Increased cardiac IL-18 mRNA, pro-IL-18 and plasma IL-18 after myocardial infarction in the mouse; a potential role in cardiac dysfunction

Per Reidar Woldbaek\textsuperscript{a,b,*}, Theis Tønnessen\textsuperscript{a,b}, Unni Lie Henriksen\textsuperscript{a}, Geir Florholmen\textsuperscript{a}, Per Kristian Lunde\textsuperscript{a}, Torstein Lyberg\textsuperscript{c}, Geir Christensen\textsuperscript{a}

\textsuperscript{a}Institute for Experimental Medical Research, Ulleval University Hospital, 0407 Oslo, Norway
\textsuperscript{b}Department of Cardiothoracic Surgery, Ulleval University Hospital, 0407 Oslo, Norway
\textsuperscript{c}Research Forum, Ulleval University Hospital, Oslo, Norway

Received 5 September 2002; accepted 6 March 2003

Abstract

Objective: Interleukin (IL)-18 has been reported to be an important predictor for mortality in ischemic heart disease. IL-18 has proinflammatory properties, induces cell death and stimulates nitric oxide production. We hypothesized that following myocardial infarction (MI) an increased myocardial IL-18 production occurs, which may be involved in the pathogenesis of post-ischemic heart failure.

Methods and Results: Seven days after induction of MI in the mouse, myocardial hypertrophy and pulmonary edema were observed. RNase protection assay of tissue from the non-infarcted left ventricular myocardium revealed an increase in IL-18 (2.0-fold; \( P < 0.001 \)) and IL-1\( \beta \) (1.6-fold; \( P < 0.001 \)) mRNA after MI. Enhanced abundance of pro-IL-18 (1.4-fold; \( P < 0.05 \)), IL-18 receptor (3.5-fold; \( P < 0.05 \)) and IL-18 binding proteins (1.6-fold; \( P < 0.05 \)) was also demonstrated, whereas cardiac IL-18 protein decreased by 25\% (\( P < 0.05 \)) following MI. However, the concentration of circulating IL-18 was significantly elevated (MI; 90.4 ± 11.7 pg/ml, sham; 47.2 ± 4.2 pg/ml; \( P < 0.001 \)). After MI, enhanced cardiac activity of the pro-IL-18 processing enzyme, caspase-1, was measured. Additionally, a 3.4-fold increase (\( P < 0.001 \)) in the activity of the IL-18 degrading enzyme, caspase-3, was found in cardiac tissue, which may explain the observed reduction of cardiac IL-18 protein abundance. Finally, IL-18 reduced shortening of electrically stimulated adult cardiomyocytes and left ventricular contractility in vivo.

Conclusions: After MI in the mouse, increased production of cardiac IL-18 mRNA and pro-IL-18, as well as circulating IL-18 occurs. Since IL-18 also reduced myocardial contractility, we suggest that IL-18 may be involved in the pathogenesis of contractile dysfunction following MI.

© 2003 European Society of Cardiology. Published by Elsevier Science B.V. All rights reserved.

Keywords: Cytokines; Gene expression; Heart failure; Infarction; Myocytes

1. Introduction

Interleukin (IL)-18 is a member of the IL-1 cytokine family and has structural similarities with IL-1\( \beta \) [1]. In unstimulated cells, IL-18 is present primarily in the inactive precursor form pro-IL-18 [2]. The intrinsic processing of pro-IL-18 by caspase-1, also known as IL-1\( \beta \)-converting enzyme (ICE), leads to secretion of mature IL-18 [3], whereas caspase-3 degrades mature IL-18 to inactive metabolites [4]. A soluble decoy receptor of IL-18, termed IL-18 binding protein (IL-18BP), acts as a natural antagonist and is considered to be a regulator of IL-18 bioactivity [5].

IL-18 is a proinflammatory cytokine [6]. It stimulates production of inducible nitric oxide synthase (iNOS) [7], tumor necrosis factor (TNF-\( \alpha \)) [8], and the combination of IL-18 and IL-12 synergistically induces interferon (IFN)-\( \gamma \) [9], which previously has been shown to depress myocardial contractility [10]. Moreover, IL-18 enhances the activity of cytotoxic T-lymphocytes (CTL) [6,11] and induces synthesis of intercellular adhesion molecule

*Corresponding author. Tel.: +47-23-016-790; fax: +47-23-016-799.
E-mail address: p.r.woldbak@ioks.uio.no (P. Reidar Woldbaek).

