Interleukin-23 Deficiency Leads to Impaired Wound Healing and Adverse Prognosis After Myocardial Infarction

Konstantinos Savvatis, MD; Kathleen Pappritz; Peter Moritz Becher, MD; Diana Lindner, PhD; Christin Zietsch, PhD; Hans-Dieter Volk, MD, PhD; Dirk Westermann, MD; Heinz-Peter Schultheiss, MD; Carsten Tschöpe, MD

Background—CD4+ cells are implicated in the healing process after myocardial infarction (MI). We sought to investigate the role of interleukin-23 (IL-23) deficiency, a cytokine important in differentiation of CD4+ cells, in scar formation of the ischemic heart.

Methods and Results—MI was performed in wild-type and IL23p19−/− mice. Thirty-day mortality, hemodynamic function 4 days after MI and myocardial inflammation, and remodeling 4 and 30 days after MI were examined. Differentiation of fibroblasts from infarcted and noninfarcted hearts into myofibroblasts was examined under basal conditions and after stimulation with interferon-γ, IL-17α and IL-23. Interleukin-23p19−/− mice showed higher expression of proinflammatory cytokines and immune cell infiltration in the scar early after MI compared with wild-type mice. A stronger interferon-γ/Th1 reaction seemed to be responsible for the increased inflammation under IL-23 deficiency. Expression of α-smooth muscle actin (α-SMA), collagen I and III was significantly higher in the heart tissue and isolated cardiac fibroblasts 4 days after MI in the wild-type mice. Interleukin-23p19−/− mice showed impaired healing compared with wild-type mice, as seen by significantly higher mortality because of ventricular rupture (40% higher after 30 days) and stronger left ventricular dilation early after MI. Stimulation of cardiac fibroblasts with interferon-γ, the main Th1 cytokine, but not with IL-23 or IL-17α, led to a significant downregulation of α-smooth muscle actin, collagen I and III and decreased migration and differentiation to myofibroblasts.

Conclusions—IL-23 deficiency leads to increased myocardial inflammation and decreased cardiac fibroblast activation, associated with impaired scar formation and adverse remodeling after MI. (Circ Heart Fail. 2014;7:161-171.)

Key Words: fibroblasts ■ interleukin-23 ■ left ventricular remodeling ■ myocardial infarction

In past years, extensive research work has shown that the immune system is centrally involved in the wound healing processes after myocardial infarction (MI). Immune cells cooperate with cardiac innate cells for clearance of cell debris, angiogenesis, and coordination of the reparative mechanisms. However, immunosuppressive therapeutic interventions in the myocardial inflammatory response have been largely disappointing, showing that not a generalized immunosuppression but rather a target-specific immunomodulation might be the right strategy. Therefore, a better understanding of the inflammatory response after MI is necessary to develop proper therapeutic strategies.

Clinical Perspective on p 171

Apart from the innate, the adaptive immune system is also activated early after MI and influences the myocardial remodeling and scar formation. CD4+ helper T cells infiltrate the myocardium after MI and are necessary for effective wound healing. Cytokines involved in the differentiation of helper T cells into their different subsets, such as interferon-γ (IFN-γ) and interleukin-17α (IL-17α), are found upregulated in the myocardium after MI. Deficiency in CD4+ cells leads to adverse remodeling and increased ventricular rupture. In our previous work, we could also show that administration of an IL-2-IgG2b fusion protein after MI could reduce myocardial inflammation and improve myocardial remodeling through upregulation of CD4+ regulatory T cells, an anti-inflammatory group of CD4+ T cells.

CD4+ helper T cells consist of several distinct subsets, which play various roles in the inflammatory response. Cytokines of the IL-12 family, mainly IL-12 and IL-23, play a central role in their differentiation. IL-12 drives mainly the Th1 response, whereas IL-23 stabilizes the Th17 cells. Both cytokines are heterodimeric cytokines and share a common subunit, the IL12p40 unit, showing their potential interactions in the coordination of the immune response. The exact
microenvironment in the inflammation area decides which subunits bind together to form either IL-12 or IL-23. Th17 and Th1 cells are implicated in the pathogenesis of atherosclerosis and are upregulated in patients with acute coronary syndromes. Furthermore, IL-23 and Th17 cells play a pathogenic role in transplant rejection after heart transplantation, as well as in viral myocarditis, where they drive myocardial inflammation and viral replication. However, little is known about the exact role of Th1 and Th17 subsets and IL-23 in the remodeling processes of the myocardium itself after MI. Therefore, we examined the effects of IL-23 in myocardial inflammation, wound healing, and survival in IL-23 knockout (IL-23KO) animals using a mouse model of MI.

