Role of Tumor Necrosis Factor-α in the Pathogenesis of Atrial Fibrosis and Development of an Arrhythmogenic Substrate

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**Background:** Although tumor necrosis factor-α (TNF-α) levels are increased in patients with atrial fibrillation (AF), its role in the pathogenesis of AF is unclear. We investigated whether direct delivery of TNF-α could induce atrial fibrosis.

**Methods and Results:** TNF-α (4 μg/kg) was injected into the tail vein of 20 male Swiss albino mice (TNF group) and saline into 20 control mice (CON group). The dose was carefully chosen to avoid any significant decrease in left ventricular (LV) function. Animals were killed after 16 weeks and their atria examined for fibrosis. We found increased atrial fibrosis in the TNF group compared with the CON group [372.8 ± 21.5 arbitrary units (a.u.) vs. 56.9 ± 6.5 a.u., respectively, mean ± SEM; P < 0.0001] and decreased connexin-40 immunofluorescence [7.5 ± 0.4 a.u. vs. 40.4 ± 1.9 a.u., respectively; P < 0.0001]. Transforming growth factor-β [TGF-β; 95.6 ± 1.8 a.u. vs. 29.4 ± 5.8 a.u.; P < 0.001], α-smooth muscle actin (α-SMA; 97.9 ± 13.0 a.u. vs. 50.1 ± 18.5 a.u.; P < 0.05) and matrix metalloproteinase 2 (MMP-2)/GAPDH levels [157.3 ± 26.4 a.u. vs. 105.8 ± 13.3 a.u.; P < 0.05] were also increased in the TNF group.

**Conclusions:** TNF-α is involved in the pathogenesis of atrial fibrosis and altered connexin-40 expression in mice through the TGF-β signaling pathway, activation of myofibroblasts and increased secretion of MMPs. Collectively, these changes may contribute to the arrhythmogenic substrate and development of AF. (*Circ J* 2013; 77: 1171–1179)

**Key Words:** Atrial fibrosis; Immunohistochemistry; Transforming growth factor-β; Tumor necrosis factor-α

To date, the potential detrimental effect and role of TNF-α in the pathogenesis of atrial fibrillation (AF), the most common sustained cardiac arrhythmia in humans, has been less well investigated. Experimental data from animal models of AF and studies of human atrial samples obtained from patients with AF show that AF is associated with progressive structural and electrical remodeling of the atria. Atrial fibrosis appears to be central and is associated with an alteration of electrical conduction and excitability, which provides the substrate for AF maintenance. It is likely that a number of distinct mechanisms and triggers result in the development of atrial fibrosis. In view of the increasing clinical burden of AF and significant comorbidities associated with the disease, a greater understanding of the mechanisms that lead to atrial fibrosis may allow for novel therapeutic strategies in the treatment of this complex arrhythmia.

Increasing evidence suggests that inflammatory biomarkers, including TNF-α, are elevated in patients with AF. In the pres-
ent study, we aimed to further understand the link between TNF-α and the development of atrial fibrosis by studying the effects of a bolus injection of TNF-α (carefully chosen to avoid any significant effects on ventricular function) on atrial structure and function in mice. We hypothesized that TNF-α is not only an inflammatory biomarker that is increased in patients with AF, but that it also plays a pathogenic role in the development of atrial fibrosis and the arrhythmogenic substrate.

**Methods**

**Animals**

Male Swiss albino mice (weighing 30–35 g) were used for the study. Handling and care of all animals was performed in accordance with guidelines adopted by the Department of Experimental Surgery, Singapore General Hospital and the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health. The study was approved by the Singhealth Institutional Animal Care and Use Committee (IACUC).

