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Inflammation and postinfarct remodeling: Overexpression of IκB prevents ventricular dilation via increasing TIMP levels

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Abstract

Objective: Nuclear factor-kappa B (NF-κB) orchestrates genes involved in inflammation and extracellular matrix (ECM) remodeling following myocardial infarction (MI). The objective of the present study was to investigate the effect of overexpression and mode of function of IκB, the natural inhibitor of NF-κB, on ECM remodeling in a rat model of MI.

Methods: MI was induced in male Sprague-Dawley rats by ligation of the left anterior descending coronary artery (LAD) and was followed by adenovirus-mediated intramyocardial transfection of IκB ($n=26$) or LacZ reporter genes ($n=26$). Sham-operated animals ($n=14$) served as controls.

Results: In transthoracic echocardiography 49 days after MI, systolic and diastolic left ventricular dimensions were reduced while fractional shortening was preserved in the treatment group. Additionally, evaluation on the isolated heart showed an attenuated downward shift of pressure–volume relationships in the IκB group compared to LacZ. NF-κB p65 DNA binding activity was diminished both at 5 and 49 days post-MI in the treatment group. Five days post-MI in the treatment group, protein levels of tumor necrosis factor (TNF)-α and interleukin (IL)-1β were significantly reduced by 72.6% and 73.2%, respectively, compared to LacZ ($p<0.05$). In parallel, matrix metalloproteinase (MMP)-2 and MMP-9 levels were reduced 5 days post-MI, with MMP-9 still being decreased 49 days post-MI ($p<0.01$). In contrast, tissue inhibitors of metalloproteinases (TIMP)-1, -2, and -3 were increased compared to LacZ ($p<0.01$ and $p<0.05$, respectively) 5 days post-MI. After 49 days, TIMP-2, -3, and -4 expressions were significantly elevated ($p<0.05$).

Conclusion: Reducing NF-κB activity via IκB overexpression after MI positively influences ECM remodeling by reducing MMP-2 and -9 levels while increasing TIMP-1, -2, -3, and -4 levels. Therefore, IκB overexpression prevents ventricular dilation and consequently preserves cardiac function.

Keywords: Myocardial infarction; Gene therapy; Nuclear factor-kappa B; Extracellular matrix remodelling

1. Introduction

The loss of viable myocytes following myocardial infarction (MI) goes hand in hand with geometric, structural and functional alterations of the myocardium called cardiac remodeling (CR). While CR is crucial

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during the first days post-MI to maintain cardiac function and increase the chance of survival, in the long run it is the leading cause of heart failure and late cardiac death. Acute MI starts a cytokine cascade in both the infarcted and non-infarcted myocardium via complement activation and reactive oxygen species [1–3]. Inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-1beta (IL-1β) and interleukin-6 (IL-6) trigger the pathophysiological alterations characteristic of CR such as replacement of the infarcted area by scar, myocyte hypertrophy, switch to fetal gene programs, myocyte loss through apoptosis, alterations of the ECM and endothelial dysfunction [1].

A hallmark of early CR post-MI is the expansion of the infarcted area as a result of the degradation of ECM molecules, caused by an imbalance in the ratio of matrix metalloproteinases (MMPs) and tissue inhibitors of matrix metalloproteinases (TIMPs) [4]. This mechanism could also be shown in chronically failing hearts and has led to the suggestion that the loss of collagen with subsequent chamber dilation is a major determinant of the pathophysiology of CR [5]. It has further been demonstrated that inflammatory cytokines decrease collagen synthesis in cardiac fibroblasts, enhance total MMP-activity and reduce TIMP expression in both myocytes and non-myocytes [6,7]. A critical regulatory element in this coordinated activation of cytokine and adhesion molecule genes is the dimeric transcription factor NF-κB [8,9]. A variety of stimuli including cytokines, ischemic and mechanical stress, reactive oxygen species, and growth factors activate IkappaB kinase, a kinase complex which leads to degradation of inhibitor kappa B (IκB) [8]. IκB dissociates from its complex with NF-κB, thereby NF-κB is activated and moves to the nucleus where it up-regulates the transcription of κB-responsive genes [9].

NF-κB activity is significantly up-regulated within minutes after MI and can be further increased by following reperfusion [10]. Blocking NF-κB activity in experimental models of acute myocardial ischemia and myocarditis reduced the expression of adhesion molecules and inflammatory cytokines and consequently preserved cardiac function [11–14]. In addition to orchestrating the inflammatory cascade post-MI, NF-κB directly regulates the transcription of MMP-1, MMP-2, MMP-3, MMP-9 and MMP-13 and TIMP-1 [15,16].

