Crucial Role of Interleukin-6 in the Development of Norepinephrine-induced Left Ventricular Remodeling in Mice

Henning Meier^{1*}, Jörg Bullinger^{1*}, Grit Marx¹, Alexander Deten^{1,3}, Lars-Christian Horn², Beate Raßler¹, Heinz-Gerd Zimmer¹ and Wilfried Briest^{1,4}

¹Carl-Ludwig-Institute of Physiology and ²Institute of Pathology, University of Leipzig, ³current address: Fraunhofer Institute for Cell Therapy and Immunology, Leipzig, ⁴current address: Laboratory of Cardiovascular Sciences, National Institute on Aging, Baltimore, MD, *These authors contributed equally to this study

Key Words

Interleukins • Extracellular matrix • Hypertrophy • Remodeling

Abstract

Background: Elevated serum concentration of interleukin (IL)-6 is a predictor for poor prognosis in congestive heart failure. It was shown previously in rats, that IL-6 expression in the left ventricle (LV) was followed by LV hypertrophy. **Methods:** Using IL-6 deficient mice (IL-6^{-/-}), we studied the role of IL-6 in a model of norepinephrine (NE)-induced LV hypertrophy. **Results:** In wild type (WT) mice, IL-6 mRNA expression and its concentration in the serum were elevated after 4 h of NE-treatment (s.c. 0.25 mg/kg·h). Further, NE-induced LV hypertrophy was detected: LV weight/body weight (LVW/BW) ratio (+12.3±3%, $p < 0.05$) and mRNA expression of atrial natriuretic peptide (ANP) in WT mice (+120±25%, $p < 0.05$) after 3 days were increased. In contrast, NE did not induce elevation of LVW/BW ratio and ANP expression in IL-6^{-/-} mice. Replacement with recombinant IL-6 restored the hypertrophy-inducing effect of NE in IL-6^{-/-} mice. As to the extracellular matrix (ECM) proteins, NE increased collagen type I and III expres-

sion only in WT mice and not in IL-6^{-/-} mice. The addition of recombinant IL-6 elevated the expression of the ECM proteins to the WT level. **Conclusion:** IL-6 is a major player in the development of NE-induced LV hypertrophy in mice.

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Introduction

Left ventricular (LV) hypertrophy is a prominent feature of evolving heart failure, whether caused by hypertension, obesity, diabetes, valve disease or coronary disease [1]. The heart attempts to maintain normal contractile function and cardiac output by undergoing a process of cardiac hypertrophy under compromising stress, such as myocardial infarction, hypertension, or valvular disease. The initial response is concentric hypertrophic growth of the heart, which is believed to have a compensatory, although dispensable, function by diminishing wall stress and thereby oxygen consumption. If the underlying pathologic stimulus is not removed, the heart eventually undergoes further LV remodeling leading to dilatation and overt heart failure. LV chamber remodeling and increased wall stiffness result from

alterations in cardiac myocytes and in the extracellular matrix (ECM) [2].

Increasing evidence has evolved that cardiomyocyte hypertrophy is initiated by endocrine, paracrine, or autocrine factors via a variety of intracellular signaling pathways to ultimately modulate transcription factors and gene expression [3-5]. For instance, angiotensin II, endothelin-1, catecholamines, and the interleukin (IL)-6 family of cytokines were shown to elicit a hypertrophic response, including increased cell size, sarcomeric reorganization, and induction of the fetal gene expression program [6].

IL-6 family members act through receptor complexes containing gp130 [7]. IL-6 binds first to the IL-6 receptor. This complex induces a homodimerization of gp130 and subsequent signal transduction [7]. Continuous activation of gp130 by overexpressing both IL-6 and IL-6 receptor led to cardiac hypertrophy [8], whereas expression of dominant-negative gp130 mutant attenuated pressure-overload-induced hypertrophy [9]. Elevated level of serum concentrations of IL-6 have been shown to predict a poor prognosis in congestive heart failure secondary to idiopathic dilated cardiomyopathy [10]. Furthermore, IL-6 serum levels are associated with a high risk of mortality in older women [11], and an increased future risk of myocardial infarction among healthy men [12].