**Study Protocol**

After the animals had acclimatized for 1 week, a single bolus dose of murine TNF-α dissolved in sterile saline/0.1% human serum albumin (HSA) was injected into a lateral caudal tail vein of 20 mice (TNF group), while an equivalent volume of saline/0.1% HSA was injected into the tail veins of 20 mice (Control (CON) group). The dose of TNF-α injection was chosen from previous studies in mice that have reliably induced systemic and hepatic inflammation and from our pilot studies (described later) to avoid any significant detrimental effects on left ventricular (LV) function. After 16 weeks, animals were killed by being placed in a box connected to a CO2 gas cylinder in which the inflow of gas could be precisely regulated. Following confirmation of death, their hearts were removed and stored in liquid nitrogen for subsequent analysis.

**Echocardiography**

Transtracheal echocardiography was performed using a Vevo 2100 (VisualSonics Inc) echocardiography machine with a linear 30 MHz probe. Mice were anesthetized in an induction chamber with isoflurane (2–1.5%) mixed with 100% oxygen for 20 min. Once fully anesthetized (unresponsive when their tail was squeezed), the animals were transferred to a heated platform (37°C) and maintained on 1.5–1.0% isoflurane through a nose cone. The heart rate was monitored continuously and maintained at 350–450 beats/min. We measured the following echocardiographic parameters at baseline and 4 and 16 weeks after TNF-α injection: LV end-diastolic and -systolic diameters (LVEDD, LVESD), ejection fraction (EF) and fractional shortening (FS). We measured left atrial (LA) diameter using 2-dimensional guided M-mode echocardiography on the parasternal short-axis view at the aortic valve level and assessed E’ using tissue Doppler imaging at the septal mitral annulus. LV dimensions and EF were determined from M-mode echocardiography at the level of the papillary muscle in the short-axis view. Mitral inflow pattern was assessed by measuring the E/A ratio. Analysis was performed offline using the Vevo 2100 workstation. All measurements were averaged over 3 consecutive cardiac cycles. LV FS was calculated as:

\[ \frac{(LVEDD - LVESD)}{LVEDD} \times 100\% \]

and LVEF was calculated as:

\[ \frac{(LVEDD^2 - LVESD^2)}{LVEDD^2} \times 100\% \].

LV mass was measured using the following formula:

\[ 1.05 \times ([LVEDD^2 + SW^2 + PW^2] - (LVEDD^3)) \]

where SW is the septal wall thickness and PV is the posterior wall thickness. The LV mass index (mg/g) was LV mass relative to body weight.

**Measurement of Plasma Cytokine Levels**

Blood (200 μl) was taken from the submandibular vein at baseline and 4 weeks after TNF-α or control saline injection. The plasma was separated by centrifugation and immediately stored at −80°C until further analysis. Plasma levels of C-reactive protein (CRP) were measured using rat/mouse CRP Single-Plex kit (Millipore Corp, Billerica, MA, USA) and plasma levels of TNF-α, IL-6, IL-10 and monocyte chemotactic protein 1 (MCP-1) measured using mouse cytokine/chemokine Panel 4-Plex (Millipore Corp). The analyses were performed as per manufacturer’s instructions.

**Histology**

Atrial cryo-sections (obtained from the LA) were fixed with Bouins Solution (Sigma Aldrich, St Louis, MO, USA) and then stained with picrosirius solution (0.1% Sirius Red in picric acid, Sigma Aldrich). To quantify the atrial collagen content, images were captured with the Olympus Microscope Camera on the Olympus BX51 microscope and the red pixel content of the atria was measured using Image-Pro Plus (Media Cybernetics, Silver Spring, MD, USA) software. Between 6 and 12 cryostat sections were obtained for each mouse.

