Etanercept or intravenous immunoglobulin attenuates expression of genes involved in post-myocardial infarction remodeling ☆

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Abstract

Objective: Persistently elevated levels of inflammatory cytokines such as tumor necrosis factor (TNF)α after acute myocardial infarction (MI) may contribute to maladaptive ventricular remodeling. The aim of the present study was to examine the effects of immunomodulatory therapy with recombinant soluble TNF receptor (TNFR:Fc) or intravenous immunoglobulin (IVIg) on left and right ventricular post-MI remodeling in rats.
Methods and results: Adult male Sprague–Dawley rats were subjected to MI by left coronary artery ligation and randomized to treatment with vehicle, TNFR:Fc, or IVIg and sacrificed after 7 days. The main findings were that: (i) TNFR:Fc- and IVIg-treated rats developed less right ventricular (RV) hypertrophy compared to vehicle-treated controls. (ii) LV and arterial pressures in post-MI rats were not affected by the TNFR:Fc or IVIg treatment. (iii) As determined by real-time RT-PCR, both treatments reduced the expression of the hypertrophy-related genes, atrial natriuretic peptide and the ratio of β/α-myosin heavy chains, and genes related to extracellular matrix remodeling (i.e., collagens I and III, matrix metalloproteinase [MMP]-2 and its tissue inhibitor TIMP-1) in the non-ischemic segment of LV and, in particular, in the RV. (iv) Treatment with IVIg, but not TNFR:Fc, reduced MMP-2 zymographic activity in the RV and the expression of genes for TNFα and monocyte chemoattractant protein-1.
Conclusion: Therapy targeted directly against TNFα (i.e., TNFR:Fc) and a more general immunomodulatory approach (i.e., IVIg) in the acute phase of MI attenuates the cardiac remodeling process and expression of genes that are involved. These findings raise the possibility that initiation of immunomodulatory therapy post-MI could be beneficial in preventing the later development of heart failure.
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1. Introduction

Acute myocardial infarction (MI) is followed by an inflammatory response characterized by complement activation, generation of reactive oxygen species and increased levels of myocardial as well as circulating inflammatory cytokines such as tumor necrosis factor (TNF)α and interleukin (IL)-1β [1]. This inflammatory process is a prerequisite for wound repair and scar formation and it may

also play a role in the development of compensatory cardiac hypertrophy [2,3]. However, while this response has obvious beneficial aspects, persistent or excessive inflammation could also have later maladaptive effects [2]. Several experimental studies have recognized that inflammatory cytokines may be potentially important mediators of ventricular remodeling [2,4]. TNFα, in particular, has received much attention and cardiac-specific overexpression of this cytokine promotes a phenotype mimicking several features of clinical heart failure (HF) including cardiac hypertrophy, ventricular dilatation, fibrosis and several biochemical and cellular defects [4]. Recent data indicate that a complex TNFα-mediated regulation of matrix metalloproteinases (MMP) and tissue inhibitors of metalloproteinases (TIMP) may significantly contribute to myocardial remodeling [5,6]. Myocardial overexpression of TNFα leads to an early increase in MMP activity, possibly causing derangement of fibrillar collagen and ventricular dilation. However, over time a decrease in MMP-activity, coincident with increased TIMP expression, leads to increased collagen content and myocardial fibrosis [6]. Thus, it has been hypothesized that immunomodulatory strategies that modify an excessive inflammatory reaction could attenuate post-MI remodeling, preserve myocardial function and limit progression to chronic HF.

Etanercept and intravenous immunoglobulin (IVIg) represent two different immunomodulatory strategies with potentially beneficial effects on cardiac performance in patients with chronic HF [7,8]. Etanercept (TNFR:Fc), a fusion protein of TNF receptor (TNFR) type II and the Fc portion of human IgG, specifically binds TNFα, potentially rendering it biologically inactive. IVIg consist of pooled human IgG and has broad immunomodulatory effects. Although the exact mechanism of action is poorly understood it may involve Fc receptor blockade, modulation of the idiotypic–anti-idiotypic antibody network, attenuation of complement mediated tissue damage and modulation of cytokine production [9].

The aim of the present study was to investigate whether intervention in the acute phase after MI in rats with these different immunomodulatory strategies (i.e., TNFR:Fc and IVIg) affected left and right ventricular remodeling, particularly focusing on the effects on expression of genes related to hypertrophy, fibrosis and inflammation.

2. Methods

2.1. Induction of myocardial infarction and treatment groups

Induction of MI was performed as previously described [10]. Briefly, adult male Sprague Dawley rats were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (8 mg/kg) intraperitoneally (IP). Animals were intubated and placed on a mechanical respirator. The chest was opened by a left thoracotomy and the heart was exposed. The left anterior descending coronary artery was ligated close to the aorta and the incision was closed in layers. In sham operations ligation of the coronary artery was not performed. MI rats were randomized to either no treatment, treatment with IVIg (rat γ-globulin, Sigma) or treatment with TNFR:Fc (etanercept; generously provided by Immunex). IVIg treatment was based on a study in heart failure patients [8] and consisted of 100 mg/250 g rat intravenously (IV) or IP on the day of MI induction and 75 mg/250 g rat IP on the 3rd day post-MI. TNFR:Fc was administered IP (300 μg/250 g rat) 2 days prior to coronary artery ligation and every 2 days thereafter for 1 week. Pre-treatment with TNFR:Fc has been used by other investigators (e.g., [11]) and was done in this study in order to achieve a maximal level at time of MI induction. TNFR:Fc dosage was based on early studies [12] and was tested in preliminary experiments. Since one of our main goals was to assess the effects of immunomodulatory therapy on the expression of genes involved in post-MI remodeling, rats were sacrificed at 1 week post-MI, a time at which several of the genes studied are significantly elevated [2,13]. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Physiological studies of hemodynamic variables