So far the effect of NF-κB inhibition after inducing ischemic injury was examined only over a short-term. We recently presented the positive hemodynamic effects of reducing NF-κB by adenoviral-mediated overexpression of its natural inhibitor IκB [17]. It was the purpose of the present study to test the hypothesis whether this reduction of NF-κB activity by adenoviral-mediated overexpression of IκB is mediated via attenuation of inflammation and matrix degradation.

2. Materials and methods

2.1. MI protocol

MI was caused in adult male Sprague-Dawley rats (395±26 g) by ligating the LAD as described in Podesser et al. [18]. Briefly, animals were anesthetized intraperitoneally with a mixture of xylazin (1 mg/100 g bodyweight) and ketamin (10 mg/100 g bodyweight) and ventilated mechanically. A left lateral thoracotomy was performed and a ligature using a 6-0 prolene was placed around the LAD beneath the left atrium. Immediately after the onset of ischemia gene transfer was performed according to the protocol described in the next section. Sham-operated rats underwent the same procedure, except no suture was placed around the LAD and no gene transfer was performed. All animals had free access to standard rat chow and water during the observation period of 7 weeks and received humane care in compliance with 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). The experiments were approved by the committee for animal research, Medical University of Vienna.

2.2. Adenovirus constructs

As described earlier, for the overexpression of IκBα, we used an adenoviral construct containing the coding sequence for human IκBα together with a nuclear localization signal driven by a cytomegalovirus promoter (rAd.IκBα) as described in Trescher et al. [17] and Wrighton et al. [19]. As control, adenoviral construct a recombinant adenovirus containing the coding sequence of β-Galactosidase (rAd.β-gal) under the control of the Rous Sarcoma Virus promoter was used which is described in Stratford-Perricaudet et al. [20].

2.3. Intramyocardial gene delivery

After MI, animals were randomised to receive either rAd.IκB (treatment group, $n=26$) or rAd.β-gal (control group, $n=26$). Sham operated animals (sham group, $n=14$) did not receive gene transfer. For intramyocardial gene delivery, the heart was exposed and the pale area of the left ventricular free wall was identified as the infarcted myocardium. A total of $1.56\times10E9$ pfu of rAd.IκB and rAd.β-gal, respectively, in a final volume of 250 μL were delivered by a 25-gauge needle into 5 sites around the ischemic area in the periinfarct zone.

2.4. In vivo hemodynamic measurements

Transthoracic echocardiography was performed before the induction of MI to obtain baseline data and 7 weeks post-MI, as described earlier [17]. Echocardiographic

studies were carried out under light anesthesia with xylazin (1 mg/100 g bodyweight) and ketamin (10 mg/100 g bodyweight) to allow spontaneous respiration. Imaging was performed by an experienced ultrasonographer blinded to the study groups using a VINGMEDSound, CFM 800 with a 7.5 MHz standard pediatric transducer.

2.5. In vitro hemodynamic measurements

In vitro hemodynamic measurements were performed by using an isolated red cell perfused working heart apparatus as described previously [21]. Briefly, after completion of echocardiographic measurements animals were heparinized (200 IU i.v.) and the beating heart was rapidly excised. Within 10 s, it was placed on the perfusion apparatus and perfusion started in the Langendorff mode (constant pressure at 60 mmHg). The perfusate consisted of a Krebs–Henseleit buffer with bovine erythrocytes at a hematocrit of 20% [18]. After cannulation of the left atrium for conversion to working heart mode, a micro-tip catheter (Millar SP-407, Millar) was placed in the left ventricle via the aortic valve for continuous measurement of LV pressure. Afterload was set at a constant pressure of about 60 mmHg, and post-MI and sham-operated hearts were paced with 220 bpm by an electric stimulator (HSE stimulator P, Hugo Sachs Elektronik) and allowed to stabilize for about 15 min. To determine the influence of increasing afterload on these hearts pressure–volume relationships were performed by increasing the afterload in 10 mmHg increments while cardiac output (identical to left atrial flow (LAF), mL/min; Flowmeter Narcomatic RT-500, Narco Biosystems) and LV systolic pressure were monitored [22]. External heart work was calculated by multiplying cardiac output and left ventricular systolic pressure. All hemodynamic parameters were registered continuously as mean values, derived from respective pressure and flow tracings, and monitored by an online data acquisition system.

2.6. Infarct size and tissue collection

After evaluation on the Langendorff apparatus hearts were immediately placed in iced saline. Total heart weight was determined, the atria were removed from the ventricles, the right ventricle was separated from the left ventricle, the infarcted region was dissected from the non-infarcted LV, and each part was weighed and immediately frozen in liquid nitrogen. Infarct size was expressed as the ratio of the infarct region to total LV mass.