**Immunofluorescence Studies**

For the connexin immunolabeling, the atria were fixed in OCT compound. The 5-μm cryostat sections of atrial tissue were fixed with 2% para-formaldehyde for 10 min and washed with phosphate-buffered saline (PBS). The sections were then permeabilized with 0.1% Triton X-100 for 10 min. For blocking of non-specific binding, the sections were incubated with 5% bovine serum albumin for connexin-43 and 5% donkey serum for connexin-40 for 1 h and then incubated with immunofluorescent goat anti-connexin-40 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and rabbit anti-connexin-43 (Abcam, UK) overnight at 4°C. After rinsing with PBS, sections were then incubated for 2 h with secondary antibody FITC-labelled goat anti-rabbit IgG (Abcam, UK) and Alexa Fluor 488 conjugated donkey anti-goat (Molecular Probes, Eugene, OR, USA). After washing with PBS, the sections were then counterstained with DAPI (Molecular Probes) for nuclei and co-stained with 70% glycerol. Images were then captured using a Zeiss Axioscope light microscope (Carl Zeiss, Jena, Germany) and the images were acquired by a Zeiss AxioCam digital camera using AxioVision software (Carl Zeiss).

Quantitative image analysis of connexin expression was performed using Image-Pro Plus software (Media Cybernetics). For each set of observations, the surfaces of 5 randomly chosen areas were studied at a magnification of x40.

**Immunohistochemistry**

Immunohistochemical staining of transforming growth factor-β1 (TGF-β1), α-smooth muscle actin (α-SMA), Smad3 and phospho-Smad3 was performed on atrial cryostat sections. The tissue sections were rinsed in PBS with Tween 20 (PBST) and incubated in 2.5% normal horse serum (Vector Laboratories, Burlingame, CA, USA), for 30 min. After blocking, the slides were rinsed in PBST and then incubated for 30 min at room temperature with a primary antibody diluted in normal horse

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**LIEW R et al.**
serum. The primary antibodies used were rabbit anti-human TGF-β (Santa Cruz Biotechnology), specific mouse monoclonal anti-α-SMA (Santa Cruz Biotechnology), rabbit polyclonal antibody against ChIP-grade Smad3 (Abcam, UK) and rabbit anti-phospho-Smad3 antibody (Abcam, UK). For α-SMA detection, additional blocking of 2.5 h in mouse-on-mouse IgG blocking reagent (Vector Laboratories) was done before the addition of primary antibody onto tissue sections. After rinsing with PBST, sections were incubated with secondary antibody for 30 min. The secondary antibodies used are anti-rabbit Ig ready-to-use (ImPress Kit, Vector Laboratories) and anti-mouse Ig ready-to-use (ImPress Kit). After rinsing with PBST, the 3,3'-diaminobenzidine reagent was then applied to the sections, which were washed and counterstained with hematoxylin. The sections were then mounted and coverslipped with dibutyl phthalate and xyylene mounting medium (Sigma Aldrich). Images were captured with the Olympus Microscope Camera on the Olympus BX51 microscope and the red pixel content of the atria was measured using Image Pro-Plus (Media Cybernetics) software.

**GAPDH and Matrix Metalloproteinase (MMP) Assays**

Mouse atrial protein extracts were prepared by homogenizing the tissue in PBS with protease inhibitor. Because of the small amount of atrial tissue available from each mouse, left and right atrial samples were required for these experiments. Protein concentrations were determined by Bradford assay using Protein Assay Kit (Bio-Rad). Following this, samples were reduced with 2-mercaptoethanol and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membrane. Detection with primary antibodies (anti-MMP-2, anti-MMP-9; Santa Cruz Biotechnology) was performed overnight at 4°C followed by HRP-conjugated secondary antibodies at room temperature for 1 h. Blot images were developed by using Supersignal West Pico chemiluminescent substrate (PIERCE #34080) and captured by an Alpha Innotech CCD camera. The blots were stripped and re-probed with anti-GAPDH antibody (Abcam, UK). Results were quantified using densitometry tools of Fluorchem HD2 software (Alpha Innotech).

**Determination of Optimal TNF-α Dose**

We performed initial studies before the main experiments to determine the optimal dose of TNF-α. In order to test our hypothesis, we wanted to induce an inflammatory state with potential detrimental effects on atrial structure and function without significantly affecting LV function or causing overt heart failure, because this could act as a significant confounding factor and cause a secondary alteration in atrial structure and function. We had previously demonstrated that a single bolus injection of 8 μg/kg TNF-α in mice resulted in an increase in LV size and a decrease in systolic function after 28 days.14 We therefore used 2 lower doses of TNF-α (2 and 4 μg/kg) in 18 mice (6 for each dose and 6 controls, which received saline injection). Echocardiographic measurements of LV size and function and LA diameter were assessed at baseline and after 4 weeks.