At 1 week post-MI rats were anesthetized as described above. Prior to sacrifice hemodynamic measurements were performed in a subgroup of rats in each treatment using the following protocol. A cut down was performed on the right carotid artery, which was cannulated with a Mikro Tip® catheter transducer (Millar Instruments, Houston TX). ECG, aortic and LV pressures were digitized and recorded with A/D conversion (DI-720 Series, DATAQ instruments, Akron OH) onto a computer. DATAQ software enable the identification of left ventricular end diastolic pressure (LVEDP) from the position of the peak R wave of the ECG, as well as peak LV systolic pressure (LVSP) and LV dP/dt. Thirty consecutive beats were averaged for LVEDP, LVSP and maximum $+dP/dt$ and $-dP/dt$. Mean arterial pressure (MAP) was determined from aortic pressure measurements during 30 consecutive beats. Using Prism software (GraphPad, San Diego CA) the time constant for isovolumic relaxation (tau) was calculated by fitting an exponential curve to the pressure decay beginning at maximal $-dP/dt$ as described previously by the investigator [14].

2.3. Tissue preparation and determination of heart dimensions

Following hemodynamic measurements while rats still under anesthesia, the heart was excised and weight of

whole heart, right+left ventricle (RV+LV) and free wall of RV alone were determined. The LV was divided by a cross section at the middle of the infarct. The apical part was frozen for cryostat sectioning. The basal half was further dissected to obtain two tissue portions of each, i.e., the infarct and non-infarct (septum). In a subset of rats, the aorta was clamped and hearts were arrested by injection of cold cardioplegic solution into the left and right ventricle cavities (NaCl 4 g/L, KCl 4.48 g/L NaHCO$_3$ 1.0 g/l, glucose 2 g/L and heparin 10,000 U/L). The arrested heart was removed and placed in the cold cardioplegic solution and then dissected for geometric measurements in order to obtain dimensions of septal and infarct wall thickness and LV cavity. Cross sections of the apical half were stained with hematoxillin and eosin (H&E). By 7 days post-MI the infarct region consists of necrotic tissue surrounded by a rim of dense concentration of non-myocytes. The infarct borders between the non-myocytes and the surviving myocytes were clearly defined. Infarct size was defined as the fraction it constituted of the cross section of the LV at the papillary muscle level. It was estimated from the proportion of the endocardial length of the LV using NIH Image software. Endocardial length was also used to calculate a theoretical radius of the LV as an indication of LV chamber size in hearts arrested with cardioplegic solution. Likewise, infarct and septum thickness were determined by multiple measurements along the infarct wall or the septum. Collagen was visualized in LV cross section using picrosirius red staining. Collagen area fraction was determined in digitized tissue images of the septum using NIH Image software [15].

2.4. Analysis of gene expression

Total RNA was extracted from infarct, septal, or RV regions of 1 week post-MI hearts using RNeasy system modified for extraction from tissue (Qiagen) and DNase-treated (RQ1 DNase; Promega). Quantification of gene expression was performed using the ABI Prism 7000 (Applied Biosystems) [16]. Primers were designed using the Primer Express software version 2.0 as suggested by the manufacturer Applied Biosystems (Table 1). To avoid amplification of genomic DNA, one of the primers were designed to span an exon–exon junction. cDNA was prepared from 1 µg total RNA using the High Capacity cDNA Archive kit (Applied Biosystems). Real-time quantitative RT-PCR was performed using qPCR Master Mix for SYBR Green I (Eurogentec) and 300 nM sense and anti-sense primers. All samples were run in triplicate. Standard curves prepared from a pool of cDNA were run on the same plate and the relative standard curve method was used to calculate the relative gene expression [17]. After every analysis, a melting point analysis was performed. This yielded a single and concise peak, confirming the specificity of the PCR. Expression of 18S Ribosomal RNA (Applied Biosystems)

