy was mediated by the cross-talk between cardiomyocytes and cardiac fibroblasts via IL-6 family cytokines, mainly CT-1 and LIF. The discrepancy of the poor existence of AT-1 receptor in cardiomyocytes and the high effectiveness of ACE inhibitors or AT-1R antagonist on cardiac hypertrophy might be easily explained by these cross-talk of the paracrine factors between cardiac fibroblast and cardiomyocytes. Further studies are needed to clarify the precise understanding of paracrine cross-talk between these cells.

Acknowledgment—We acknowledge Kio Nakamaru for technical assistance.

REFERENCES
1. Baker, K. M., Booz, G. W., and Dostal, D. E. (1992) Annu. Rev. Physiol. 54, 227–241
2. Sadoshima, J., Xu, Y., Stayter, H. S., and Izumo, S. (1993) Cell 75, 977–984
3. Lindpaintner, K., and Ganten, D. (1994) Circ. Res. 74, 905–921
4. Sadoshima, J., and Izumo, S. (1990) Circ. Res. 67, 413–422
5. Crabos, M., Roth, M., Hahn, A. W., and Erne, P. (1994) J. Clin. Invest. 93, 2372–2378
6. Schorb, W., Booz, G. W., Dostal, D. E., Conrad, K. M., Chang, C. K., and Baker, S. (1995) Circ. Res. 77, 1245–1254
7. Villarreal, F. J., Kim, N. N., Ungarg, G. D., Printz, M. P., and Dillmann, W. H. (1993) Circulation 88, 2849–2861
8. Kim, N. N., Villarreal, F. J., Printz, M. P., Lee, A. A., and Dillmann, W. H. (1995) Am. J. Physiol. 269, E426–E437
9. Harada, M., Itoh, H., Nakagawa, O., Ogawa, Y., Miyamoto, Y., Kuwahara, K., Ogawa, E., Ito, T., Yamashita, J., Masuda, I., Yoshimasa, T., Tanaka, I., Saito, Y., and Nakao, K. (1997) Circulation 96, 3737–3744
10. Gibbons, G. H., Pratt, R. E., and Ilac, V. J. (1992) J. Clin. Invest. 90, 456–461
11. Delafontaine, P., and Lou, H. (1993) J. Biol. Chem. 268, 16866–16870
12. Inoue, H., Hira, Y., Adachi, S., Tanaka, M., Tsuchin, M., Kuo, A., Nogami, A., Marumo, F., and Hiroe, M. (1993) J. Clin. Invest. 92, 395–403
13. Gray, M. O., Long, C. S., Kalinyak, J. E., Li, H. T., and Kallin, J. S. (1998) Cardiovasc. Res. 40, 35–42
14. Fisher, S. A., and Aisher, M. (1995) Am. J. Physiol. 268, C910–C917
15. Lee, A. A., Dillmann, W. H., McCulloch, A. D., and Villarreal, F. J. (1995) J. Mol. Cell. Cardiol. 27, 227–235
16. Moriyama, Y., Fujihayashi, M., Fujikawa, Y., Kaneko, T., Xia, C., Imai, E., Kamada, T., Ando, A., and Ueda, N. (1995) J. Am. Soc. Nephrol. 6, 95–101
17. Judd, A. M., and MacLeod, R. M. (1994) Endocrinology 135, 1245–1254
18. Pennica, D., King, K. L., Shaw, K. J., Luiz, R., Rullamas, J., Luoh, S. M., Darbonne, W. C., Knutson, D. S., Yen, R., Chien, K. R., Baker, J. B., and Wood, W. I. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 1142–1146
19. Pennica, D., Shaw, K. J., Wanzen, T. A., Moore, M. W., Shelton, D. L., Zienockez, Z., Rosenhali, A., Taga, T., Paoni, N. F., and Wood, W. I. (1995) J. Biol. Chem. 270, 10915–10922
20. Kodama, H., Fukushima, K., Pan, J., Makino, S., Baba, A., Hori, S., and Ogawa, O. (1999) Heart Vascul. Cardiol. 13, 199–208
21. Fukuda, K., and Izumo, S. (1998) J. Mol. Cell. Cardiol. 30, 2069–2080
IL-6 Family Mediates AngII-induced Cardiac Hypertrophy

23. Wellert, K. C., Taga, T., Saitou, M., Narazaki, M., Kishimoto, T., Glembocki, C. C., Vernaillia, A. B., Heath, J. K., Pennica, D., Wood, W. L., and Chien, K. R. (1996) J. Biol. Chem. 271, 9535–9545
24. Ikeda, S., Awane, Y., Kusumoto, K., Wakimasu, M., Watanabe, T., and Fujino, M. (1994) J. Pharmacol. Exp. Ther. 270, 728–733
25. Kunisada, K., Hirota, H., Fujio, Y., Matsui, H., Tani, Y., Yamauchi-Takahara, K., and Kishimoto, T. (1996) Circulation 94, 2626–2632
26. Sheng, Z., Knowlton, K., Chen, J., Hoshijima, M., Brown, J. H., and Chien, K. R. (1997) J. Biol. Chem. 272, 5783–5791
27. Fujio, Y., Kunisada, K., Hirota, H., Yamauchi-Takahara, K., and Kishimoto, T. (1997) J. Clin. Invest. 99, 2888–2905
28. Long, C. S., Henrich, C. J., and Simpson, P. C. (1991) Cell Regul. 2, 1081–1095
29. Long, C. S., Hartogensis, W. E., and Simpson, P. C. (1993) J. Mol. Cell. Cardiol. 25, 915–925
30. Nishida, M., Springhorn, J. P., Kelly, R. A., and Smith, T. W. (1993) J. Clin. Invest. 91, 1934–1941
31. Eid, H., de Bold, M. L., Chen, J. H., and de Bold, A. J. (1994) J. Cardiovasc. Pharmacol. 24, 715–720
32. Pfeffer, J. M., Pfeffer, M. A., Mirsky, I., and Braunwald, E. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 3310–3314
33. Baker, K. M., Chernin, M. I., Wixson, S. K., and Aceto, J. F. (1990) Am. J. Physiol. 259, H324–H332
34. Funakoshi, Y., Ichiki, T., Ito, K., and Takeshita, A. (1999) Hypertension 34, 118–125
35. Kuwahara, K., Saito, Y., Harada, M., Ishikawa, M., Ogawa, E., Miyamoto, Y., Hamanaka, I., Kamitani, S., Kajiyama, N., Takahashi, N., Nakagawa, O., Masuda, I., and Nakao, K. (1999) Circulation 100, 1116–1124
36. Yamazaki, T., Komuro, I., Kodoh, S., Zhao, Y., Shirou, I., Mizuno, T., Takano, H., Hiroi, Y., Ueki, K., Tobe, K., Kadokawa, T., Nagai, R., and Yazaki, Y. (1995) Circ. Res. 77, 258–265
37. Hirota, H., Yoshida, K., Kishimoto, T., and Taga, T. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4862–4866