\( \text{doi:10.1016/S0008-6363(03)00339-0} \)
Both CTL and ICAM-1 have been reported to be associated with development of cardiac dysfunction [13,14]. Recently, Pomerantz et al. [15] have shown in an ischemia/reperfusion model that inhibition of caspase-1 reduces contractile dysfunction in isolated human atrial trabeculae. Furthermore, IL-18 has been reported to be an important predictor for mortality in ischemic heart disease [16]. However, cardiac production and processing of IL-18 as well as its role in development of cardiac dysfunction following myocardial infarction (MI) still remain undefined. The biological actions of IL-18, as well as its structural and functional relationship with IL-1β, makes it conceivable that IL-18 contributes to development of the post-ischemic heart failure syndrome. Therefore, the characterization of the IL-18 processing during heart failure may allow development of new therapeutic strategies. In this context, we hypothesized that following MI a local cardiac de novo synthesis and elevated levels of circulating IL-18 occur, and that IL-18 reduces myocardial contractile function. Based on this hypothesis, we have examined cardiac synthesis and processing of IL-18 by caspase-1 and -3, and circulating levels of IL-18 1 week after induction of MI in the mouse. In addition we examined the cardiac tissue localization of IL-18 and the abundance of IL-18 receptors (R) and IL-18 binding proteins (BP). Finally, the effects of IL-18 on cardiomyocyte shortening and left ventricular contractility were assessed.

2. Methods

2.1. Animal preparation

Eighty-seven male BALB-C mice, 5–6 weeks old, were anesthetized and operated as previously described [17]. Briefly, a left thoracotomy was performed and a silk suture (8–0) was placed in the interventricular groove directly underneath the left atrium. Myocardial blanching was observed to ensure that the left coronary artery was occluded. Sham operated mice (sham) underwent the same surgical procedure except that no ligature was placed.

After 7 days the mice were sacrificed with excision of heart, lungs, liver and spleen. Heart and lungs were weighed immediately, and rapidly snap frozen in liquid nitrogen. Before freezing, left ventricles from MI-mice were divided into infarcted area and remaining non-infarcted area, which were easily distinguished macroscopically. Care was taken to avoid that the border-zone was included in the non-infarcted area. From sham hearts only left ventricular (LV) myocardium was used. Blood samples were collected following puncture of the right ventricle. The investigation conforms 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).

2.2. Cytokine and caspase mRNA measurements

The gene expression of IL-12p35, IL-12p40, IL-10, IL-1β, IL-1 receptor antagonist (Ra), macrophage migration inhibitory factor (MIF), IL-18, IFN-γ, IL-6 and of caspase-8, -3, -6, -11, -2, -7, -1 and -14 in the non-infarcted region of the LV myocardium was analyzed using commercially available kits (RiboQuant, Pharmingen, San Diego, CA) and antisense RNA multiprobes (mCK2b and mAPO-1, Pharmingen) according to the manufacturer’s protocol.

The densities of the RNase protection assay bands were determined with a phosphor imaging system (Fujiﬁlm Bio-imaging Analyser BAS-1800, Fuji Photo Film Co., Tokyo, Japan) and quantified using the software Image Gauge 3.12 (Fuji Photo Film Co., Ltd., Tokyo, Japan). The L32 bands were quantified separately after 3 h, whereas the cytokine bands were determined 16 h later. The mRNA expression data presented were normalized to the mean value of the two L32 bands. There were no significant differences between L32 signals in sham and MI-mice.