**Methods**

**Animals, Surgical Procedures, and Hemodynamic Measurements**

Male C57BL/6j mice (8–12 weeks old) were purchased from Charles River Laboratories, Sulzfeld, Germany. IL-23p19−/− (IL-23KO) mice on the C57BL/6j background (wild type [WT]) were a kind gift from Gentec Inc (San Francisco, CA), and were housed in the Research Facility for Experimental Medicine, Charité Universitätsmedizin, Berlin, Germany. MI was induced by permanent occlusion of the left anterior descending artery as previously described. Histologic measurements with a microconductance catheter are described elsewhere. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health and was approved by the local ethics committee (LaGeSo).

**Immunohistology**

Frozen tissue samples were embedded in Tissue-Tek (Sakura Finetek, Torrance, CA) and cut into 5-μm thick sections. Staining for following antigens was performed: CD4 (BD Biosciences, Heidelberg, Germany), CD11b (Mac-1; BD Biosciences, Heidelberg, Germany), γδ TCR (GeneTex, Irvine, CA), α-smooth muscle actin (SMA; Abcam, Cambridge, United Kingdom), collagen I (Merck Millipore, MA), collagen III (Merck Millipore, MA), neutrophils (Abcam, Cambridge, United Kingdom). Analysis of stained sections was made in a blinded fashion by digital image analysis on a Leica DMRB microscope (Leica Microsystems, Wetzlar, Germany) at a ×200 magnification, as previously described. The number of cells is expressed as cells per millimeter squared and amount of the different proteins as the percentage of the area of the examined section, which was red stained (area fraction %).

**Isolation and Culture of Murine Cardiac Fibroblasts**

For the characterization of adult murine cardiac fibroblasts tissue from the infarcted, noninfarcted, and sham-operated left ventricle was obtained 4 days after operation. Obtained tissue was cut in small pieces, and then they were treated with 10 ng/mL IFN-γ (Peprotech, Hamburg, Germany), 10 ng/mL IL-23 (Miltenyi Biotech, Bergisch Gladbach, Germany), or 10 ng/mL IL-17a (Peprotech, Hamburg, Germany) for ≤24 hours. The untreated control cells were incubated for the same time without addition of IFN-γ, IL-23, or IL-17a. The experiments were repeated 3× with n=6 per group.

**Assessment of Fibroblast Wound Healing Capacity**

For assessment of the healing capacity of cardiac fibroblasts, a scratch healing assay was used. Fibroblasts from sham hearts stimulated with IFN-γ and untreated fibroblasts were used as described above. A wound was made by scratching across each well with a 1000-μL pipette tip. Healing capacity of the fibroblasts was assessed as the percentage of the initial scratched area at 0 hours that was repopulated by fibroblasts after 6 and 24 hours.

**RNA Isolation and Gene Expression Analysis**

Total RNA from tissue was isolated using the Trizol method as described previously. Gene expression analyses were performed with the 7900 TaqMan System (Applied Biosystems). After gene expression assays were used: Col1A1 (Mm01302043_g1), Col3A1 (Mm00802331_m1), Acta2 (Mm00725412_s1), tumor necrosis factor-α (Mm00443258_m1), IL-1β (Mm0043228_m1), IL-10 (Mm00439616_m1), IL-6 (Mm00446190_m1), transforming growth factor-β (Mm00441724_m1), monocyte chemoattractant protein-1 (MCP-1; Mm99999056_m1), MCP-3 (Mm00443113_m1), RORγt (Mm03682796_m1), IFN-γ (Mm00817788_m1), IL-23 (Mm00519942_m1). The expression of IL-17α was examined with a semiquantitative method with following custom-made primers: forward 5'-TTCATGTGGTGGTCCAGCTTTC-3' and reverse 5'-CCTCAGACTACCTCAACCGTTC-3'. The quantification of the relative mRNA expression was performed using CDKNIb (Mm00431867_g1) for each sample as an internal control and expressed in the 2-ΔCt formula. For the comparison of the effect of different stimulation experiments the mRNA expression was normalized to the WT-sham using the 2-ΔCt method.

**Zymography of Matrix Metalloproteinase Activity**

Gelatin zymography from frozen heart tissue was performed to determine the gelatinolytic activities of matrix metalloproteinase-2 and matrix metalloproteinase-9 in the scar and the noninfarcted area as previously described.