2.7. Transcription factor activity assay

Tissue was solubilized as described by Abraham et al. [23]. The DNA binding activity of the tissue lysates (30 μg/well) were measured using the BD™ TransFactor NFκB p65 Kit (BD Biosciences, Palo Alto, CA, USA). The TransFactor Kit was performed according to the manufacturer's protocol. The absorbance of the plate was measured at 655 nm with a microplate reader (Anthos Labtec Instruments Reader 2001, Austria).

2.8. Western blotting

Tissue was solubilized as described above. Tissue lysates (50 μg/lane) were separated by SDS–PAGE prior to electrophoretic transfer onto Hybond C super (Amersham, Uppsala, Sweden). The blots were probed with antibodies against IêB (Cell Signaling Technology), TNF-α, IL-1β (R&D Systems, Inc.), MMP-2, -9, TIMP-1, -2, -3 and -4 (Chemicon, Temecula, CA, USA) prior to incubation with horseradish peroxidase-conjugated secondary antibodies (Amersham). Proteins were immunodetected on the membrane using chemiluminescence (Supersignal-West-Femto, Pierce, Rockford, IL, USA) and specific protein bands were quantified using Easy plus Win 32 software (Herolab, Wiesloch, Germany). Ponceau staining (Amersham Biosciences) confirmed the successful transfer and was used to normalize the blots to avoid misinterpretation loading differences.

2.9. Statistical methods

Data are presented as mean±standard deviation (S.D.). Student's t test or one-way ANOVA was applied to compare changes in different groups, as appropriate. If a significant F value was obtained, comparison among the means was performed using post-hoc Tukey's HSD test and Fisher's test, respectively. For all statistical procedures, SPSS statistical analysis software (version 10.0 for windows) was used. Statistical significance was considered at $p<0.05$.

3. Results

3.1. Mortality

Three animals in the IκB-treated group and 2 animals in the LacZ infarct group did not survive the first 48 h post-MI, none of the sham-operated animals died. After 5 days, 6 sham-operated animals and 8 IκB-infarct and 8 LacZ infarct rats were sacrificed for the evaluation of NF-κB activity, IκB, TNF-α, IL-1β, MMP-2, -9 and TIMP-1, -2, -3 and -4 expressions. 8 sham-operated and 15 IκB- and 16 LacZ-transfected animals completed the study protocol of 7 weeks.

3.2. Morphometric data

Bodyweight and tibia length were similar in all groups (Table 1). Infarct size did not differ between IκB-treated and LacZ-transfected rats. Only animals with an infarct size of more than 30% LV weight were included in further analysis, 2 animals in the LacZ-infarct group and 1 animal in the IκB-infarct group were excluded because of infarct size less than 30% of LV mass. Total heart weight and LV/body weight ratio were significantly increased in MI hearts compared to

Table 1
Morphometric and echocardiographic data 49 days post-MI

	Sham-operated ($n=8$)	Infarct+LacZ ($n=14$)	Infarct+IκB ($n=14$)
Infarct size, % total LV weight	N/A	42.4±8.3	41.8±8.2
Body weight, g	463±25	455±18	461±29
Tibia length, mm	46.7±1.3	46.8±0.9	47.1±1.1
Total heart weight, g	1.88±0.26	2.32±0.21	2.32±0.13
LV weight, g	1.16±0.09	1.43±0.11***	1.52±0.07***
LV-to-body weight ratio	2.5±0.1	3.1±0.2***	3.3±0.2***
LV diameter diastolic, mm	5.2±0.9	8.5±0.5***	6.6±0.5**,***
LV diameter systolic, mm	2.8±0.5	6.7±0.2***	5.0±0.8**,***
FS, %	41±5	19±4***	29±1*,***

Values are given as mean±S.D.
* $p<0.05$ vs. LacZ.
** $p<0.01$ vs. LacZ.
*** $p<0.05$ vs. sham-operated.

sham-operated hearts, while there was no difference between the two infarct groups.

3.3. IκB overexpression and NF-κB activity

At 5 days post-MI, the level of IκB determined by Western blotting showed a 5.8-fold increase in the IκB-transfected hearts compared to MI hearts transfected with the LacZ gene ($p<0.05$, Fig. 1A). IκB overexpression led to a significant reduction in NF-κB DNA binding activity 5 days post-MI compared to LacZ ($p<0.01$, Fig. 1B). NF-κB activity was still reduced 49 days post-MI ($p<0.05$).