Norepinephrine (NE) is well known to cause LV hypertrophy in rats [13] and mice [14] connected with a remodeling of the ECM. We have recently shown that NE stimulated the expression of interleukin (IL)-6 in rat myocardium [15] and in isolated cardiac fibroblasts [16]. Since a NE-induced successive elevation of gene expression including IL-6, receptors of IL-6, signal transducer and activator of transcription (STAT)3, and IL-6 dependent gene C/EBP δ was demonstrated [17], we hypothesized, that IL-6 mediated the NE-induced hypertrophic response. The role of IL-6 in cardiac hypertrophy was addressed by using the experimental model of NE-induced hypertrophy in IL-6 knock-out mice. The expression of the main components of the ECM, collagen type I and III and their chaperone colligin was analyzed to characterize the NE-induced remodeling of the ECM.

Materials and Methods

Animal Experiments

IL-6-deficient (IL-6^{-/-}) mice (generated by M. Kopf [18]; >8 generations backcrossed to BALB/c) and

IL-6^{+/+} BALB/c mice (wild-type; purchased from Charles River, Sulzfeld, Germany) were used. The IL-6^{-/-} genotype was determined by PCR (5'-ttc cat cca gt tgcc ttc ttg g-3' and 5'-ttc tca ttt cca cga ttc cca g-3', or neo 5'-ccg gag aac ctg cgt gca atc c-3'). Norepinephrine (NE; Sigma, Deisenhofen, Germany) was subcutaneously administered at a dose of 0.25 mg/kg·h by Alzet mini-osmotic pumps (Alza, Palo Alto, CA, USA) for 4 hours, 1, and 3 days, respectively. This NE dose induced a compensated LV hypertrophy after 3 days of treatment [14]. Animals with an unfilled mini-osmotic pump replacement served as controls. The lack of functional IL-6 in IL6^{-/-} was replaced by subcutaneous injection of recombinant mouse IL-6 (R&D Systems, Wiesbaden, Germany; 80 μ l of carrier free IL-6 dissolved in 0.9% NaCl, 22 μ g/kg) at the start of NE-treatment and after 12 h. Animals with injection of physiological sodium chloride (80 μ l, twice) served as controls for cytokine injection. Animal care and use were in accordance with the *Guide for Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996) and were approved by the appropriate State agency of Saxony. The animals were allowed to move freely in their cages with access to tap water and standard chow diet, *ad libitum*.

The hearts were rapidly excised from animals anaesthetized with thiopental sodium (Trapanal® 100 mg/kg i.p., Byk Gulden, Konstanz, Germany). The RV free wall was trimmed away. The left ventricle (LV) was weighed after freezing in liquid nitrogen.

Tibia length was measured. Left ventricle weight was related independently to body weight at the beginning of the experiment and tibia length.

Hemodynamic measurements

Heart function was measured with ultraminiature catheter pressure transducers in closed-chest spontaneously breathing anaesthetized animals as previously described [19]. Briefly, the LV catheter (1.4F model SPF-671, Millar Instruments Inc., Houston, TX, USA) was placed in the right carotid artery and advanced upstream to the aorta and into the LV. Heart rate (HR), LVP (pressure processor amplifier 13-4615-52, Gould-Nicolet, Erlensee, Germany), and the rate in rise and fall of LVP (LV dP/dt_{max}, LV dP/dt_{min}, differentiator amplifier 13-4615-71, Gould-Nicolet) were recorded continuously on a PC at a sampling rate of 2 kHz using PCI-6023E multifunction analogue and digital conversion card and DASyLab V7.00 software (National Instruments, Mönchengladbach, Germany). Hemodynamic parameters

were analyzed using IOX V1.7.0.18 software (EMKA Technologies, Paris, France).

RNase protection assay (RPA)

7.5 µg total RNA, isolated by TRIZOL™ (Invitrogen), were used for the detection of cytokine mRNA (Pharmingen custom made probe set containing: IL-12p35, IL-12p40, IL-10, IL-1α, IL-1β, IL-1Ra, LIF, IL-6, IFNγ, MIF, L32 and GAPDH), 5 µg of total RNA were used for the detection of IL-6 receptor mRNA (Pharmingen probe set mCR4), 2.5 µg total RNA were used for the detection of mRNA of extracellular matrix (ECM) proteins and of natriuretic peptides (probe set mECM1 [20]) and finally 7.5 µg total RNA were used for the detection of MMP and TIMP mRNA (probe set mECM2 [20]) in the RNase protection assay (RPA) as previously described [20].

IL-6 ELISA

An Enzyme-Linked Immuno-Sorbent Assay (ELISA, R&D Systems, Wiesbaden, Germany) was applied for the determination of mouse IL-6 in 50 µl of the serum (detection range: 7-500 pg/ml). Incubation and further procedures were performed according to the manufacturer's instruction. The absorbance was measured at 450 nm with wavelength correction at 570 nm using a Spectra count (Packard Instruments Illinois, USA). IL-6 concentrations were calculated using the program I-smart (Packard Instruments Illinois, USA) with a 4-parameter-regression curve fitting.