**Statistical Analysis**

Results are expressed as mean±SEM and were analyzed using Student’s t-test or 1-way analysis of variance (ANOVA) and the Bonferroni post-test. A value of P<0.05 was considered significant.

| Table 1. Pilot Study Echocardiographic Parameters at Baseline and 4 Weeks After TNF-α Injection (2 or 4 μg/kg) |
|-----------------|-----------------|-----------------|
| **Baseline**    | **2 μg/kg TNF** | **4 μg/kg TNF** |
| LVEDD (mm)      | 4.2±0.11        | 4.4±0.10        | 4.4±0.10        |
| LVESD (mm)      | 2.7±0.32        | 2.8±0.06        | 2.9±0.32        |
| FS (%)          | 34.3±6.8        | 35.7±1.3        | 33.9±3.2        |
| EF (%)          | 56.5±8.6        | 58.7±1.7        | 56.2±4.1        |
| E:A ratio       | 1.3±0.08        | 1.5±0.08        | 1.4±0.16        |
| LAD (mm)        | 1.65±0.05       | 1.66±0.04       | 1.70±0.18       |
| **After 4 weeks** | **LVEDD (mm)** | **LVESD (mm)** | **FS (%)** |
| LVEDD (mm)      | 4.3±0.25        | 4.4±0.11        | 4.6±0.31        |
| LVESD (mm)      | 2.7±0.30        | 2.7±0.06        | 2.9±0.32        |
| FS (%)          | 32.7±5.7        | 35.6±0.5        | 34.0±5.7        |
| EF (%)          | 54.5±7.4        | 58.6±0.7        | 56.1±7.4        |
| E:A ratio       | 1.4±0.20        | 1.5±0.23        | 1.3±0.09        |
| LAD (mm)        | 1.85±0.22       | 1.71±0.03       | 1.9±0.12        |

There were no significant differences between groups in any of the parameters at baseline or 4 weeks after TNF-α injection at either dose. Values are mean±SEM. Values are mean±SD, n=6 mice in each group.

CON, control mice; EF, ejection fraction; FS, fractional shortening; LAD, left atrial diameter; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; TNF-α, tumor necrosis factor-α; TNF, TNF-α treated mice.

Results

**Pilot Study to Determine TNF-α Dose**

We performed initial pilot studies to determine whether tail injections of 2 or 4 μg/kg TNF-α produced any significant effects on LV size (LVEDD, LVESD) or function (EF, FS or E/A ratio), which may represent important confounding factors on subsequent atrial changes. We found that there were no baseline differences in LV size or function between control mice and those that had been injected with 2 μg/kg or 4 μg/kg TNF-α. Furthermore, there were no differences in any of these parameters after 4 weeks (Table 1). Because the data suggested that neither 2 nor 4 μg/kg TNF-α had any significant
Table 2. Comparison of Echocardiographic Measurements in CON (Saline) and TNF-α (4 μg/kg) Mice in Main Study