**Western Blot**

Left ventricular (LV) samples of the infarcted, noninfarcted, and sham-operated area of the heart were homogenized in lysis buffer containing protease and phosphatase inhibitors. Collagen I, GAPDH, and the total and phosphorylated forms of the signal-regulated kinase (ERK)-1/2 and Stat3, involved in the IL-23 signaling, were detected with specific antibodies (Cell Signaling Technology) as previously described.

**Statistical Analysis**

Statistical analysis was performed using Graphpad Prism 5.0 (Graphpad Software, La Jolla, CA). Data are expressed as mean±SEM. Data were tested for normality with the Kolmogorov–Smirnov and the Shapiro–Wilk tests. Statistical differences between groups were assessed with 1-way ANOVA and the Bonferroni post hoc test for normally distributed data or the Kruskal–Wallis test with the Dunn post hoc test for nonparametric data. Because of the small number of 8 to 10 animals per group, a nonparametric approach was used. Survival analysis was performed with the Kaplan–Meier method and survival curves were compared with the log-rank test. Differences were considered to be statistically significant at a 2-sided value of P<0.05.
Results

LV Function After MI

IL-23KO mice showed significant dilation of the left ventricle 4 days after MI. LV end-diastolic volume was 35% and end-systolic volume 33% higher in the IL-23KO mice compared with their WT controls ($P=0.02$ and $0.05$, respectively). Accordingly, LV end-diastolic pressure was higher in the IL-23KO mice ($P=0.0016$ versus WT; Table).

Effects of IL-23 on Mortality After MI

Deficiency of IL-23 led to a 40% higher mortality in the knockout mice compared with that in the WT mice ($P=0.04$). All deaths in both strains were because of ventricular rupture in the subacute phase after MI (Figure 1). IL-23KO mice showed a higher rate of ventricular rupture. No further deaths were observed in any of the 2 groups between days 8 and 30 after MI.

Role of IL-23 in Early Myocardial Inflammation After MI

The mRNA amount of IL-23R was significantly upregulated by 4.2× in the infarcted area ($P=0.0003$) compared with the sham-operated animals and by 1.5× compared with the noninfarcted area ($P=0.017$) in WT animals 4 days after MI. The IL-23KO mice showed a 40% lower mRNA expression in the infarcted area ($P=0.003$) and a 50% lower mRNA expression in the noninfarcted area ($P=0.0076$) compared with the WT mice (Figure 2A).

Gene expression of IL-1β was 60% higher in the infarcted area in the IL-23KO mice compared with WT ($P=0.006$) and that of IL-10 in the infarction zone was reduced by 40% because of IL-23 deficiency ($P=0.0379$ versus WT). Gene expression of IL-1β and IL-10 in the noninfarcted area was the same between the 2 strains (Figure 2C and 2D). Analysis of tumor necrosis factor-α, transforming growth factor-β, and IL-6 showed no significant difference in the mRNA expression between the 2 strains (Figure 2b, 2E, and 2F). Gene expression of the chemokines MCP-1 and MCP-3 did not differ in the scar; however, the IL-23KO mice showed a 1.8-fold increase of MCP-1 and a 1.7-fold increase of MCP-3 mRNA in the noninfarcted area ($P=0.049$ and $P=0.049$, respectively; Figure 2G and 2H).

Immunohistologic analysis of immune cell infiltration in the myocardium 4 days after MI revealed a 2.9-fold increase of CD4+ cells ($P=0.013$ versus WT), a 1.4-fold increase in neutrophils ($P=0.036$ versus WT), and an 1.4-fold increase in CD11b cells ($P=0.021$ versus WT) in the infarction zone in the IL-23KO mice 4 days after MI, with no significant differences in the noninfarcted area of the LV (Figure 3A–3C).