3.4. In vivo hemodynamics

Seven weeks post-MI systolic and diastolic LV, diameters were increased in both infarct groups compared to the sham-operated animals (Table 1). Nevertheless, LV dilation was significantly attenuated in the IκB-treated hearts compared to the LacZ-transfected hearts for diastolic (6.6±0.5 mm vs. 8.5±0.5 mm, $p<0.01$) and systolic (5.0±0.8 mm vs. 6.7±0.2 mm, $p<0.01$) values. Consequently, fractional shortening was reduced in the MI hearts compared to the sham-operated hearts (41±5% vs. 19±4% and 29±1%, $p<0.01$); in LacZ-transfected hearts, this reduction was more pronounced than in the IκB treatment group (19±4% vs. 29±1%, $p<0.01$).

3.5. In vitro hemodynamics

At 49 days post-MI, at any given afterload, the recovery of cardiac output was significantly reduced in both MI groups compared to sham operated hearts. However, IκB-treated hearts showed a significantly higher recovery of

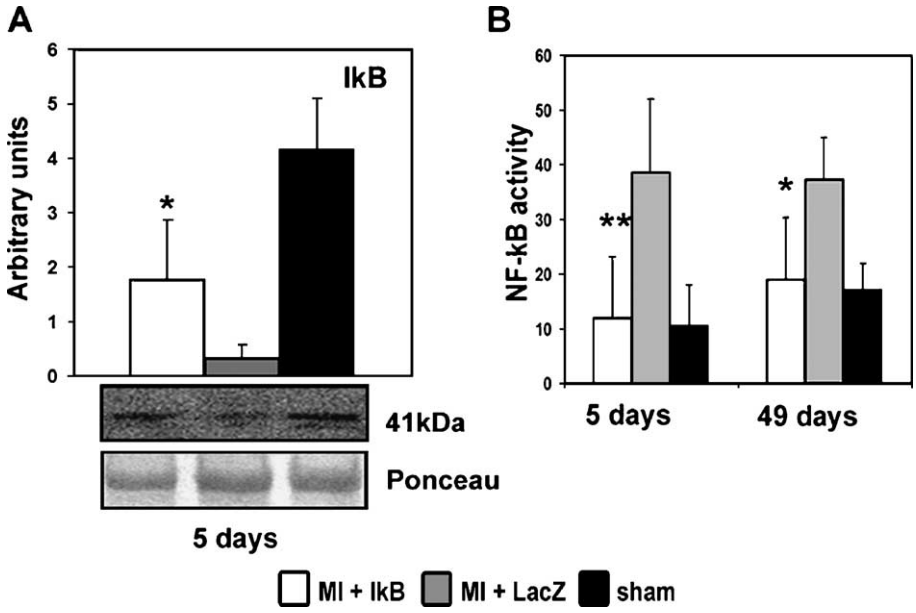

Fig. 1. Effect of direct intramyocardial transfer of the IκB gene to the periinfarct zone immediately post-MI: (A) IκB expression levels in the periinfarct zone 5 days post-MI and gene transfer in the three groups; (B) NF-κB activity in the respective samples in the periinfarct zone 5 and 49 days post-MI. *$p<0.05$; **$p<0.01$ vs. LacZ.