MMP-2 activity

The Biotrak MMP-2 activity assay system (Amersham Biosciences Europe, Freiburg, Germany) provided quantitative determination of MMP-2 activity in the cardiac tissue after homogenization as previously described [21].

Histological analysis

In another series, the hearts were excised and fixed in 4% buffered formaldehyde, embedded in paraffin. 5 µm sections were stained with hematoxylin-eosin (HE). An expert pathologist evaluated the staining of the sections to rate the severity of histological changes. He was blinded to the origin of the sections. The description of the histological changes represents the characteristic features of all preparations. For presentation, we chose typical examples, which were observed in all slices of the same subgroup.

Fig. 1. Changes in IL-6 expression after norepinehrine (NE)-treatment in mice. IL-6 (A) mRNA expression in the left ventricle and IL-6 concentration in the serum (B) of mice treated with NE (0.25 mg/kg·h) for different indicated time periods were compared with sham operated controls. Relative abundance of mRNA expression obtained by RPA detection with 7.5 µg of total RNA was related to GAPDH mRNA expression ($\times 10^{-3}$) as arbitrary units (AU). IL-6 concentration was obtained from ELISA detection. Values are means $\pm$ SEM. * $P < 0.05$ vs. time-corresponding sham; number of measurements in parentheses.

Statistical analysis

All data were analyzed and expressed as mean $\pm$ SEM. A multiple-sample comparison (ANOVA and the multiple range test as post hoc test using the criterion of the least significant differences) was applied to test the differences between the groups' different modes and time intervals of treatment for significance. A value of $p < 0.05$ was considered to be significant.

Results

Cytokine expression

The expression of IL-6 mRNA was elevated by norepinehrine (NE) in the left myocardium of WT mice after 4 h (Fig. 1A). This early elevation of IL-6 has also

Fig. 2. Effect of norepinephrine (NE) on the LV weight/body weight (LVW/BW) ratio (A) and on atrial natriuretic peptide (ANP) expression (B) from wild type (WT) and from IL-6^{-/-} mice. The results of NE-treatment (0.25 mg/kg·h for 3 days) were compared with sham operated controls. The lack of functional IL-6 in IL-6^{-/-} mice was replaced by subcutaneous injection of recombinant mouse IL-6 (22 µg/kg; +IL6) at the start of NE-treatment and after 12 h, respectively. Mice with injection of physiological sodium chloride (80 µl, twice) served as controls (+NaCl) for cytokine injection. Relative abundance of mRNA expression obtained by RPA detection with 2.5 µg of total RNA was related to GAPDH mRNA expression (x10⁻³) as arbitrary units (AU). Values are means ± SEM. *P<0.05 vs. corresponding sham; †P<0.05 vs. NE WT; ‡P<0.05 vs. NE+NaCl IL-6^{-/-}; number of measurements in parentheses.

been observed in rats [15]. It was accompanied by more than 10-fold increase of the IL-6 concentration in the serum (Fig. 1B).

LV hypertrophy

NE infusion (0.25 mg/kg·h) for 3 days elevated the LV weight (LVW, Table), the LVW/body weight (LVW/BW)-ratio (Fig. 2A) and the expression of atrial natriuretic peptide (ANP, Fig. 2B) in WT mice. This hypertrophic response was absent in IL-6^{-/-} mice. The BW of IL-6^{-/-} mice was higher than the BW of WT mice (table), reduced in both by NE. The LVW/tibia-ratio was also higher in IL-6^{-/-} mice in sham and after NE treatment, respectively (Table). The specificity of the lack of the hypertrophic

Fig. 3. Histological changes of the heart in norepinephrine (NE)-treated wild type (WT) and IL-6^{-/-} mice (C and D, respectively) in comparison to sham operated controls (A, B) were shown by representative hematoxylin and eosin staining in heart section of a mice treated 3 days with NE (0.25 mg/kg per h). Hypoxic damage (open arrow) and inflammation (closed arrow) of the cardiac tissue was seen in WT (C) and IL-6^{-/-} mice (D) (original magnification 40x).

response in IL-6^{-/-} mice was verified by replacement of the lacking IL-6 with recombinant mouse IL-6, subcutaneously injected at the start of the NE infusion and after 12 h (22 µg/kg). Application of IL-6 did not change the LVW/BW ratio or ANP expression in IL-6^{-/-} mice. NE elevated the LVW/BW-ratio as well as the ANP expression in IL-6^{-/-} mice after replacement of IL-6 to the same extent as in WT mice (Fig. 2A and 2B, respectively).