2.3. Protein immunoblot analysis

Homogenates of non-infarcted and infarcted LV myocardium, lung, liver and spleen tissues were prepared as previously described by Semb et al. [18] with minor modifications. Briefly, tissues were homogenized with a Polytron homogenizer in a HEPES buffer containing protease inhibitors. Protein concentrations were determined by the bicinchoninic acid assay (Pierce 23235). Protein homogenates (20 μg) were separated by standard sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) (8, 12 or 15%), transferred to polyvinyl difluoride membranes, blocked overnight in 10% non-fat dry milk at 4 °C and incubated with primary antibodies. The following antibodies were used: mature IL-18: anti-human IL-18 (RDI-IL18abR, Research Diagnostics Inc; 15% SDS-gel), pro-IL-18: anti-mouse IL-18 (sc-6179, Santa Cruz Biotechnology, Inc; 15% SDS-gel), IL-18Rα: anti-mouse IL-18Rα (AF-856, R&D Systems; 8% SDS-gel), IL-18BP: anti-mouse IL-18BPc and IL-18BPd (sc-9464, Santa Cruz Biotechnology, Inc; 12% SDS-gel) and caspase-1 p20: anti-mouse caspase-1 p20 (sc-1218, Santa Cruz Biotechnology, Inc; 12% SDS-gel). Antibody binding was detected by the ECL-system (Amersham Life Sciences). Recombinant mouse IL-18 (B 002-5) and mouse IL-18BPd (122-BP, both R&D Systems) were used as positive controls.

2.4. Enzyme-linked immunosorbent assay

The level of serum IL-18 was measured by a mouse IL-18 sandwich enzyme-linked immunosorbent assay (ELISA) kit from Medical and Biological Laboratories.
Table 1

<table>
<thead>
<tr>
<th>Body, heart and lung weights</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (Sham) g</td>
<td>30</td>
</tr>
<tr>
<td>BW (MI) g</td>
<td>40</td>
</tr>
<tr>
<td>LW (Sham) g</td>
<td>30</td>
</tr>
<tr>
<td>LW (MI) g</td>
<td>42</td>
</tr>
<tr>
<td>HW (Sham) g</td>
<td>30</td>
</tr>
<tr>
<td>HW (MI) g</td>
<td>42</td>
</tr>
</tbody>
</table>

Data represent means±S.E.M. MI, myocardial infarction; BW, body weight when sacrificed 7 days after primary operation; HW, heart weight; LW, lung weight. * P<0.05 vs. Sham, ** P<0.001 vs. Sham.

(Japan), according to the manufacturer’s protocol. The detection limit for IL-18 using this kit is 25.0 pg/ml.

2.5. Caspase-1 and caspase-3 activity

Homogenates of non-infarcted and infarcted LV myocardium were prepared as previously described by Fauvel et al. [19]. Activities of caspase-1 and caspase-3 were determined using CaspACE™ Assay System (Promega) according to the manufacturer’s protocol. Briefly, homogenates (100 µg) were incubated at 37 °C with fluorescent substrates Ac-YVAD-AMC and Ac-DEVD-AMC for caspase-1 and caspase-3, respectively, and the protease activities were calculated from the increase in fluorescence.

2.6. Immunohistochemical analysis

Immunohistochemical analysis was performed on hearts from sham (n=2) and MI-mice (n=2). Kidney tissue (n=2) served as positive control. The excised hearts were placed in ice-cold phosphate-buffered saline (pH 7.4), embedded in Tissue-Tek™ O.C.T. compound (Sakura Finetek, Torrance, CA, USA) and rapidly frozen in dry ice-cooled isopropanol and stored at −70 °C until further processed. Then 5-µm thick sections were cut, collected on Superfrost®Plus slides (Menzel-Glaser, Braunschweig, Germany) and fixed for 5 min in acetone at −20 °C and air-dried. After rinsing in Tris–buffered saline (TBS, pH 7.6) containing 0.05% Tween 20, sections were incubated with the DAKO Peroxidase Blocking Reagent (DAKO, Glostrup, Denmark) for 15 min at 37 °C followed by TBS rinsing. Sections were incubated for 30 min at 37 °C with rabbit polyclonal antibodies against IL-18 (H-173) Santa Cruz Biotechnology, Inc. (1:10). After three washes in TBS the primary antibody was labeled for 30 min using the DAKO EnVision™+ Peroxidase, Rabbit K4010 system and 3,3′-diaminobenzidine tetrahydrochloride as the chromogen. Sections were counterstained with hematoxylin for 1–2 min, mounted and examined in a Leitz Aristoplan microscope.