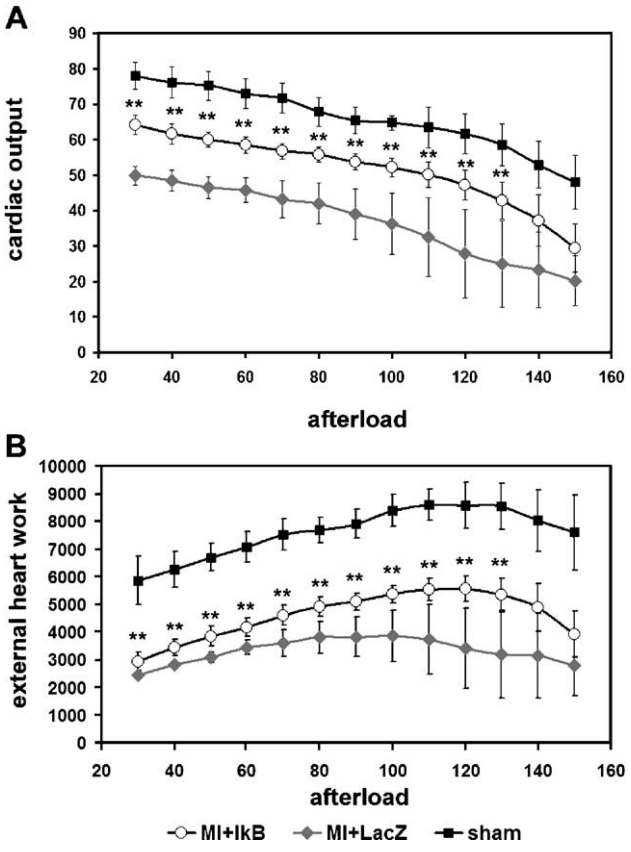

Fig. 2. Effect of IκB overexpression on cardiac function 7 weeks post-MI: (A) cardiac output [ml min^{-1}] is depicted as a function of afterload [mmHg]; (B) external heart work [dyn cm min^{-1}] as function of afterload. Both infarct groups shifted downward compared to the sham-operated hearts ($n=8$) indicating heart failure. This downward shift was less pronounced in the IκB-treated hearts ($n=14$) compared to LacZ ($n=14$). **$p<0.01$ IκB vs. LacZ.

cardiac output within the physiologic range (30 mmHg–130 mmHg, $p<0.01$) compared to the LacZ group (Fig. 2).

External heart work showed a similar picture: in both infarct groups external heart work was reduced compared to the sham-operated control hearts, nevertheless IκB-treated-MI hearts showed a significantly higher preservation of LV function within the physiologic range (30 mmHg–130 mmHg, $p<0.01$) compared to the LacZ group.

3.6. Effect of NF-κB blockade on inflammatory cytokines

At 5 days post-MI, up-regulation of TNF-α in the non-infarcted myocardium evaluated by immunoblotting was reduced by 72% compared to the LacZ-transfected MI hearts ($p<0.05$, Fig. 3). We were unable to detect measurable levels of TNF-α protein in the sham-operated control hearts. IL-1β expression showed a similar picture: IκB overexpression inhibited the increase of IL-1β expression by 73% ($p<0.05$). As seen with TNF-α, there was no IL-1β expression detectable in the sham-operated hearts.

At 49 days post-MI, IL-1β protein was measurable only at a very low level and did not show any significant difference between the two infarct groups while TNF-α protein could not be detected any more.

3.7. Effect of NF-κB blockade on MMPs and TIMPs

At day 5 post-MI, blocking NF-κB activity led to a significant reduction of MMP-9 expression in the non-infarcted myocardium of the treatment group compared to the LacZ-transfected MI hearts ($p<0.05$, Fig. 4). This decrease in MMP-9 levels was accompanied by a significant increase in the expression of its corresponding inhibitor,

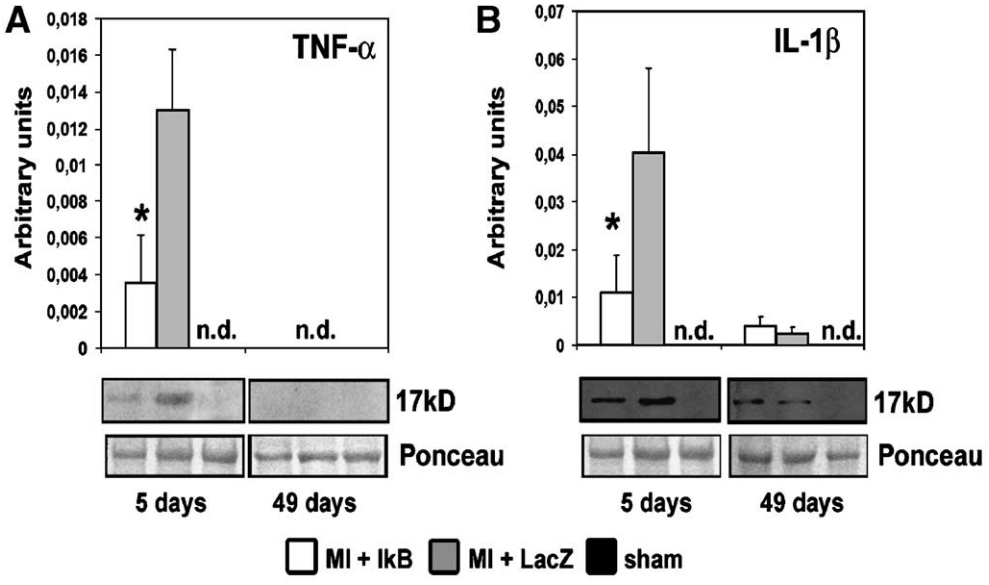

Fig. 3. Effect of IκB overexpression on inflammatory cytokines in the non-infarcted LV myocardium 5 and 49 days post-MI: (A) TNF-α expression; (B) IL-1β expression. *$p<0.05$ IκB vs. LacZ; n.d.=not detected.