NE treatment was accompanied with hypoxic damage, specified by change in myocardial texture, cellular structure and inflammation in the cardiac tissue in WT and IL-6^{-/-} mice (Fig. 3). There were no differences observed between WT and IL-6^{-/-} mice.

Heart function

The measurements of LV function revealed an elevation of heart rate (HR), LV systolic pressure (LVSP), diastolic aortic pressure (DAP) as well as the contractility (LV dP/dt_{max}) in WT mice after 3 days of NE treatment (Table). NE-stimulation did not change LV end-diastolic pressure in WT mice or in IL-6^{-/-} mice. LVSP and DAP were elevated by NE also in IL-6^{-/-} mice. However, HR as well as LV dP/dt_{max} were increased to a higher extent in IL-6^{-/-} mice than in WT mice (Table).

Table. Changes in body weight (BW), left ventricular weight (LVW) and functional parameters of the LV in IL-6^{-/-} mice in comparison to wild type mice (WT) after 3 days of norepinephrine (NE; 0.25 mg/kg·h) treatment. Animals with an unfilled mini-osmotic pump replacement for 3 days served as controls (sham). Values are mean ± SEM; * P < 0.05 vs. corresponding sham; † P < 0.05 vs. corresponding WT; ‡ P < 0.05 vs. baseline; § P < 0.05 vs. NE-WT; number of measurements in parentheses.

	WT sham (n = 11)	WT NE (n = 14)	IL-6 ^{-/-} sham (n = 11)	IL-6 ^{-/-} NE (n = 8)
BW, g	24.3 ± 0.44	25 ± 0.63	27.3 ± 0.67†	26.9 ± 0.69†
BW2 (end of treatment), g	24.3 ± 0.51	22.3 ± 0.63*‡	27.2 ± 0.63†	24.8 ± 1.04*†
LVW, mg	71 ± 2.45 (n = 8)	82 ± 1.98* (n = 10)	79.8 ± 2.66† (n = 10)	79.2 ± 3.91† (n = 6)
Tibia, mm	17.0 ± 0.14	17.2 ± 0.19	17.7 ± 0.15†	17.8 ± 0.10†
LVW/tibia, mg/mm	4.03 ± 0.14 (n = 10)	4.77 ± 0.11* (n = 13)	4.48 ± 0.15† (n = 5)	4.58 ± 0.27† (n = 4)
Heart rate, bpm	416 ± 11.8	615 ± 35.4*	416 ± 16.5	749 ± 38.1*§
LV systolic pressure, mmHg	110 ± 3.9	133 ± 4.4*	103 ± 5.1	147 ± 5.5*
LV enddiastolic pressure, mmHg	4.6 ± 0.8	4 ± 0.4	2.8 ± 0.3	3.7 ± 0.5
Diastolic aortic pressure, mmHg	78 ± 3.4	91 ± 5.5*	74 ± 3.4	104 ± 4.3*
LVdp/dt _{max} , mmHg/s	8670 ± 786.8	14407 ± 1211*	7210 ± 647.7	22769 ± 3727*§

Fig. 4. Changes in the expression of extracellular matrix proteins after norepinephrine (NE)-treatment for 3 days in wild type (WT) and IL-6^{-/-} mice. Colligin (A), collagen I (B) and collagen III (C) mRNA expression in the left ventricle from wild type (WT) and from IL-6^{-/-} mice was analyzed by RPA detection. The results of NE-treatment (0.25 mg/kg·h) were compared with sham operated controls. The lack of functional IL-6 in IL-6^{-/-} mice was replaced by subcutaneous injection of recombinant mouse IL-6 (22 µg/kg; +IL6) at the start of NE-treatment and after 12 h, respectively. Mice with injection of physiological sodium chloride (80 µl, twice) served as controls (+NaCl) for cytokine injection. Relative abundance of mRNA expression with 2.5 µg of total RNA was related to GAPDH mRNA expression (x10⁻³) as arbitrary units (AU). Values are means ± SEM. *P<0.05 vs. corresponding sham; †P<0.05 vs. NE WT; ‡P<0.05 vs. NE+NaCl IL-6^{-/-}; number of measurements in parentheses.