Table 1
Characteristics of the real-time PCR assays used in the study

Target	Sequence (5′→3′)	Acc. nr.
ANP	(+)-ATCTGATGGATTTCAAGAACC	NM_012612
	(−)-CTCTGAGACGGGGTTGACTTC	
α-MHC	(+)-CTCCATCTCTGACAACGCCTATC	NM_017239
	(−)-CTCCGGATTCTCCAGTGATGA	
β-MHC	(+)-GGAGCTGATGCACCTGTAGACA	X15939
	(−)-AGTGCGGACACGGTCTGAA	
Collagen I	(+)-GAGGGCGAGTGCTGTCCTT	Z78279
	(−)-GGTCCCTCGACTCCTATGACTTC	
Collagen III	(+)-TGAAGGAAATAGCAAATTCACTTACAC	X70369
	(−)-TCAAAGACTGTCTTGCTCCATTC	
Interleukin-1β	(+)-CACCTCTCAAGCAGAGCACAG	M98820
	(−)-GGGTTCCATGGTGAAGTCAAC	
MCP-1	(+)-CTCACCTGCTGCTACTCATTCACT	M57441
	(−)-TGCTGCTGGTGATTCTCTTGTAGT	
MMP-2	(+)-TGGGACAAGAACCAGATCACATA	X71466
	(−)-GCCCGAGCAAAAGCATCAT	
TGF-β$_1$	(+)-AAGAAGTCACCCGCGTGCTA	X52498
	(−)-TGTGTGATGTCTTTGGTTTTGTCA	
TIMP-1	(+)-AGGGCTACCAGAGCGATCACT	U06179
	(−)-AAGGTATTGCCAGGTGCACAA	
TNFα	(+)-ATCATCTTCTCAAAACTCGAGTGAC	X66539
	(−)-TTGAGATCCATGCCATTGG	

The table shows the sequence of primers used in the real-time PCR assays. (+), forward primers; (−), reverse primers; Acc. nr., GenBank accession number. ANP, atrial natriuretic peptide; MHC, myosin heavy chain; MCP, monocyte chemo-attractant protein; MMP, matrix metalloproteinase; TGF, transforming growth factor; TIMP, tissue inhibitor of MMP; TNF, tumor necrosis factor.

Table 2
Effects of TNFR:Fc and IVIg on post-MI rat heart

	Sham	MI	MI-TNFR:Fc	MI-IVIg
BW 7 days post-MI [g]	287 ± 2.2	263 ± 4.6**	270 ± 4.9*	256 ± 3.6***
BW change [%]	7.5 ± 0.5	−0.6 ± 1.4***	1.9 ± 1.6*	−0.3 ± 1.2**
Heart weight [g]	0.92 ± 0.01	1.04 ± 0.02***	1.00 ± 0.02*	0.97 ± 0.02[††]
Heart weight/BW [%]	0.32 ± 0.003	0.40 ± 0.01***	0.37 ± 0.005***,[†]	0.38 ± 0.01***
LVW [g]	0.68 ± 0.01	0.67 ± 0.01	0.68 ± 0.02	0.64 ± 0.01
LVW/BW [mg/g]	2.37 ± 0.02	2.53 ± 0.04*	2.51 ± 0.03	2.52 ± 0.05
LV size (radius, mm)[a]	1.92 ± 0.12	2.98 ± 0.14***	2.89 ± 0.16***	3.14 ± 0.20***
RVW [g]	0.15 ± 0.004	0.21 ± 0.01***	0.17 ± 0.01*,[††]	0.17 ± 0.01*,[††]
RVW/BW [mg/g]	0.51 ± 0.01	0.79 ± 0.03***	0.65 ± 0.02**,[†††]	0.69 ± 0.035***,[†]
Infarct size [%]	N/A	52 ± 1.5	51.5 ± 1.1	54 ± 1.4
Infarct thickness [mm][a]	N/A	1.47 ± 0.4	1.15 ± 0.15	0.96 ± 0.081[†]
Septum thickness [mm][a]	2.6 ± 0.16	2.40 ± 0.24	2.56 ± 0.2	2.78 ± 0.3
Collagen area fraction %[b]	1.5 ± 0.08	1.8 ± 0.22	1.9 ± 0.14	2.0 ± 0.17

Data are presented as mean ± S.E.M.

$n = 14$–23 per experimental groups except where noted.

MI, myocardial infarction; BW, body weight; BW change, change at day 7 relative to pre-operative weight; LVW, left ventricle weight; RVW, right ventricle weight.

*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs. sham-operated rats; [†]$p < 0.05$ and [††]$p < 0.01$ and [†††]$p < 0.001$ vs. untreated MI rats.

[a] $n = 3$–7 per experimental group.

[b] $n = 5$–11 per experimental group.

was also analyzed by real-time RT-PCR and used for normalization.

2.5. Gelatin zymography

Proteins were extracted from pulverized RV tissue and resuspended in ice-cold lysis buffer [PBS containing protease inhibitor cocktail w/o EDTA (Gibco) with 1% Triton X-100 and 0.1% Tween 20] at a ratio of 0.1 ml per 10 mg weight tissue. Extracts were incubated on ice for 15 min and then centrifuged at $12,000 \times g$ for 15 min at 4°C. The supernatants were retained and protein concentrations in the samples were measured with the BCA method (Pierce). The protein extracts (75 μg) were separated on 10% SDS–PAGE containing 1% gelatin (Novex, Invitrogen) as previously described [18]. All samples were pre-activated by 1 mM aminophenylmercuric acetate (Sigma) for 1 h prior to separation. The gels were scanned by a Kodak 440 CF imaging station (Nen™) and the gelatinolytic activity of MMP-2 (pro-

and active) was determined using the software Total Laboratory v.1.10 (Phoretix). Comparisons were made after normalization against one test sample run on every zymogram. MMP standard (Sigma) consisting of MMP-2 and −9 was run to ensure identification of MMP activity. Values given for MMP-2 activity were obtained after adding up the pro- and active MMP-2 activities.