2.7. Measurements of cardiomyocyte function

Adult ventricular myocytes were isolated from hearts of male Wistar rats as previously described by Holt et al. [20]. Myocytes on laminated coverslips were placed in an open perfused cell bath and superfused with HEPES
Reidar Woldbaek et al. / Cardiovascular Research 59 (2003) 122–131
Tyrodes (HT) solution containing (mmol): NaCl 140, HEPES 5, glucose 5.5, MgCl₂ 0.5, CaCl₂ 1.8, KCl 5.4; pH 7.4. The myocytes were stimulated at 0.5 Hz with a bipolar pulse applied by platinum electrodes. The temperature was 32.5±0.5°C. Myocyte shortening was analyzed using a video-edge detector (model 103, Crescent Electronics, Sandy, UT). Prior to IL-18 stimulation, a stable period of myocyte contractions was recorded for 4 min. Then, the myocytes were superfused with HT solution containing IL-18 (100 ng/ml) for 4 min followed by 4 min with the HT solution. Fractional shortening (%), maximal shortening velocity (μm/s) and maximal relaxation velocity (μm/s) were measured in 8 myocytes from four separate isolations.

2.8. Measurements of left ventricular contractility

To assess the maximal positive derivative of the left ventricular pressure (LV+dP/dt), a 1.4 F Millar micro-tipped transducer catheter (Model SPR-671, Millar Instruments, Houston, TX, USA) was inserted into the right carotid artery and advanced into the left ventricle. For intravenous infusion, a 0.6 mm silicon tube connected to a micro-infusion pump (Model CMA/100, CMA/Microdialysis, Solna, Sweden) was positioned in the right internal jugular vein. A volume of 0.2 ml 0.9% NaCl (n=4) or 0.2 ml NaCl containing 500 ng mouse IL-18 (B 002-5, R&D Systems; n=4) was infused during a time period of 5 min. LV+dP/dt was recorded before, and 5 and 15 min after infusion. Data from 10 consecutive beats were recorded in DASYLab version 5.1 software (Datalog, National Instruments Company, Mönchengladbach, Germany) and analyzed using a program designed in a commercially available software package (MATLAB®, The MathWorks, Inc., Natick, MA, USA).

2.9. Statistical analysis

The data are expressed as means±S.E.M. Statistical analysis was performed using Student’s t-test, Friedman test and Repeated measures ANOVA on Ranks when appropriate (SigmaStat version 2.0, Jandel Scientific GmbH, Erkrath, Germany). A P<0.05 was considered statistically significant.

3. Results

3.1. Characterization of the animals

Mean body weights, heart weights and lung weights in sham and MI-mice are shown in Table 1. Before surgery there was no significant difference in body weight between sham and MI-mice. When sacrificed, 7 days after the primary operation, we observed significant lower body weight in MI-mice. MI-mice showed significantly increased lung and heart weights, indicating post-MI pulmonary edema and cardiac hypertrophy.

3.2. Cardiac gene expression

Gene expression of IL-12p35, IL-12p40, IL-10, IL-1β, IL-1Ra, IL-18 and IL-6 in sham hearts and in non-infarcted LV-myocardium from mice after MI, are shown in Fig. 1. We found a 2.0-fold increase in cardiac IL-18 gene expression in MI-mice compared with sham. A 1.6-fold upregulation of IL-1β mRNA and a pronounced 13.2-fold increase in IL-6 mRNA were measured in MI-mice. Moreover, a 9.1-fold increase in IL-1Ra mRNA was found following MI. No differences in the cardiac IL-12p35, IL-12p40 and IL-10 gene expression were observed in MI-mice compared with sham. IFN-γ and MIF mRNA were below the limit of detection in the LV myocardium.

3.3. Pro-IL-18 and mature IL-18 protein

The relative levels of pro-IL-18 and mature IL-18 proteins in hearts from sham, and in the non-infarcted and infarcted regions of the LV myocardium from MI-mice are shown in Fig. 2A–D. Following MI, there was a 1.4- and 2.1-fold increase in pro-IL-18 protein content in the non-infarcted (Fig. 2A) and infarcted (Fig. 2B) LV myocardium, respectively. The mature IL-18 protein content decreased by 25% in the non-infarcted (Fig. 2A) and by 79% in the infarcted (Fig. 2D) LV myocardium. Reduced mature IL-18 protein content was also found in the non-infarcted region of the LV 3 days after induced MI (data not shown). We detected relatively high levels of mature IL-18 in cardiac tissue, whereas the 18 kDa band representing mature IL-18 was absent in lung, liver and spleen tissue both in sham and MI-mice (Fig. 2E). Pro-IL-18 protein was demonstrated in liver and spleen tissue (Fig. 2F).