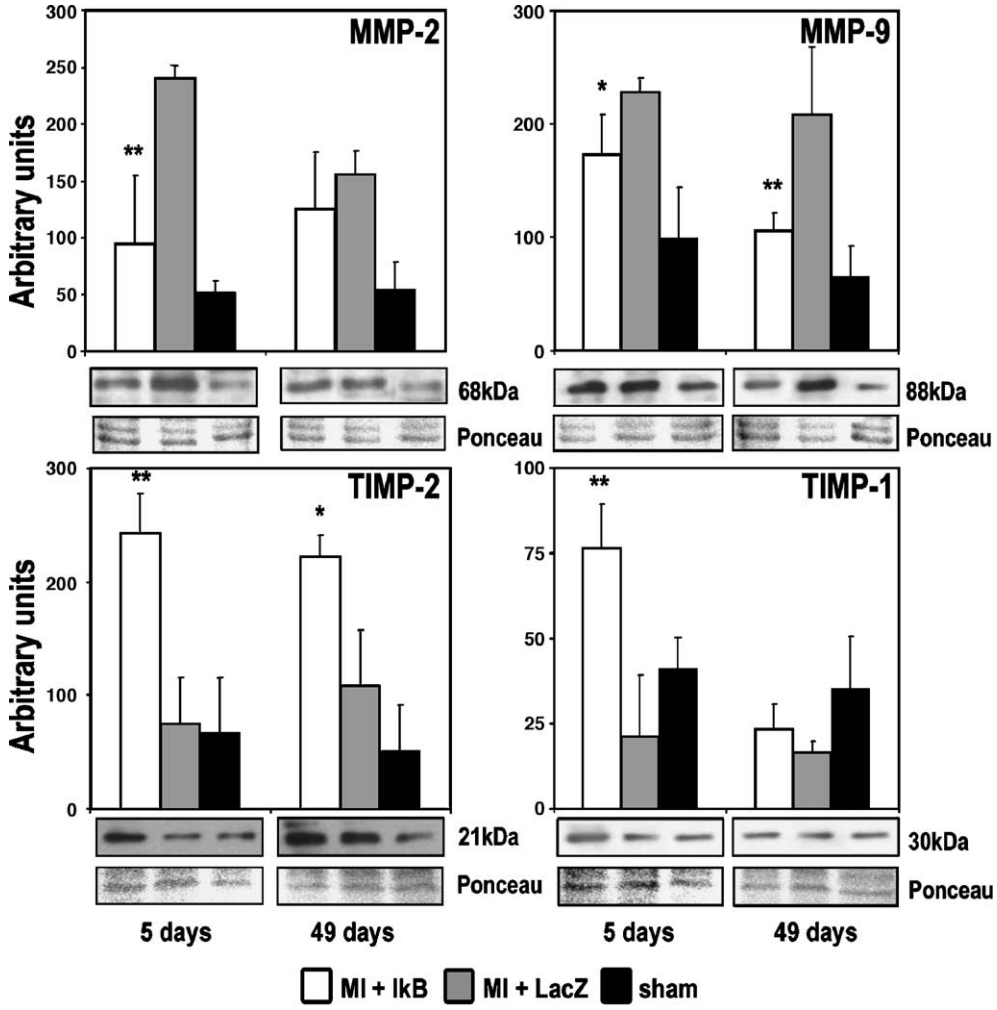

Fig. 4. Effect of IκB overexpression on MMP-2 and -9 and TIMP-1 and -2 protein levels in the non-infarcted LV myocardium 5 and 49 days post-MI. *$p<0.05$, **$p<0.01$ IκB vs. LacZ.