2.6. Statistical analysis

Data are given as mean ± S.E.M. Significant difference between treatment groups was demonstrated for the effect on rat heart dimensions and hemodynamic variables (Tables 2 and 3) by ANOVA followed by post-test Bonferroni analyses to determine differences between experimental groups. Gene expression data were not normally distributed. Thus, comparisons between the groups were analyzed by the non-parametric Kruskal–Wallis test followed by Mann–Whitney post-test. Significant difference was determined as $p < 0.05$.

Table 3
Hemodynamic variables

	Sham	MI	MI-TNFR:Fc	MI-IVIg
n	11	8	11	5
MAP (mm Hg)	108 ± 3	83 ± 5**	82 ± 6***	85 ± 4*
LVEDP (mm Hg)	6.5 ± 0.8	12.4 ± 1.0**	12.9 ± 1.6**	12.3 ± 1.3*
LVSP (mm Hg)	127 ± 4	94 ± 5***	96 ± 6***	95 ± 4**
+dP/dt (mm Hg/s)	4744 ± 212	3773 ± 302*	3352 ± 224***	3240 ± 82**
−dP/dt (mm Hg/s)	−4306 ± 194	−2959 ± 240***	−2872 ± 205***	−2703 ± 188***
tau (ms)	18.1 ± 0.5	24.5 ± 3.5	20.4 ± 1.2	25.2 ± 3.1

Data presented as mean ± S.E.M. MAP, mean arterial pressure; LVEDP, left ventricular end-diastolic pressure; LVSP, left ventricular systolic pressure; +dP/dt, maximal rate of increase of LV systolic pressure; −dP/dt, maximal rate of decline of LV systolic pressure; tau, time constant of isovolumic pressure decline.

*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs. sham-operated rats; no differences were observed between MI and MI-TNFR:Fc or MI-IVIg rats.

3. Results

3.1. Survival

Of the 15 rats that underwent sham operation, all survived surgery. One rat died 2 days post-operative and 14 survived (93%) until sacrifice at 7 days. Sixty-six untreated rats underwent coronary artery ligation and of these 16 (24%) died during or shortly after surgery. Of the 50 that survived surgery, 30 received no treatment and 23 (77%) survived until sacrifice on day 7. The remaining 20 surgical survivors were treated with IVIg and 16 (80%) survived for 7 days. Thirty-six rats received TNFR:Fc (beginning 2 days before surgery) and 6 (17%) died during or shortly after surgery. Of the 30 remaining rats, 18 survived for 7 days until sacrifice (60%). Although the death rate with TNFR:Fc treatment was greater than in the MI and IVIg-treated rats the differences were not statistically significant.

3.2. Body weight, tissue weight, and infarct size

The effects of intervention with TNFR:Fc and IVIg after acute MI on multiple histomorphometric measures are summarized in Table 2. The increase in HW/BW ratio observed post-MI relative to sham in both untreated and treated rats was due primarily to an increase in RV/BW ratio. Both TNFR:Fc and IVIg-treated rats had significantly lower RV/BW weight ratios as compared to untreated MI rats. Although tissue weight is only one indicator of ventricular remodeling and can be affected by other factors, the decrease in weight suggests attenuation of RV hypertrophy. In contrast, LV weight and LV/BW weight ratios were unchanged in all experimental groups (except for untreated MI). Importantly, neither intervention with TNFR:Fc nor IVIg seemed to affect the infarct size. Hence, it is unlikely that the attenuation of RV hypertrophy is merely due to differences in the size of the MI caused by treatment. Infarct thickness was significantly reduced in the IVIg-treated rats. LV chamber size was significantly increased following MI but was unaffected by treatments. Finally, septal thickness and collagen area fraction in the septum were unaffected by induction of MI with or without treatments.

3.3. Hemodynamic variables

Hemodynamic variables are summarized in Table 3. MAP, LVSP and positive dP/dt were reduced post-MI. LVEDP was significantly increased. Negative dP/dt was significantly deceased post-MI and although the time constant of isovolumic relaxation (tau) was prolonged this difference did not reach statistical significance. Notably, neither of the treatments significantly affected any of the hemodynamic variables in comparison to the untreated post-MI rats.