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th></th>
<th>After 4 weeks</th>
<th></th>
<th>After 16 weeks</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>TNF</td>
<td>P value</td>
<td>CON</td>
<td>TNF</td>
<td>P value</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>38.7±2.5</td>
<td>38.4±1.1</td>
<td>0.76</td>
<td>46.8±3.5</td>
<td>45.8±2.8</td>
<td>0.62</td>
</tr>
<tr>
<td>LV mass index (mg/g)</td>
<td>3.68±0.34</td>
<td>3.61±0.50</td>
<td>0.79</td>
<td>3.82±0.39</td>
<td>3.50±0.62</td>
<td>0.34</td>
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<tr>
<td>LVEDD (mm)</td>
<td>4.33±0.09</td>
<td>4.29±0.23</td>
<td>0.71</td>
<td>4.46±0.25</td>
<td>4.52±0.25</td>
<td>0.67</td>
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<tr>
<td>LVESESD (mm)</td>
<td>2.89±0.35</td>
<td>2.85±0.30</td>
<td>0.81</td>
<td>2.97±0.44</td>
<td>2.99±0.36</td>
<td>0.93</td>
</tr>
<tr>
<td>FS (%)</td>
<td>33.3±7.4</td>
<td>33.8±4.3</td>
<td>0.88</td>
<td>33.8±6.5</td>
<td>34.2±5.6</td>
<td>0.91</td>
</tr>
<tr>
<td>EF (%)</td>
<td>55.1±9.3</td>
<td>56.1±5.7</td>
<td>0.83</td>
<td>55.8±8.5</td>
<td>56.5±471</td>
<td>0.89</td>
</tr>
<tr>
<td>E:A ratio</td>
<td>1.38±0.14</td>
<td>1.35±0.19</td>
<td>0.76</td>
<td>1.45±0.21</td>
<td>1.29±0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>LAD (mm)</td>
<td>1.70±0.10</td>
<td>1.85±0.16</td>
<td>0.11</td>
<td>1.92±0.19</td>
<td>1.93±0.13</td>
<td>0.94</td>
</tr>
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</table>

Values are mean±SEM. Values are mean±SD, n=6 mice in each group. Abbreviations as in Table 1.

Table 3. Plasma Cytokine Levels at Baseline and 4 Weeks After Injection of TNF-α or Saline (CONs)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th></th>
<th>After 4 weeks</th>
<th></th>
<th>P value</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON (n=7)</td>
<td>TNF (n=8)</td>
<td></td>
<td>CON (n=7)</td>
<td>TNF (n=8)</td>
<td></td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>4.7±0.6</td>
<td>4.2±0.4</td>
<td>0.48</td>
<td>4.1±0.2</td>
<td>4.7±0.4</td>
<td>0.19</td>
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<tr>
<td>IL-6 (pg/ml)</td>
<td>28.8±8.9</td>
<td>22.5±3.1</td>
<td>0.49</td>
<td>35.8±5.2</td>
<td>25.0±3.7</td>
<td>0.10</td>
</tr>
<tr>
<td>IL-10 (pg/ml)</td>
<td>26.4±4.0</td>
<td>28.2±3.8</td>
<td>0.75</td>
<td>35.8±5.2</td>
<td>25.0±3.7</td>
<td>0.10</td>
</tr>
<tr>
<td>MCP-1 (pg/ml)</td>
<td>34.2±7.2</td>
<td>35.9±7.7</td>
<td>0.87</td>
<td>31.6±12.8</td>
<td>36.8±6.8</td>
<td>0.74</td>
</tr>
<tr>
<td>CRP (ng/ml)</td>
<td>68.8±4.4</td>
<td>67.9±3.2</td>
<td>0.88</td>
<td>60.0±1.5</td>
<td>64.8±2.1</td>
<td>0.10</td>
</tr>
</tbody>
</table>

There were no significant differences between the 2 groups (CON and TNF) in any of the cytokines at baseline or at 4 weeks, or any significant change in cytokine levels between baseline and 4 weeks. Values are mean±SEM. CRP, C-reactive protein; IL, interleukin; MCP, monocyte chemotactic protein. Other abbreviations as in Table 1.

Figure 1. Representative echocardiographic images of murine cardiac structure and function. (A) 2D image of the left ventricle (LV) from parasternal long-axis view. (B) 2D-guided M-mode tracing of LV diameter from short-axis view at papillary muscle level. (C) Transmitral Doppler flow from modified 4-chamber view showing E and A waves. (D) 2D-guided M-mode tracing of left atrial (LA) diameter from short-axis view at aortic valve level. Ao, aorta; AV, aortic valve; LVEDD, LV end-diastolic diameter; LVESD, LV end-systolic diameter; RV, right ventricle.
Effects of TNF-α on Echocardiography-Derived Parameters of Cardiac Structure and Function

There were no significant differences between the 2 groups in any of the echocardiographic parameters measured at baseline.
4 weeks or 16 weeks (Table 2). After 16 weeks, the TNF mice had significantly lower body weights than the CON mice (P=0.05), although there was no significant difference in the LV mass index between the 2 groups. Representative echocardiographic images are shown in Figure 1.