Table. Left Ventricular Function 4 Days After Myocardial Infarction

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT-sham</th>
<th>IL-23KO-sham</th>
<th>WT-MI4d</th>
<th>IL-23KO-MI4d</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats per minute</td>
<td>546±19</td>
<td>542±21</td>
<td>450±17*</td>
<td>437±24*</td>
</tr>
<tr>
<td>LVP$_{max}$, mm Hg</td>
<td>98.5±6</td>
<td>93.4±2.7</td>
<td>72.2±3.3*</td>
<td>70.7±4.1*</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>4.6±0.4</td>
<td>4.3±0.5</td>
<td>6.8±0.48*</td>
<td>12.2±0.6*†</td>
</tr>
<tr>
<td>$dP/dt_{max}$, mm Hg/ms</td>
<td>7241±384</td>
<td>7661±521</td>
<td>3676±398*</td>
<td>3309±384*</td>
</tr>
<tr>
<td>LVEDV, μL</td>
<td>−6298±650</td>
<td>−5886±337</td>
<td>−3085±194*</td>
<td>−3184±356*</td>
</tr>
<tr>
<td>τ, ms</td>
<td>10.1±0.4</td>
<td>10.3±0.5</td>
<td>13.7±0.4*</td>
<td>13.8±0.6*</td>
</tr>
<tr>
<td>PHT, ms</td>
<td>21.3±3</td>
<td>27.8±4</td>
<td>61.5±5.8*</td>
<td>62.5±8.4*</td>
</tr>
<tr>
<td>LVESV, μL</td>
<td>21.3±3</td>
<td>27.8±3.7</td>
<td>57.2±4.5*</td>
<td>76.2±6*†</td>
</tr>
<tr>
<td>LVEDV, μL</td>
<td>61.2±5.6</td>
<td>59.4±4.4</td>
<td>73.1±6.3*</td>
<td>98.5±4.5*†</td>
</tr>
<tr>
<td>SV, μL</td>
<td>40.3±3.6</td>
<td>35.6±1.7</td>
<td>26.3±6.9*</td>
<td>20.7±2.8*</td>
</tr>
<tr>
<td>EF, %</td>
<td>65.5±3</td>
<td>60.7±2.4</td>
<td>28.2±4.5*</td>
<td>22.1±3.7*</td>
</tr>
<tr>
<td>CO, μL/min</td>
<td>2211±1756</td>
<td>18658±665</td>
<td>9994±2532*</td>
<td>10568±1470*</td>
</tr>
</tbody>
</table>

IL-23KO mice showed already 4 days after MI a LV dilation with increase of the LVEDV and LVESV and increased LVEDP. Parameters are presented as mean±SEM. CO indicates cardiac output; $dP/dt_{max}$, maximal rate of left ventricular pressure increase; $dP/dt_{min}$, maximal rate of left ventricular pressure fall; EF, ejection fraction; HR, heart rate; IL-23KO; interleukin-23 knockout; IL-23KO-MI4d, interleukin-23 knockout-myocardial infarction 4 days; LVP$_{max}$, maximal left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; LVEDV, left ventricular end-diastolic volume; LVESV, LV end-systolic volume; PHT, pressure half-time; SV, stroke volume; WT, wild type; and WT-MI4d, wild type-myocardial infarction 4 days.

*P<0.05 vs respective sham group.
†P<0.05 vs respective WT group. n=8–10 mice per group.

Figure 1. Effect of interleukin (IL)-23 deficiency on survival after myocardial infarction (MI). Kaplan–Meier analysis of survival and subjects at risk at various time points after MI. IL-23KO indicates IL-23 knockout; and WT, wild type.
Finally, we found no significant difference in the number of γδ-T cells between the WT and IL-23KO mice (Figure 3D).

Effect of IL-23 on Balance Between Th1/Th17 Reaction
We examined the myocardial gene expression of signature cytokines, IFN-γ for the Th1 and IL-17α for Th17 cells, and the gene expression of their transcription factors, Tbet and RORγt, respectively, 4 days after MI. IL-23KO mice showed a significant 3.3-fold higher gene expression of IFN-γ in the infarcted area ($P=0.046$) and a 2.4-fold higher gene expression in the noninfarcted LV ($P=0.025$) versus the WT mice. However, WT mice showed a significant upregulation of IL-17α in both the infarcted and the noninfarcted LV.

Figure 2. Gene expression of cytokines and chemokines 4 days after myocardial infarction (MI). A, Interleukin (IL)-23 receptor was significantly upregulated in the wild-type (WT) mice compared with IL-23 knockout (IL-23KO) mice. B to H, IL-23KO mice showed a stronger inflammation, indicated by higher gene expression of IL-1β and lower IL-10 mRNA in the scar and higher monocyte chemoattractant protein (MCP)-1 and MCP-3 mRNA in the noninfarcted area. There was no significant difference in the mRNA expression of TNF-α, IL-6 or TGF-β between the 2 strains. *$P<0.05$ vs respective WT group; #$P<0.05$ vs left ventricle (LV) from the same group; and §§$P<0.05$ vs respective sham group. n=8 to 10 per group. TGF indicates transforming growth factor; and TNF, tumor necrosis factor.