3.4. TNFR:Fc and IVIg attenuate the post-MI upregulation of cardiac gene expression

3.4.1. Expression of fetal genes

Re-expression of a fetal gene pattern is a hallmark of post-MI ventricular remodeling [19]. Thus, to further examine the effects of treatment with TNFR:Fc and IVIg in the acute phase after MI, we analyzed the gene expression of atrial natriuretic peptide (ANP) and α and β-myosin heavy chain (MHC) in the infarction area, the non-ischemic part of LV and in the RV. Characteristic of post-MI remodeling, we found markedly upregulated expression of these fetal genes in the regions examined (Fig. 1). In keeping with our finding of lower RVW/BW ratios in the rats receiving immunomodulatory therapy, the gene expression of ANP and the ratio of gene expression of β-MHC to α-MHC in the RV was lower in IVIg (both $p < 0.05$) and TNFR:Fc ($p < 0.05$ and $p = 0.10$, respectively) treated comparing untreated MI rats (Fig. 1). The expression of these genes tended to be decreased also in the non-ischemic part of LV with both treatments, but only reached statistical significance for β-MHC/α-MHC with TNFR:Fc treatment (Fig. 1). There was no change with treatment in the infarcted region.
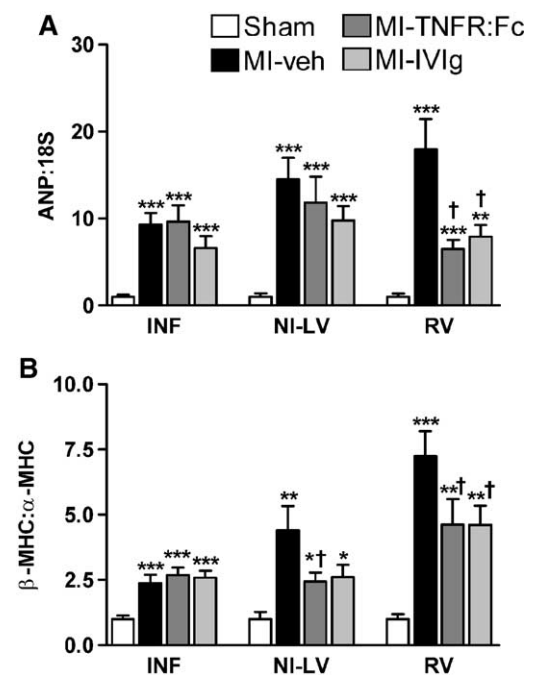

Fig. 1. Gene expression of (A) atrial natriuretic peptide (ANP) and (B) ratio of gene expression of β-myosin heavy chain (MHC) to α-MHC in the infarcted area (INF), non-ischemic part of left ventricle (NI-LV) and right ventricle (RV) of sham-operated rats (open bars, $n = 6$), untreated rats with myocardial infarction (MI-veh) (filled bars, $n = 17$), MI rats treated with TNFR:Fc (dark grey bars, $n = 11$) and MI rats treated with intravenous immunoglobulins (IVIg) (light grey bars, $n = 13$). Gene expression was determined by real-time quantitative RT-PCR and normalized to the expression of 18S ribosomal RNA. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$ vs. sham operated rats. $^{\dagger}p < 0.05$ vs. untreated MI rats.

3.4.2. Expression of genes related to fibrosis

MMPs are a driving force behind extracellular matrix remodeling after MI and may contribute to ventricular dilation. Moreover, the levels and activities of MMPs and their inhibitory proteins (TIMPs) and the level of collagen synthesis determine the overall amount of fibrosis seen in the heart post-MI [20]. When comparing sham rats with untreated MI rats, we found significantly increased gene expression of pro-α-collagen I or III as well as MMP-2 and its endogenous inhibitor TIMP-1 in the infarction area, non-ischemic part of LV and in the RV (Fig. 2). Notably, neither IVIg nor TNFR:Fc down-regulated pro-α-collagen I or III mRNA levels in the infarction area (Fig. 2A and B). However, rats treated with TNFR:Fc and IVIg had significantly lower gene expression of pro-α-collagen III in non-ischemic LV than untreated MI rats (Fig. 2A and B). Moreover, there was a reduced induction of both pro-α-collagen I and III gene expression in RV that was significant with IVIg, but not with TNFR:Fc treatment (Fig. 2A and B). As depicted in Fig. 2C and D, immunomodulating treatment also reduced the increase in mRNA levels of MMP-2 and TIMP-1 in both non-ischemic LV and RV, but not in the infarction area as compared to untreated MI rats, although not all the comparisons reached statistical significance.

3.4.3. Expression of genes related to inflammation

We also examined whether immunomodulatory therapy altered myocardial cytokine gene expression. The expression of genes encoding monocyte chemoattractant peptide (MCP)-1, interleukin (IL)-1β, transforming growth factor (TGF)-β_1 and TNFα were significantly higher in untreated MI rats than in sham rats in all examined regions. TNFα gene expression, however, tended to be increased by 50% in these regions, while the other genes were increased by 2-fold or greater (Fig. 3). Although neither TNFR:Fc nor IVIg treatment significantly affected the gene expression of TNFα, MCP-1, IL-1β and TGF-β_1 in the infarction area or in the non-ischemic LV, IVIg, but not TNFR:Fc, significantly reduced the TNFα and MCP-1 gene expression of in RV (Fig. 3).