Extracellular matrix

Sustained activation of metabolism of the extracellular matrix (ECM) was indicated by the persistent increase in the mRNA expression of colligin, type I and type III collagen (Fig. 4), of the tissue inhibitor of matrix metalloproteinase (TIMP)-1 (Fig. 5A) and the increased activity of matrix metalloproteinase (MMP)-2 (Fig. 5B) after 3 days of NE-treatment in WT mice. The amount of colligin mRNA, a chaperone for collagens, was twice as high as that of type I or type III collagen in the LV of sham WT mice and in the LV of sham IL-6^{-/-} mice. In contrast, the concentration of colligin in rat myocardium was only the half of the collagen I concentration [22]. The change in the collagen type III/type I ratio is a good predictor for a pathological remodeling of the ECM. The mRNA of collagen type III was elevated to a higher degree by NE than type I and colligin. The ratio of type III/type I collagen was near 1 in WT mice and IL-6^{-/-} mice. After NE treatment it was elevated to 1.4 in WT mice and to 1.5 in IL-6^{-/-} mice (P<0.05, respectively). The NE-induced mRNA elevation of colligin (Fig. 4A)

as well as of type I and type III collagen (Fig. 4B and 4C, respectively) was significantly attenuated in IL-6^{-/-} mice. This effect was due to the deficiency of IL-6, since replacement of IL-6 elevated all mRNAs in IL-6^{-/-} mice by NE to the same extent as the NE-induced elevation in WT mice.

Fig. 5. Changes in the expression of the inhibitor of matrix metalloproteinase (TIMP)-1 and in gelatinolytic activity after norepinephrine (NE)-treatment for 3 days in wild type (WT) and IL-6^{-/-} mice. TIMP-1 (A) mRNA expression and MMP-2 (B) activity in the left ventricle from wild type and from IL-6^{-/-} mice were analyzed by RPA detection or by an activity assay, respectively. The results of NE-treatment (0.25 mg/kg·h) were compared with sham operated controls. The lack of functional IL-6 in IL-6^{-/-} mice was replaced by subcutaneous injection of recombinant mouse IL-6 (22 µg/kg; +IL6) at the start of NE-treatment and after 12 h, respectively. Mice with injection of physiological sodium chloride (80 µl, twice) served as controls (+NaCl) for cytokine injection. Relative abundance of mRNA expression with 7.5 µg of total RNA was related to GAPDH mRNA expression ($\times 10^{-3}$) as arbitrary units (AU). The MMP-2 activity was determined by a specific ELISA and is given as the ratio to extracted cardiac protein. Values are means $\pm$ SEM. * $P < 0.05$ vs. corresponding sham; number of measurements in parentheses.

The NE-induced elevation of TIMP-1 mRNA was only detectable in WT mice and not in IL-6^{-/-} mice (Fig. 5A). After replacement of IL-6 NE induced an elevation of TIMP-1 mRNA in IL-6^{-/-} mice that was comparable to that in WT mice. In contrast, the MMP-2 activity was elevated in IL-6^{-/-} mice by NE to the same extent than in WT mice (Fig. 5B). The replacement of IL-6 prevented the NE-induced elevation of the MMP-2 activity in IL-6^{-/-} mice.

Discussion

Our data demonstrate a critical role for IL-6 in the development of LV hypertrophy in mice. The induction of IL-6 was an early signal in the development of hypertrophy since it was elevated at 4h (Fig. 1A) by NE treatment. The effect was demonstrated after 4–12 h in rats [15] and after 6h in the surviving LV after myocardial infarction in mice [19] as well. This was followed by the induction of leukemia inhibitor factor (LIF), another member of the IL-6 cytokine family, which was elevated in the myocardium of end-stage heart failure as well [23, 24]. There was more IL-6 than LIF mRNA detectable in human myocardium from heart failure patients [24] and in rats with terminal heart failure [25]. The gp130-mediated signaling was involved in overload induced cardiac hypertrophy in rats [26]. IL-6 was also elevated in this overload model. The parallel increase of IL-6 in the serum by NE treatment (Fig. 1B) implicated that the heart could be the source of this elevated IL-6 serum concentration. The increased IL-6 plasma level after acute

myocardial infarction was discussed as a spillover from cardiac tissue too, which was facilitated by catecholamine stimulation [27]. The lack of the development of cardiac hypertrophy in IL-6^{-/-} mice was also seen in the model of angiotensin (Ang) II stimulation [28], however it was not seen after myocardial infarction [29] or moderate physical exercise [30]. Therefore we have to consider that cardiac gp130-mediated signaling pathway to hypertrophy [7] seems to be not universal. The AngII stimulation depends on classic IL-6 signaling via the membrane-bound cognate IL-6 receptor, whereas the AngII-mediated hypertension is mediated by agonistic complexes of IL-6 with the soluble IL-6 receptor that bind to gp130 receptor, a mechanism referred to as IL-6 trans-signaling [28].