Figure 3. Immunohistochemical analysis of immune cell infiltration in the myocardium 4 days after myocardial infarction (MI). A to D, Interleukin-23 knockout (IL-23KO) mice showed a significant higher infiltration with CD4+ T cells, CD11b+ cells, and neutrophils in the scar. However, no difference was observed in the number of the γδ-T cells, which are also stimulated by IL-23 to produce IL-17α. E, Representative images of CD4+ cells and neutrophils in the border zone in infarction-operated wild type (WT) and IL-23KO mice 4 days after MI (magnification, x100; scale bar, 100 μm). *$P<0.05$ vs respective WT group; #$P<0.05$ vs left ventricle (LV) from the same group; and §§$P<0.05$ vs respective sham group. n=8 to 10 per group.
noninfarced area, whereas the IL-23KO failed to upregulate IL-17α after MI. Accordingly, mRNA amount of Tbet in the scar was significantly higher in the IL-23KO mice compared with that in the WT mice ($P=0.0078$ versus WT), whereas gene expression of RORγt was significantly lower ($P=0.0012$ versus WT), indicating a stronger activation of the Th1 over Th17 immune reaction under IL-23 deficiency (Figure 4A–4D).

**Myocardial Stat3 and ERK1/2 Activation**

To investigate the effects of IL-23 on the myocardium, we examined by Western blot analysis the phosphorylation of Stat3, the main transcription factor over which IL-23r signals 4 days after induction of MI. A significantly enhanced phosphorylation of Stat3 was observed in both the infarcted and the noninfarcted area compared with sham-operated hearts. However, we found a 2.8-fold increase in the phosphorylation of Stat3 in the infarcted area in the WT compared with that in the IL-23KO mice ($P=0.0056$). Likewise, a significantly higher activation of Stat3 by 2.6× was observed in the noninfarcted area of the WT compared with that in the noninfarcted area of the IL-23KO mice ($P=0.0009$; Figure 5A).

The phosphorylation status of ERK1/2 was significantly upregulated in the infarction area compared with healthy hearts in WT mice. We observed no significant activation of ERK1/2 in the IL-23KO mice neither in the infarcted nor in the noninfarcted area. In comparison, activation of ERK1/2 in the infarcted area was 1.5× higher in the WT than in the IL-23KO mice ($P=0.028$; Figure 5B).

**Myocardial Wound Healing and Remodeling**

We analyzed myocardial wound healing parameters and myofibroblast differentiation early after MI to understand the mechanisms leading to increased rupture. Gene expression of both Collα1 and Col3α1 in the infarcted area 4 days after MI was 20% higher in the WT mice compared with that in the IL-23KO ($P=0.021$ and $0.029$, respectively; Figure 6A). We performed a zymography to assess the gelatinolytic activity of matrix metalloproteinase-2 and matrix metalloproteinase-9 4 days after MI. We observed no difference in their activity between the 2 strains (Figure 5C and 5D). Gene expression of Acta2 as a marker of myofibroblast differentiation in the scar was 25% higher in the WT than in the IL-23KO mice ($P=0.026$; Figure 6A).

Increased mRNA expression of Colα1 in the infarcted area in WT mice persisted until 30 days after MI and was significantly higher by 1.6× ($P=0.04$) and that of Col3α1 by 1.7× ($P=0.042$) compared with that in the IL-23KO mice. Accordingly, protein abundance of collagen I in the infarcted area was higher in the WT mice by 3.6× ($P=0.002$) compared with that in the IL-23KO mice, whereas collagen III protein levels were the same in both groups (Figure 6A and 6C). Western blot analysis of collagen I confirmed the data from immunohistochemistry and showed a 1.9× higher amount of protein in the scar 4 days and 1.7× 30 days after MI ($P=0.0312$ and $P=0.0048$, respectively; Figure 6D). Higher gene expression of Acta2 persisted also until 30 days after MI (1.6-fold higher in the scar in the WT mice versus IL-23KO; $P=0.0028$; Figure 6A). Similarly, protein amount of α-SMA in the infarcted area was 2× higher in the WT than in the knockout mice 30 days after MI ($P=0.028$; Figure 6B and 6C). No significant difference could be observed in the noninfarcted area between the 2 strains.

**Myofibroblast Differentiation Under Basal Conditions**

Cardiac fibroblasts from heart 4 days after MI were examined. Basal mRNA expression of Acta2 in WT scar fibroblasts was 1.9× higher than in IL-23KO fibroblasts ($P<0.0001$).