3.4. Serum concentrations of IL-18

Serum concentrations of IL-18 in sham and in MI-mice are shown in Fig. 2G. The serum concentration of IL-18 was significantly elevated in MI-mice (90.4±11.7 pg/ml) compared to sham (47.2±4.2 pg/ml).

3.5. Immunohistochemical localization of IL-18

Specific IL-18 staining was detected in smooth muscle cells and endothelial cells both in cardiac (Fig. 2H) and kidney (Fig. 2I) tissues. No specific staining was seen in cardiomyocytes or macrophages. No obvious differences were observed between tissues from MI-mice and sham.

3.6. Cardiac IL-18Ra protein

The relative levels of the α-chain of the IL-18R protein
Fig. 3. Representative Western blots and bar graphs showing the relative abundance of the α-chain of the IL-18 receptor (IL-18Rα) protein from sham hearts (S; n=5) and from non-infarcted (non-inf.; A) and infarcted (inf.; B) left ventricle (LV) from mice after myocardial infarction (MI; n=5). (C, D) IL-18 binding protein (IL-18BP), 25 kDa and 65 kDa, in sham hearts (S; n=5) and in non-inf. LV from MI-mice (n=5). Data represent means±S.E.M. *P<0.05 vs. Sham.

in hearts from sham and in non-infarcted and infarcted LV myocardium from MI-mice are shown in Fig. 3. Following MI, there was a 3.5-fold increase in the IL-18Rα protein content in the non-infarcted region (Fig. 3A) and a 2.8-fold increase in the infarcted region (Fig. 3B) of the LV myocardium.

3.7. Cardiac IL-18BPs

The relative protein content of IL-18BP isoform c and d in hearts from sham and in non-infarcted LV myocardium from MI-mice are shown in Fig. 3. Following MI, there was a 1.4- and a 1.7-fold increase in the protein content of the IL-18BP (unglycosylated) ~25 kDa (Fig. 3C) and IL-18BP (glycosylated) ~65 kDa (Fig. 3D), respectively. Moreover, higher levels of IL-18BP were found in cardiac tissue compared with spleen and liver tissue (data not shown).

3.8. Cardiac production of caspases and caspase activity

Caspase-8, -3, -6, -11, -2, -7, -1 and -14 mRNA expression and the relative protein abundance of caspase-1

Table 2
Cardiac caspase mRNA

<table>
<thead>
<tr>
<th>Caspase</th>
<th>Sham (n=10)</th>
<th>MI (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caspase-8</td>
<td>1.02±0.07</td>
<td>1.47±0.08*</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>0.78±0.06</td>
<td>2.04±0.17**</td>
</tr>
<tr>
<td>Caspase-6</td>
<td>1.02±0.07</td>
<td>1.22±0.06</td>
</tr>
<tr>
<td>Caspase-11</td>
<td>1.19±0.13</td>
<td>2.13±0.27*</td>
</tr>
<tr>
<td>Caspase-2</td>
<td>1.15±0.11</td>
<td>2.05±0.14**</td>
</tr>
<tr>
<td>Caspase-7</td>
<td>1.02±0.08</td>
<td>1.17±0.06</td>
</tr>
<tr>
<td>Caspase-1</td>
<td>0.91±0.08</td>
<td>1.11±0.13</td>
</tr>
<tr>
<td>Caspase-14</td>
<td>1.38±0.22</td>
<td>1.95±0.25</td>
</tr>
</tbody>
</table>

The cardiac caspase mRNA expression data represent means±S.E.M. normalized to ribosomal L32. MI, myocardial infarction. *P<0.05 vs. Sham, **P<0.001 vs. Sham.
subunit p20 in sham hearts and in non-infarcted LV myocardium from MI-mice are shown in Table 2, and in Fig. 4A and B. There was no significant increase in the cardiac gene expression of the IL-18 processing enzyme, caspase-1, following MI. However, a 2.1-fold increase in the amount of the active protein caspase-1 p20 was found in MI-mice. Additionally, MI-mice showed a 4.0- and a 2.2-fold increase in caspase-1 like activity (Fig. 4C) in the non-infarcted and infarcted region of the LV myocardium, respectively. Moreover, a 3.3-, 1.5- and a 2.1-fold increase