3.5. Activity of matrix metalloproteinase-2

Since immunomodulatory therapy significantly reduced MMP-2 gene expression in non-ischemic LV and particularly in RV, we sought to further elucidate this issue by examining MMP-2 activity as assessed by gelatinolytic activity in RV (Fig. 4). In keeping with the decreased gene expression of MMP-2, IVIg-treated rats ($n=13$) had significantly lower total MMP-2 activity in the RV
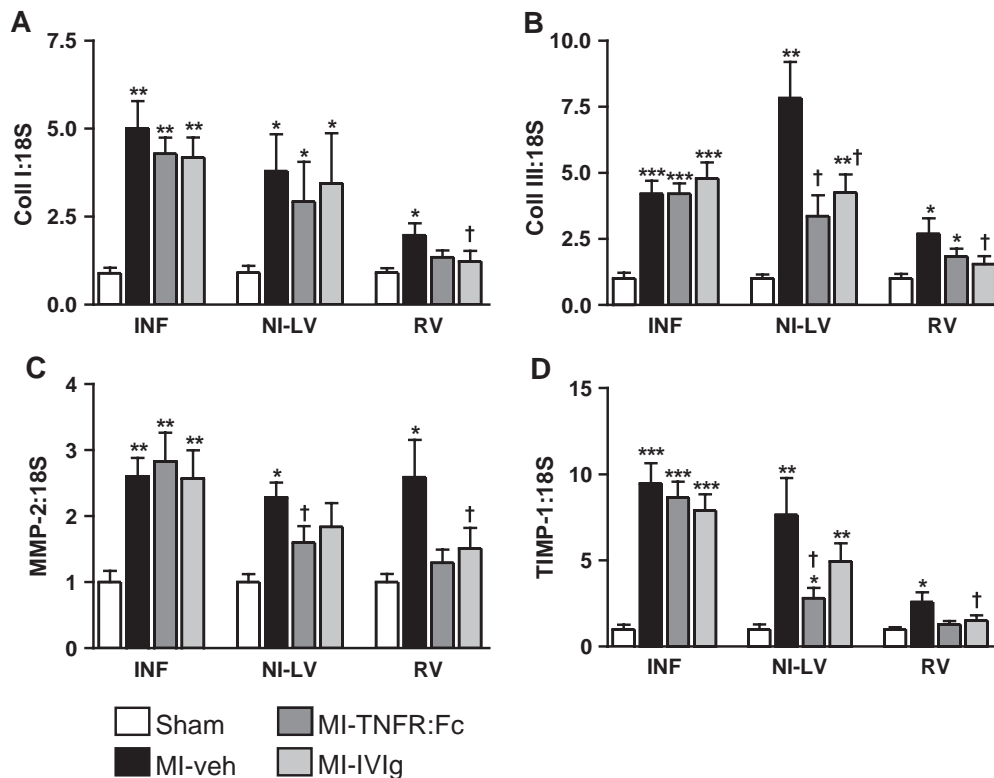

Fig. 2. Gene expression of (A) α1-collagen type I (Coll I), (B) α1-collagen type III (Coll III), (C) matrix metalloproteinase (MMP)-2 and (D) tissue inhibitor of MMP (TIMP)-1 in the infarcted area (INF), non-ischemic part of left ventricle (NI-LV) and right ventricle (RV) of sham-operated rats (open bars, $n=6$), untreated rats with myocardial infarction (MI-veh) (filled bars, $n=17$), MI rats treated with TNFR:Fc (dark grey bars, $n=11$) and MI rats treated with intravenous immunoglobulins (IVIg) (light grey bars, $n=13$). Gene expression was determined by real-time quantitative RT-PCR and normalized to the expression of 18S ribosomal RNA. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ vs. sham operated rats. †$p<0.05$ vs. untreated MI rats.
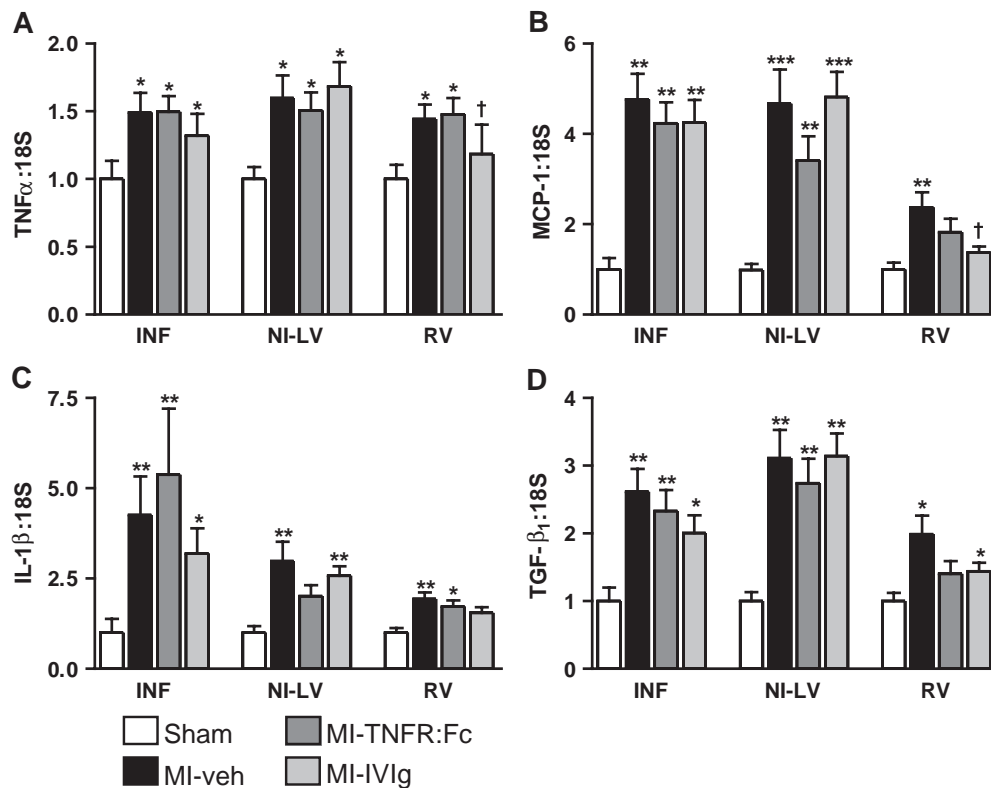
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Fig. 3. Gene expression of (A) tumor necrosis factor (TNF)α, (B) monocyte chemoattractant protein (MCP)-1, (C) interleukin (IL)-1β and (D) transforming growth factor (TGF)-β_1 in the infarcted area (INF), non-ischemic part of left ventricle (NI-LV) and right ventricle (RV) of sham-operated rats (open bars, $n=6$), untreated rats with myocardial infarction (MI-veh) (filled bars, $n=17$), MI rats treated with TNFR:Fc (dark grey bars, $n=11$) and MI rats treated with intravenous immunoglobulins (IVIg) (light grey bars, $n=13$). Gene expression was determined by real-time RT-PCR and normalized to the expression of 18S ribosomal RNA. *$p<0.05$, **$p<0.01$ and ***$p<0.001$ vs. sham operated rats. $^{†}p<0.05$ vs. untreated MI rats.