---

**Figure 4.** A to D, Gene expression of the transcription factors Tbet and RORγt and the cytokines interferon (IFN)-γ and interleukin (IL)-17α 4 days after myocardial infarction. Deficiency in IL-23 diverses the immune reaction into the Th1 direction with an upregulation of Tbet and IFN-γ production in both the noninfarcted area and the scar. Absence of IL-23 abrogated the Th17 reaction in the IL-23 knockout (IL-23KO) mice. *$P<0.05$ vs respective wild-type (WT) group; #$P<0.05$ vs left ventricle (LV) from the same group; and §$P<0.05$ vs respective sham group. n=8 to 10 per group.
Furthermore, WT fibroblasts isolated from the scar produced 1.4× more Col1a1 mRNA (\(P=0.0147\)) and 1.5× more Col3a1 RNA (\(P=0.0039\)) than IL-23KO fibroblasts (Figure 7A–7D).

**Effects of IFN-\(\gamma\), IL-23, and IL-17\(\alpha\) on Cardiac Fibroblasts**

WT fibroblasts were isolated from heart and stimulated with 10 ng/mL of IL-23, IFN-\(\gamma\), or IL-17\(\alpha\). Both IL-23 and IL-17\(\alpha\) showed no effects on the mRNA expression of Acta2, Col1a1, and Col3a1 after 24 hours of stimulation on healthy fibroblasts (data not shown). However, we observed significant effects of IFN-\(\gamma\) on fibroblast differentiation. Therefore, we stimulated fibroblasts isolated from sham and infarcted hearts with IFN-\(\gamma\). Stimulation with IFN-\(\gamma\) led to a significant downregulation of the gene expression of Col1a1, Col3a1, and Acta2 after 24 hours stimulation in fibroblasts isolated from sham-operated hearts or from the scar. The effects of IFN-\(\gamma\) were not so strong on fibroblasts isolated from the noninfarcted LV, where only a reduction in Col3a1 mRNA expression was significant after 24 hours of stimulation (Figure 7E).
Figure 6. Gene and protein expression of collagen I, III, and α-smooth muscle actin (SMA) after myocardial infarction (MI). A and B, Wild-type (WT) mice showed a higher deposition of collagen I and III already 4 days after MI, which persisted till day 30 after MI. This was associated with increased presence of myofibroblasts in the scar early after MI, which persisted in the late stage, as measured by α-SMA. C, Representative immunohistochemical images of collagen I, III, and α-SMA from the border zone in WT and interleukin-23 knockout (IL-23KO) mice 30 days after MI (magnification, ×100; scale bar, 100 μm). D, Western blot analysis of collagen I 4 and 30 days after MI confirmed the increased collagen I abundance in the scar in the WT mice compared with the IL-23KO mice. *P<0.05 vs respective WT group; #P<0.05 vs left ventricle (LV) from the same group; and §P<0.05 vs respective sham group. n=8 to 10 per group.
IFN-γ Effects on Wound Healing Capacity of Cardiac Fibroblasts

We analyzed the effects of IFN-γ on the migratory capacity of cardiac fibroblasts using an in vitro wound healing model. Stimulation of cardiac fibroblasts with 10 ng/mL IFN-γ decreased their migratory capacity by 5% after 24 hours compared with control untreated fibroblasts as assessed by the area they covered 24 hours after induction of a scratch in the culture well (P=0.0054; Figure 8).

Discussion

The salient finding of our study is that IL-23 deficiency leads to an impaired scar formation in the ischemic heart. IL-23 controls the direction of the myocardial immune response early after MI and inhibits the Th1 reaction in the scar with its deleterious IFN-γ production. Through indirect effects on myofibroblast differentiation and migration in the infarcted area, IL-23 deficiency led to adverse myocardial remodeling, ventricular rupture, and increased mortality.