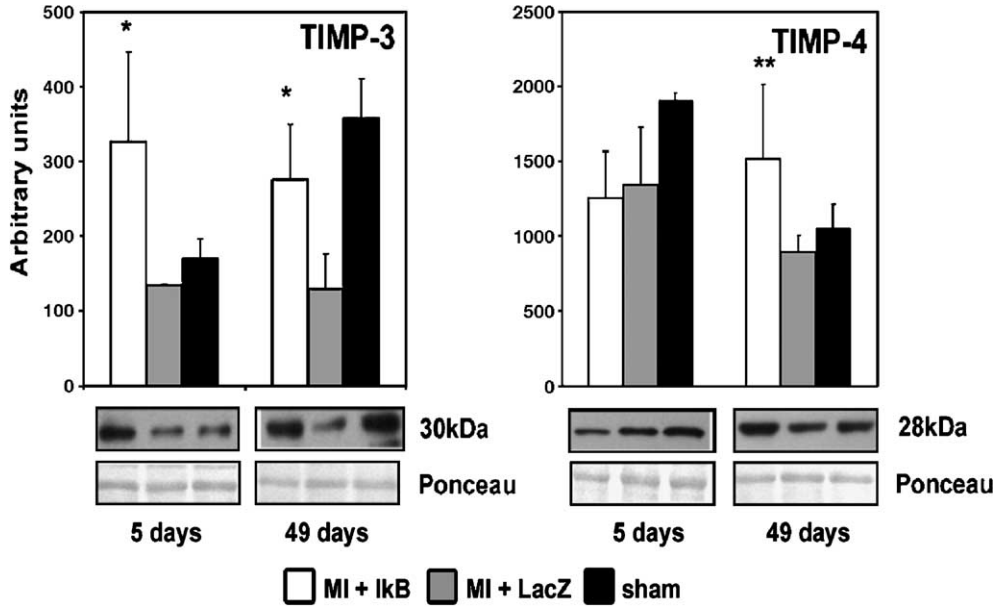

Fig. 5. Effect of IκB overexpression on TIMP-3 and -4 protein levels in the non-infarcted LV myocardium 5 and 49 days post-MI. *$p<0.05$ IκB vs. LacZ.

TIMP-1 compared to LacZ ($p<0.01$). The expression of MMP-2 and the corresponding inhibitor TIMP-2 showed a similar picture on day 5 ($p<0.01$).

Seven weeks post-MI MMP-9 expression was still decreased in IκB-treated hearts ($p<0.01$) while TIMP-1 levels did not show any difference between the two infarct groups. MMP-2 and TIMP-2 expression showed exactly the reverse picture: There was no significant difference in MMP-2 expression between the two groups while its corresponding inhibitor TIMP-2 was increased ($p<0.05$). Mean protein levels of TIMP-3 were significantly elevated in IκB-treated hearts at both 5 and 49 days post-MI compared to LacZ infarct group ($p<0.05$, Fig. 5). At 49 days post-MI, TIMP-3 levels were restored to the levels of the sham-operated control hearts.

Five days post-MI, TIMP-4 levels showed no difference between the two infarct groups whereas 49 days post-MI a significantly higher expression could be detected in the IκB-treated hearts compared to LacZ ($p<0.05$, Fig. 5).

4. Discussion

The present study focused on the long-term results of gene therapeutic-induced inhibition of NF-κB and demonstrated that a single therapeutic intervention at the time of acute MI improves the balance between proteolytic (MMPs) and matrix stabilizing factors (TIMPs) and thereby reduces LV chamber dilation and preserves LV function over a long-term observation period. The present study did not focus on the acute effect of NF-κB blockade with a possible reduction of infarct size. It was important to us to ensure that MIs were comparable between groups and hemodynamically relevant in order to cause ventricular remodeling. Therefore a cut-off point of 30% infarct-size of LV mass was chosen, although we were aware of the fact that we would probably eliminate the additional positive effect of reducing infarct size by inhibition of NF-κB activity.

Myocardial injury following ischemia is partially caused by an inflammatory reaction resulting from NF-κB activation. It is possible to attenuate the inflammatory cascade by blocking NF-κB activation, resulting in a significant improvement of cardiac function [11–14]. In an experimental model of MI in rats similar to ours, Morishita et al. used NF-κB *cis* element decoys to reduce NF-κB activation and observed an attenuation of the inflammatory response from 2 up to 48 h post-MI [13]. In contrast to decoy oligonucleotides, which show an immediate onset of blocking NF-κB activation, the expression profile of the adenovirus has a characteristic 48 h delay in the onset of transcription with an expression peak after 5 to 7 days [24]. As expected, in our study there was no transgene detectable 24 h after transfection (data not shown). Reduced activity of TNF-α and IL-1β – two major players in the inflammatory cascade being directly regulated by NF-κB – could be observed 5 days post-MI.

A delay in the onset of attenuating the inflammatory response post-MI might be a prerequisite for the success of anti-inflammatory strategies to prevent postinfarct remodeling. Experimental and clinical failure of different approaches blocking the inflammatory cascade in the first hours after the ischemic injury demonstrated the importance of inflammation for infarct stabilization and wound healing [25]. Inhibition of TNF-α activity in a rat model of MI led to an improvement of diastolic and systolic function and to a reduction of matrix turnover [26,27]. In contrast, the administration of anti-IL-1β antibodies immediately after coronary artery ligation in mice increased the occurrence of ventricular rupture, promoted left ventricular dilation and hypertrophy of the non-infarcted myocardium [28]. These results indicate a protective role of IL-1β in stabilizing the infarcted area in the acute phase immediately after MI, whereas a sustained increase in IL-1β levels during subsequent remodeling may be detrimental.