comparing to untreated MI rats ($n=17$) (1.4 ± 0.17 vs. 0.9 ± 0.13; $p<0.05$). In contrast, TNFR:Fc treatment did not reduce total MMP-2 activity in RV (1.2 ± 0.21 after TNFR:Fc therapy).

4. Discussion

Post-MI remodeling is an important cause for the development of chronic HF. In the present study we demonstrate that
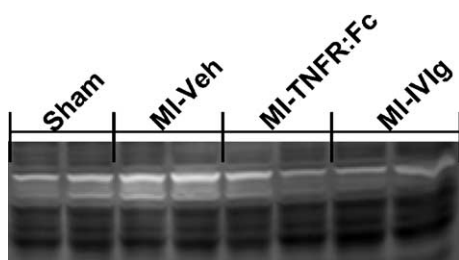

Fig. 4. Representative zymogram depicting gelatinolytic activity of pro- (upper band) and active (lower band) matrix metalloproteinase (MMP)-2 in right ventricular tissue from sham-operated rats, untreated rats with myocardial infarction (MI-Veh) and MI rats treated with recombinant soluble tumor necrosis factor p75 (MI-TNFR:Fc) and intravenous immunoglobulin (MI-IVIg).

early intervention targeted against the inflammatory response following MI attenuates the left and right ventricular remodeling processes. In rats subjected to acute MI, two different immunomodulatory treatment regimens, i.e., TNFR:Fc and IVIg, significantly reduced the development of RV hypertrophy. Furthermore, both treatment modalities also reduced the induction of genes related to the remodeling process such as hypertrophy, fibrosis and inflammation in the non-ischemic parts of LV and, in particular, in the RV. Moreover, LV hemodynamic variables were unaffected by the immunomodulatory treatment suggesting that the TNFR:Fc and IVIg-induced changes in RV were not secondary to changes in LV hemodynamics or wall stress. These findings raise the possibilities that immunomodulating therapy could delay or inhibit the development of HF after MI by inhibiting the remodeling process.

The post-MI inflammatory response is a prerequisite for effective wound healing, restriction of infarct expansion and scar formation [1]. Hence, independent of the effects on the non-ischemic myocardium, anti-inflammatory treatment of MI may potentially have both harmful (by impairing the repair process) and beneficial (by limiting infarct size) effects on myocardial integrity and function. However, while TNFα has been shown to play an important role in regulation of infarct size [21,22], the size of the MI was not influenced by

either TNFR:Fc or IVIg in this study. Moreover, immune modulation did not significantly affect the expression of the genes examined in the infarction area. These results indicate that the two treatments did not adversely affect the infarction repair process and that the observed effects on ventricular remodeling and gene expression cannot be accounted for by changes in the size or composition of the infarct. Although LV cavity size increased post-MI it was unaffected by treatments. LV weight, septal width were not changed post-MI and were unaffected by immunomodulatory therapy. The absence of LV hypertrophy, however, is not unexpected, particularly at the 7-day post-MI. The lack of change in overall LV weight at this time is likely due to an increased septal weight that is compensated by a reduction of infarcted LV free wall weight. Moreover, due to ventricular dilation, septum thickness does not necessarily increase after an MI.

Most studies examining gene expression after MI in animal models have focused on the LV. In the present study, by examining the infarcted area, non-ischemic parts of LV and the RV, we found that MI induced an almost similar effect on the expression of fetal genes and genes related to extracellular matrix and inflammation in all areas despite their being exposed to differences in loading, ischemia and paracrine stimuli. However, although immunomodulatory therapy had significant effects on the expression of several of these genes in non-ischemic segments of the LV, the effects in the RV were more prominent. The reasons for these differences are at present unclear, but potentially reflect that the stimuli responsible for the changes occurring in the heart post-MI vary between the right and left ventricles. Thus, while ischemia and local inflammatory activation may be the most important stimulus for enhanced gene expression in the infarcted area, indirect mechanisms such as diastolic stretch and paracrine signaling may be of more importance in non-ischemic parts of LV and the RV. Measurements of LVEDP, LVSP and maximum $+\mathrm{d}P/\mathrm{d}t$ and $-\mathrm{d}P/\mathrm{d}t$ demonstrated that the post-MI impairment of LV function remained unchanged by immunomodulatory treatment. Thus, it is unlikely that the changes observed in RV during therapy were secondary to improved LV function. However, endocrine influence may be of particular importance for the alterations in RV. A recent study in animal models showed that while etanercept decreased plasma cytokine levels, there was no decrease in IL-6 and MCP-1 within the myocardium [23]. Thus, it is tempting to hypothesize that modulation of systemic inflammation may be of more importance for gene expression in the right than in the left ventricle post-MI. Alternatively, the immunostimulation in the RV could be of lesser intensity than in the LV and, thus, easier to overcome by treatment. Whatever the mechanisms involved in regulating RV gene expression, our findings also underscore the limitations of these treatment modalities by showing more modest effects on the gene expression in LV.