IL-6^{-/-} mice had a higher BW than WT mice (Table), since the lack of IL-6 disturbs carbohydrate and lipid metabolism [31]. The higher BW explains also the increase of the LVW/tibia-ratio in IL-6^{-/-} mice (Table). The decrease of the BW was detectable in WT and IL-6^{-/-} mice (Table) and can be explained by the catabolic effect of NE. IL-6 was required for NE-induced LV hypertrophy (Fig. 2). The lack of elevated ventricle mass in IL-6-deficient mice after NE-stimulation was accompanied by a more pronounced positive chronotropic and inotropic effect of NE in these IL-6^{-/-} mice in comparison to the effect in WT mice (Table). The effects of chronic high catecholamine levels, with myocyte enlargement, pathologic ECM remodeling, resulting fibrosis and progression to heart failure may be reduced

by preventing the IL-6 increase in the initial phase. In fact, this was the case in IL-6^{-/-} mice (Fig. 2, 4). Higher heart rates and contractility lead to an increase in oxygen consumption and to higher energy expenditure in the heart. The ability of IL-6^{-/-} mice to a more pronounced elevation in heart rate and LV contractility (Table) may be explained by greater energy reserves. IL-6^{-/-} mice have increased circulating triglyceride levels [31]. Furthermore, IL-6 by itself seems to induce oxygen consumption. It was shown that intracerebroventricular IL-6 injections induced an acute increase in oxygen consumption in the brain [31]. Therefore IL-6 in WT mice may induce oxygen consumption that cannot be used for the demand of the increased heart function. Another explanation for the higher heart rates and contractility in IL-6^{-/-} mice may be a greater amount of the faster V1 isomyosin [32] in these mice. However, in this case we would expect a different baseline function as well.

The NE-induced hypertrophy in mice was accompanied by sustained activation of ECM metabolism which was also shown in NE-induced hypertrophy in rats [22]. The expression of collagen type I and type III, the main components of the ECM, as well as colligin, a chaperon of collagen, and the LV weight was reduced but not abolished in IL-6^{-/-} mice by NE-stimulation (Fig. 4). Furthermore, the ratio of type III/type I collagen was increased in WT mice as well as in IL-6^{-/-} mice. The shift to collagen type III could be a sign for the adapted non pathological phase of LV remodeling. This notion is supported by the increased proportion of collagen type III in aging Wistar Kyoto rats in comparison to spontaneously hypertensive rats (SHR) during the development of cardiac hypertrophy [33]. In SHR the significant higher amount of collagen is due to a higher proportion of collagen type I. The stiffer ventricle is characterized by a predominance of collagen type I with its fibril forming quality which is the result of pathological remodeling of ECM [34-36].

An important regulation point of the remodeling of ECM is the control of MMP activity by a group of specific MMP inhibitors called tissue inhibitors of matrix metalloproteinases (TIMPs) [37]. However, the change in MMP- activity after NE-treatment (Fig. 5B) could not be explained by the change in TIMP expression. The only significant change in TIMP expression was detected for TIMP-1 in WT mice after NE stimulation (Fig. 5A). It was accompanied by an increase and not by a reduction in MMP-2 activity. It was shown that TIMP-1 may exert nontraditional effects during NE-treatment [14]. The NE-induced change in MMP-2 activity was not affected like the development of hypertrophy and the expression of collagen in IL-6^{-/-} mice. Therefore, we have to conclude that MMP-2 activity may be regulated IL-6 independently, although the replacement of IL-6 abolished the increase of the MMP-2 activity in IL-6^{-/-} mice.

In conclusion, we provide evidence that IL-6 is crucial for the development of NE-induced LV hypertrophy. NE-induced elevation of LV weight detected by increased LVW/BW ratio and increased mRNA expression of ANP was prevented in mice lacking IL-6. The absence of hypertrophy in IL-6^{-/-} mice after NE-treatment was associated with reduced collagen mRNA expression in these mice. Replacement of IL-6 restored the effect of NE on cardiac hypertrophy and ECM.

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