One of the most deleterious effects of the inflammatory response is ventricular dilation resulting from the disruption of the collagen network. Inflammatory factors like TNF-α and IL-1β lead to the activation of MMPs as could be shown both in vivo and in vitro [5,6,29]. At the same time, the expression of TIMPs is down-regulated [7]. An imbalance in the expression of this proteolytic and inhibitory factors leads to the disruption of the ECM and destroys the structural integrity of the LV with consecutive dilation and impairment of ventricular function. Interventions by selective and broad-spectrum MMP-inhibitors to prevent cardiac remodeling in different heart failure models showed mixed results. Application of a broad-spectrum MMP inhibitor in mice after LAD ligation reduced both diastolic and systolic LV diameter with preserved fractional shortening 4 days after MI [30]. Similar results concerning LV remodeling could be achieved in MMP-9-deficient mice after MI [31]. In contrast, other studies reported an impairment of the normal wound-healing response with deleterious effects on myocardial function due to the loss of MMP-activity in the acute phase post-MI [32,33].

In the present study, we chose MMP-2 and -9 since they play a central role in cardiac remodeling, and their transcription is directly regulated by NF-κB [15,16]. According to our results, NF-κB inhibition post-MI helps to maintain the balance of disrupting and stabilizing factors by two distinct pathways: (1) a reduction of the active NF-κB molecule leads directly to a reduced expression of MMP-2, -3, -9, as the transcription of the genes encoding for these MMPs is regulated by NF-κB (2) via attenuating the inflammatory response with lower tissue levels of TNF-α and IL-1β – both activators of MMPs – proteolytic activity is downregulated [6,7].

Recently, TIMP-3 has been identified as a key-factor in the regulation of cardiac remodeling and its reduction has been associated with the transition of compensated to end-stage heart failure [34]. This reduction is accompanied by elevated gelatinase activity, matrix turnover and significant

changes in matrix content and structure. In an experimental model, TIMP-3 deficiency leads both to impaired cardiac function and alterations of cardiac structure [35]. In contrast, recovery of cardiac structure and function in failing hearts is accompanied by a restoration of TIMP-3 levels [36].

The profound consequences of TIMP-3 reduction can be explained by its broad spectrum within the ECM. Unlike TIMP-1, -2 and -4, it binds to the ECM, where it neutralizes MMPs, inhibits TNF-α converting enzyme (TACE) and influences cell survival [37,38]. It is thus very likely that the consequences of reduced TIMP-3 levels go beyond the entire MMP inhibition with promoting cell survival via a Fas-associated death-domain-dependent mechanism and reducing TNF-α secretion and activation. In our study, TIMP-3 expression was significantly elevated in the treatment group both 5 days and 49 days post-MI and thereby supports the important role in post-MI remodeling.

Of the currently known TIMPs, TIMP-4 is expressed in higher abundance within the myocardium compared to other tissues. Hence it is very likely that different cardiac disease states are accompanied or even caused by changes in TIMP-4 levels. Schulze et al. showed a central role of TIMP-4 in acute ischemia–reperfusion injury with a negative correlation between the recovery of myocardial function and TIMP-4 levels [39]. The importance of TIMP-4 abundance not only in acute ischemia but for matrix turnover in postinfarct remodeling was reported in a rat model of MI [40]. A region- and type-specific analysis of MMP and TIMP expression in post-infarct remodeling revealed that decreased TIMP-4 levels are correlated to the extent of LV remodeling [41].

Success and failure of attempts targeting inflammation and ECM-remodeling show the importance of finding the right “therapeutic window”, and of getting more insight in the adaptive and maladaptive process driven by the cytokine cascade in the development of heart failure. The key to success in a disease as complex as heart failure does not lie in singling out a particular pathway for complete elimination but lies in modulating several effector pathways.

To the best of our knowledge this was the first study to show that reducing NF-κB activation and thereby modulating inflammatory response and ECM remodeling in the acute phase post-MI has a positive long-term effect on the development of CR. The unique property of this gene therapeutic approach lies within the fact that a single intervention at the time of the acute ischemic event is sufficient to attenuate subsequent postinfarct remodeling. Preventing the development of heart failure after acute MI by the gene therapeutic approach as presented here, however, is well-matched to the current toolbox of gene transfer and cardiology. The proof-of-principle of this therapeutic concept and the importance of the timeframe might offer a preventive strategy for patients with acute MI at risk of developing chronic heart failure.

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