IL-23 Controls the Differentiation of CD4+ T Cells After MI

Activation of the adaptive immune system after MI is important for the orchestration of the wound healing process in the heart. IL-23 is centrally involved in the survival and stabilization of Th17 cells but also indirectly by inhibiting the development of Th1 cells. Activation of innate immune cells in the early stages after myocardial infarction is followed by migration of adaptive immune cells and especially CD4+ T cells. Our data show that 4 days after MI the
The Th1 reaction and IFN-γ production are traditionally thought to lead to deleterious effects in several immune-mediated diseases. Similar adverse effects of Th1 cells have been demonstrated in the progression of atherosclerosis, development of vascular inflammation, and adverse vascular remodeling. IFN-γ is mainly driving the development of atherosclerotic plaques and vascular inflammation and is associated with adverse prognosis. IFN-γ and IL-17α–producing cells are both upregulated in patients with acute coronary syndromes, implying an activation of Th1 and Th17 cells, respectively. Furthermore, these cells infiltrate the coronary arteries and affect the function of smooth muscle cells. In a recent clinical study in patients with coronary artery disease, low levels of IL-17α after an acute coronary syndrome were associated with a worse prognosis and increased rate of death or reinfarction. Accordingly, our data demonstrate a regulatory and anti-inflammatory role for Th17 after MI, which seems to be associated with inhibition of the deleterious Th1 reaction and IFN-γ production. However, a recent study by Yan et al demonstrated a protective effect of IL-23 deficiency after MI, associated with a decreased myocardial inflammation in the IL-23KO mice. These contradicting results may, in part, be because of different genetic background of the WT mice because the authors exhibited a mortality rate twice as high as in our own study. Furthermore, selection of different time points in our study makes direct comparisons difficult because the inflammatory reaction after MI is a continuously changing process.

**IL-23 Deficiency Reduces Stat3 and ERK1/2 Activation**

Stat3 is the main transcription factor over which signaling of the IL-23 receptor occurs. Apart from its role in the differentiation of Th17 cells, Stat3 is known to play a protective role in the heart. IL-23 deficiency led to a significantly lower activation of Stat3 in the myocardium in our study. Decreased Stat3 activation is associated with an adverse prognosis after MI by reducing the myocardial capillary growth and interstitial matrix deposition. Furthermore, Stat3 activation is protective in viral myocarditis. Importantly, however, Stat3 activation leads to increased differentiation of fibroblasts into myofibroblasts, associated with increased production of α-SMA and collagen gel contraction, which might in part explain the impaired wound healing induced by IL-23 deficiency in our model.

Furthermore, we observed a significant phosphorylation of ERK1/2. ERK1/2 activation is associated with protective effects in myocardial ischemia, mainly because of its antiapoptotic function and effects in hypertrophy and growth. Moreover, ERK1/2 seems to act downstream of Stat3 signaling and be partially responsible for the actions of Stat3 in the myocardium. Another important aspect of ERK1/2 activation is that it seems to be taking part in the IL-23 functions on cell differentiation and moreover to induce IL-23 production in a positive feedback manner, potentiating possibly the effects of IL-23 in our model. Finally, ERK1/2 activation also takes part in the activation of fibroblasts to myofibroblasts, an effect we could also observe in our study.
Deficiency of IL-23 Leads to Impaired Wound Healing and Adverse Remodeling

We observed a decreased collagen deposition in the scar already in the early stages after MI, which persisted throughout the whole observation time. Impaired scar formation in the early stages led to an increased rate of LV rupture in the IL-23KO mice. Isolation of cardiac fibroblasts from the scar 4 days after MI showed a higher gene expression of collagen I, III, and α-SMA in the WT mice, implying a stronger differentiation into myofibroblasts in the WT mice. Furthermore, protein amount and gene expression of α-SMA 30 days after MI was stronger in the WT mice as a marker for myofibroblast persistence in the scar for a longer time than in the IL-23KO mice. Formation of a collagenous scar is important for preventing infarct expansion and LV dilation and is mainly mediated by myofibroblasts.31 Myofibroblasts migrate into the scar after their activation, express α-SMA, and produce large amounts of collagen I and III already few days after MI.32 Presence of myofibroblasts and persistence in the scar is of utmost importance and early removal of myofibroblasts leads to impaired wound healing with adverse remodeling, LV dilation, and LV rupture.31,33

IFN-γ Reduces the Activation of Myofibroblasts

The interaction between cardiac fibroblasts and immune cells and the transition from the inflammatory phase to wound healing and formation of a stable scar is gaining continuous attention.34 We identified IFN-γ as the main coordinating cytokine in our model. Stimulation of cardiac fibroblasts with IFN-γ led to a significant downregulation of α-SMA, collagen I and III. These effects were retained in healthy cardiac fibroblasts as well as in fibroblasts isolated from the scar. Interestingly, IFN-γ affected less fibroblasts isolated from the noninfarcted LV. Furthermore, IFN-γ-stimulated fibroblasts showed a decreased migratory ability in the wound healing scratch experiment. However, the effects on the migratory capacity were less pronounced than the effects on the activation status of fibroblasts, which implies that IFN-γ acts mainly through induction of differentiation in the fibroblasts and that their migratory capacity might be controlled mainly by other factors in our study. However, stimulation with IL-23 or IL-17α had no significant effects on the expression of α-SMA or collagen by cardiac fibroblasts. However, a positive effect of IL-17α on the migratory capability and proliferation of fibroblasts has been described previously and cannot be excluded in our study.35