![Fig. 4. RNAse protection assay showing gene expression of caspase-8, -3, -6, -11, -2, -7, -1 and -14 in hearts from sham (S; n=10) and in non-infarcted myocardium from mice after myocardial infarction (MI; n=10). (B) Representative Western blot and relative abundance of caspase-1 p20 protein in sham hearts (n=5) and in non-infarcted left ventricle (non-inf. LV) from MI-mice (n=5). (C, D) Caspase-1 and caspase-3 like activity in sham hearts (n=8) and in the non-inf. (n=7) and infarcted (inf.; n=7) LV from MI-mice. L32, ribosomal gene. * P<0.05 vs. Sham.](image)
in caspase-3, caspase-8 and caspase-11 mRNA, respectively, were found in MI-mice. Caspase-3 like activity (Fig. 4D) increased by 3.4- and 1.8-fold in the non-infarcted and infarcted region of the LV myocardium, respectively.

3.9. Effects of IL-18 on cardiomyocyte shortening and left ventricular contractility

Fig. 5A shows shortening of one adult cardiomyocyte during control conditions without IL-18 and during superfusion with IL-18. Following IL-18 exposure, a 19\% decrease in fractional shortening was measured when compared to contractions without IL-18 (8.68±1.08 vs. 7.02±0.98\%; \( P < 0.05 \)). This depressed contractile response was completely reversed after removal of IL-18.

Fig. 5B shows the in vivo effect of IL-18 on left ventricular contractility (LV+\( \frac{dP}{dt} \)). Before infusion, there was no significant difference between the mice receiving NaCl and those receiving NaCl with IL-18. However, 15 min after IL-18 infusion a significant decrease in LV+\( \frac{dP}{dt} \) compared to the control group receiving NaCl, was demonstrated. At this time point, LV+\( \frac{dP}{dt} \) was 5652±844 mmHg/s in the IL-18 group and 9442±630 mmHg/s in the control group (\( P < 0.05 \)). After 5 min, a tendency towards a LV+\( \frac{dP}{dt} \) decrease in the IL-18 group was measured (5746±1035 mmHg/s vs. 7101±1046 mmHg/s), however the level of statistical significance was not reached.

4. Discussion

In the present study, we report for the first time increased IL-18 gene expression and elevated levels of pro-IL-18 in cardiac tissue 1 week after induction of MI in the mouse. Moreover, the levels of circulating IL-18, cardiac IL-18R\( \alpha \) and IL-18BP were found to be increased, and IL-18 reduced shortening in isolated cardiomyocytes and decreased left ventricular contractility in vivo. The content of mature IL-18 in cardiac tissue was reduced after MI together with a substantial increase in the activity of the pro-IL-18 and IL-18 processing enzymes caspase-1 and -3.

The increase in IL-18 gene expression was associated with enhanced expression of IL-1\( \beta \), but we did not observe increased expression of IL-12. IL-18 was originally discovered as a factor that induces IFN-\( \gamma \) production from Th1 lymphocytes in the presence of IL-12 [9]. One week following MI, our study revealed no detectable IFN-\( \gamma \) mRNA in the non-infarcted left ventricular myocardium. In a similar model of MI in rats, Varda-Bloom et al. [21] showed no significant increase in lymphocyte proliferation as early as 1 week after MI. Thus, a lack of significant lymphocyte proliferation in the non-infarcted myocardium at this time point may explain why we found no detectable gene expression of IFN-\( \gamma \) in our study. Therefore, our results cannot confirm that there is a synergistic action of IL-18 and IL-12 to induce IFN-\( \gamma \) synthesis in cardiac tissue 1 week after MI. However, an increase in IL-18 may act in synergy with IL-18 and potentiate its cardiovascular effects. IL-1\( \beta \) is related to IL-18 by structure, receptors, signaling molecules and functions [1,6]. It has been shown that IL-1\( \beta \) reduces myocyte shortening, possibly via a NO-dependent pathway [22]. A synergistic effect of IL-1\( \beta \) and IL-18 may therefore represent a powerful cardiodepressive pathway in heart failure.
IL-18 is first synthesized as a 24 kDa precursor protein termed pro-IL-18 [3]. We observed increased amounts of pro-IL-18 both in the infarcted and non-infarcted myocardial tissues, which is in accordance with the observed increase in IL-18 mRNA levels. Pro-IL-18 is processed intracellularly by caspase-1 [3], also termed ICE, which is an enzyme consisting of p10 and p20 subunits that become activated after cleavage with caspase-8 [23] and -11 [24] or autoproteolytically [25]. Following MI, we observed an increase in gene expression of both caspase-8 and -11, as well as elevated protein levels of the caspase-1 p20. Moreover, the caspase-1 activity was substantially increased, more in the non-infarcted than in the infarcted region of the myocardium. Thus, our findings indicate increased processing of pro-IL-18 by caspase-1 to mature IL-18 in cardiac tissue after MI.