The beneficial effects of anti-TNFα therapy have previously been demonstrated in animal models with myocardial overexpression of TNFα [24,25]). Recently, Sugano et al. demonstrated beneficial effects of myocardial transfer of an expression vector encoding soluble TNFR type I after MI [21]. However, we demonstrate for the first time salutary effects of systemic administration of TNFR:Fc in a rat model for MI. TNFR type I seems to be the most important in mediating cardiac remodeling, while TNFR type II rather may mediate cardioprotective responses [26]. However, although we used soluble TNFR type II, this should block binding of TNFα to both types of receptors. Although this could potentially attenuate beneficial effects mediated by the type II receptor, the primary effect would appear to be inhibition of maladaptive responses of TNFα that are mediated through the type I receptor.

Inflammatory cytokines may promote myocardial remodeling and development of heart failure by a range of mechanisms that include direct stimulation of cardiomyocyte hypertrophy and apoptosis, as well as interaction with Ca^{2+}-dependent processes and changes in β-adrenergic signal transduction [4]. In the present study, we have focused on the ability of immunomodulating therapy to counteract changes in expression of genes associated with myocardial hypertrophy, collagen metabolism and inflammation during post-MI remodeling. Future studies will have to characterize in more detail the molecular mechanisms of action of these drugs and their effects on the functional and structural recovery after MI.

Unrestricted MMP activation seems to play an important pathogenic role in myocardial remodeling after MI [27]. MMP-2 is ubiquitously distributed in cardiac myocytes and fibroblasts. Since it shows persistent upregulation after MI, it may be of particular importance in the remodeling process. The finding that targeted deletion of MMP-2 prevents early LV rupture and late remodeling after experimental MI in mice supports this hypothesis [28]. In the present study we show that immunomodulatory therapy with both IVIg and TNFR:Fc decreased MMP-2 gene expression and activity in RV, and also to some degree reduced gene expression in the non-ischemic part of LV. These changes in MMP gene expression and activity were accompanied by reduced expression of genes for collagen I and III in RV and also for collagen III in the non-ischemic part of LV. The changes in collagen and MMP levels may result both in reduced ventricular dilation and less fibrosis, although in the present study we have no dimensional or histological data that support this notion. However, most previous studies that have reported an increase in septal fibrosis in the post-MI rat heart have looked at later time points (e.g., [29]) and it is likely that at the 7-day point the changes are not yet of sufficient magnitude to be detected. Nevertheless, the control of MMP activation in the failing myocardium has been suggested as a new and potentially significant therapeutic target for treating cardiac remodeling [27], and our findings suggest that immunomodulatory therapy with IVIg or TNFR:Fc may represent a therapeutic option to achieve this purpose.

Although timing of treatment prior to MI is not realistic clinically, our results show similar effects with both forms of therapy suggesting that IVIg, which was given post-MI, is at least as potent as TNFR:Fc, which was given prior to MI, as an immunomodulatory agent to prevent post MI HF. The difference in timing may limit our ability to dissect the potential role of immune activation following MI. However, our recent work [30] demonstrated that immune activity characterized by increased cytokine concentration and macrophages invasion is elevated >2 days post MI during which time TNFR:Fc and IVIg are already present. Whereas TNFR:Fc had no effect on the myocardial expression of inflammatory genes, IVIg significantly suppressed the expression of MCP-1 and TNFα in the RV. This lack of anti-inflammatory effects within the myocardium during TNFR:Fc therapy has also been reported by others [23], and could be a possible mechanism for the failure of anti-TNF therapy which has recently been reported in human heart failure [31,32]. While TNFR:Fc is targeted specifically against TNFα, IVIg represents a broad immunomodulating therapy, involving several potentially important mechanisms such as a profound effect on the cytokine network, shifting the balance towards anti-inflammatory net effects [9], and the present study suggest that such an approach also could be beneficial after MI.

The disappointing results from the anti-TNFα outcome trials in chronic HF have raised questions about the future of immunomodulating therapy in this disorder [31,32]. However, our results suggest that targeted therapy against TNFα as well as other immunomodulatory approaches such as IVIg may have beneficial effects on the development of post-MI cardiac remodeling. Importantly, while the patients in the anti-TNFα trials all had chronic HF, we intervened in the acute phase of MI. Recently, using mice overexpressing TNFα, Li et al. demonstrated that intervention with an MMP inhibitor had beneficial effects in young mice, but not in older mice with established HF [33]. Early initiation of immunomodulation post-MI could therefore represent an interesting strategy for immune-based therapy through which inhibition of remodeling could reduce the likelihood of progressing to HF in the future.

Acknowledgments

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