Study Limitations

Our study was performed in a knockout mouse model in which deletion of the IL23p19 gene led to a loss of IL-23 through-out the experiment. A time- or dose-specific action of IL-23 can therefore not be excluded in our study; however, our data indicate that IL-23 acts mainly in the early stages after MI. Furthermore, we examined cardiac inflammation, myocardial wound healing, and myofibroblasts differentiation 4 days after MI, which we found to be the important time point for impaired wound healing; therefore, time-dependent changes in inflammation and wound healing parameters at other time points cannot be excluded.

Conclusions

IL-23 seems to play a key role in the regulation of the myocardial inflammatory response after MI and its interaction with cardiac fibroblasts. IL-23 deficiency leads to an aggravated myocardial inflammation and impaired wound healing in the ischemic heart associated with high LV rupture rate and adverse remodeling.

Acknowledgments

We would like to thank K. Puhl and G. Zingler for their excellent technical assistance.

Sources of Funding

This study was supported by the “Deutsche Forschungsgemeinschaft” (SFB/TR 19, Project Z3 and B5 to Dr Carsten Tschöpe), the Berlin-Brandenburg Center for Regenerative Therapies (Platform F to Dr Carsten Tschöpe), and the Hellenic Society of Cardiology (Research grant to Dr Savvatis). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the article.

Disclosures

None.

References


Mus AM, Cornelissen F, Asmawidjaja PS, van Hamburg JP, Boon L, Hendriks RW, Lubberts E. Interleukin-23 promotes Th17 differentiation by inhibiting T-bet and Foxp3 and is required for elevation of αγ and interleukin-17. A complex network of cytokines, chemokines, growth factors, and cells, which cooperate in this process. The present study demonstrates that modulation of this cytokine network might provide further insights into the individual healing processes of different cardiac structures.


Hansson GK, Hermansson AM, Cornelissen F, Asmawidjaja PS, van Hamburg JP, Boon L, Hendriks RW, Lubberts E. Interleukin-23 promotes Th17 differentiation by inhibiting T-bet and Foxp3 and is required for elevation of αγ and interleukin-17. A complex network of cytokines, chemokines, growth factors, and cells, which cooperate in this process. The present study demonstrates that modulation of this cytokine network might provide further insights into the individual healing processes of different cardiac structures.


van Nieuwenhoven FA, Turner NA. The role of cardiac fibroblasts in the transition from inflammation to fibrosis following myocardial infarction. Vascul Pharmacol. 2013;58:182–188.


**CLINICAL PERSPECTIVE**

Wound healing and formation of a stable scar after myocardial infarction are of utmost importance for stabilizing the infarct zone and preventing adverse remodeling and increased mortality. The mechanisms involved are complicated and implicate a complex network of cytokines, chemokines, growth factors, and cells, which cooperate in this process. The present study identifies interleukin-23 and its downstream cytokines interferon-γ and interleukin-17α as central coordinators of wound healing process after myocardial infarction and shows that deficiency in interleukin-23 leads to catastrophic events in the stabilization of the scar and left ventricular remodeling at the early stages after myocardial infarction resulting to increased myocardial rupture and decreased myocardial function. These events involve direct effects on cardiac immune cells and cardiac fibroblasts. The activation of this cytokine network might provide further insights into the individual healing processes and the observed differences between patients after myocardial infarction. Manipulation of this cytokine system might resolve possible new targets to abrogate adverse events in ischemic cardiomyopathy.
Interleukin-23 Deficiency Leads to Impaired Wound Healing and Adverse Prognosis After Myocardial Infarction
Konstantinos Savvatis, Kathleen Pappritz, Peter Moritz Becher, Diana Lindner, Christin Zietsch, Hans-Dieter Volk, Dirk Westermann, Heinz-Peter Schultheiss and Carsten Tschöpe

Circ Heart Fail. 2014;7:161-171; originally published online December 3, 2013;
doi: 10.1161/CIRCHEARTFAILURE.113.000604
Circulation: Heart Failure is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2013 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circheartfailure.ahajournals.org/content/7/1/161

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Heart Failure can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Heart Failure is online at:
http://circheartfailure.ahajournals.org//subscriptions/