The observed increase in circulating IL-18 may derive from release of unprocessed cardiac pro-IL-18. Secretion of pro-IL-18 rather than mature IL-18 from human peripheral blood mononuclear cells in vitro has been demonstrated [2]. Processing of pro-IL-18 extracellularly or in the circulation through caspase-1-independent pathways is conceivable. Increased pro-IL-18 processing by the extracellular proteinase-3 has been demonstrated [26], and this pathway may be involved in increasing circulating IL-18 after MI.

Reduced abundance of mature IL-18 was observed both in the non-infarcted and infarcted myocardial tissues. One possible explanation for this observation is that increased caspase-3 activity causes increased degradation of mature IL-18. Caspase-3 has been shown to cleave IL-18 [4], and we observed a substantial increase in caspase-3 mRNA and caspase-3 activity both in the non-infarcted and infarcted myocardial tissue.

We measured increased levels of cardiac IL-18BPs following MI. IL-18BPs are extracellular natural antagonists to IL-18, and have been proposed to play a role in the regulation of IL-18 bioactivity [5,27]. IL-18BPs have strong binding affinity to IL-18 and block its biological activity in a 1:1 molar ratio. We also found increased levels of IL-18Rα protein both in the infarcted and non-infarcted region of the LV myocardium following MI. High number of IL-18Rs on cardiac cells may be an effective consumer of secreted mature IL-18 within the heart. Enhanced abundance of IL-18Rs may also lead to increased bioactivity of IL-18 in cardiac tissue post-MI.

Following IL-18 exposure, we demonstrated reduced shortening of cardiomyocytes in vitro and reduced left ventricular contractility (LV+dP/dt) in vivo. This indicates the presence of IL-18R on cardiac myocytes. Recently, another study [15] has shown that IL-18BP improved cardiac contractile force in an in vitro model of ischemia and reperfusion. IL-18 stimulates iNOS [7] and activates CTLs [6,11]. CTLs may initiate myocyte death [21] and may have negative inotropic effects due to a decrease in the Ca^{2+} transient [13]. Thus, IL-18 may contribute to the depressed myocardial function observed after MI mediated via iNOS or CTLs.

The increase in circulating IL-18, which recently also was observed in patients with heart failure [28,29], may also have other cardiovascular effects. Interestingly, IL-18 induces metalloproteinase (MMP)-9 synthesis [30]. MMP-9 has recently been proposed to play a crucial role in the process of LV chamber enlargement and rupture during post-MI cardiac repair [31]. Increased levels of circulating IL-18 may therefore contribute to progression of the heart failure syndrome due to its ability to induce changes in the myocardial extracellular matrix structure. Thus, inhibition of IL-18 processing, neutralization of circulating IL-18 with IL-18BPs or the use of IL-18 receptor blockers may represent new therapeutic strategies in the congestive heart failure syndrome.

In conclusion, this study shows increased cardiac synthesis of IL-18 mRNA and pro-IL-18 as well as enhanced circulating levels of mature IL-18 following MI in the mouse. Since IL-18 also reduces cardiomyocyte shortening in vitro, left ventricular contractility in vivo, and has been shown to promote cell death and inflammation, we suggest a role for IL-18 in the pathogenesis of the ischemic heart-failure syndrome. However, further studies are required to elucidate the precise mechanisms of IL-18 involvement in development of cardiac dysfunction following MI in vivo.

Acknowledgements

The authors thank Tøve Strømme, Annelaug Ødegaard, Hilde Dishington, Roy Trondsen, Morten Eriksen, Institute for Experimental Research, Ulleval University Hospital, 0407 Oslo, Norway and Tove Noren, Department of Pathology, Ullevål University Hospital, 0407 Oslo, Norway, for skilful technical assistance. This work was supported by Anders Jahre’s Fund for Promotion of Science, Professor Carl Semb’s Medical Research Fund, Norwegian Foundation for Health and Rehabilitation, Rakel and Otto Bruun’s Fund and Norwegian Research Council.

References