**Plasma Cytokine Levels**

There were no significant differences in any of the plasma cytokines measured at baseline or at 4 weeks between the TNF and CON mice (Table 3). There were also no significant changes in any of the cytokine levels between baseline and 4 weeks for the 2 groups.

**Effects of TNF-α on Atrial Structure and Fibrosis**

We found that the atria of the TNF mice showed significantly increased amounts of fibrosis, as detected using Sirius red staining, compared with atria from the CON mice (Figure 2). Mean relative fibrosis units were 372.8±21.5 arbitrary units (a.u.) for the TNF group (n=20) and 56.9±6.5 a.u. for the CON group (n=20), P<0.0001.

**Changes in Connexin-40 and -43 Immunofluorescence With TNF-α**

Connexin-40 immunofluorescence levels were significantly reduced in the atria of TNF mice compared with the CON mice (7.5±0.4 a.u vs. 40.4±1.9 a.u, respectively, n=15 in each group; P<0.0001, Figure 3). The change in the connexin-43 immunofluorescence levels between the 2 groups (33.1±2.8 a.u vs. 23.5±3.6 a.u, for TNF-α and CON groups, respectively, n=15 in each group) was of borderline significance (P=0.05).

**Effects of TNF-α on TGF-β and α-SMA Levels**

To determine whether the fibrotic actions of TNF-α were mediated via TGF-β, a prominent downstream mediator central to signaling cascades involved in the genesis of cardiac fibrosis, we measured levels of TGF-β in atrial samples from CON and TNF mice. We found that TGF-β levels were significantly increased in TNF-α treated mice compared with con-
TNF-α and Atrial Fibrosis

The main finding of our study is that a single intravenous bolus dose of TNF-α (4 μg/kg) in mice is sufficient to induce sustained atrial fibrosis, which persists 16 weeks after the initial injection. The resultant atrial fibrosis is not accompanied by any significant change in cardiac size or overall cardiac function (as detected with transthoracic echocardiography), although there is a functional change in the atrial electrical substrate as evidenced by decreased expression of connexin-40. The combination of atrial fibrosis and altered connexin expression is compatible with the development of an arrhythmogenic substrate and similar to reports from human studies involving atrial samples taken from patients with AF.

Our study therefore provides evidence for a pathogenic role of TNF-α in the development of atrial fibrosis and the arrhythmogenic substrate for AF.

**Discussion**

The main finding of our study is that a single intravenous bolus dose of TNF-α (4 μg/kg) in mice is sufficient to induce sustained atrial fibrosis, which persists 16 weeks after the initial injection. The resultant atrial fibrosis is not accompanied by any significant change in cardiac size or overall cardiac function (as detected with transthoracic echocardiography), although there is a functional change in the atrial electrical substrate as evidenced by decreased expression of connexin-40. The combination of atrial fibrosis and altered connexin expression is compatible with the development of an arrhythmogenic substrate and similar to reports from human studies involving atrial samples taken from patients with AF.

Our study therefore provides evidence for a pathogenic role of TNF-α in the development of atrial fibrosis and the arrhythmogenic substrate for AF.

**Effects of TNF-α on Smad3 Signaling**

Because TGF-β is known to act via the Smad signaling pathway to stimulate collagen production, we performed immunohistochemical measurements of Smad3 and phospho-Smad3. We found that Smad3 levels were significantly increased in TNF-α treated mice compared with controls (97.9±13.0 a.u vs. 50.1±18.5 a.u, respectively, n=13 in each group; P<0.05, Figure 4B). Smad3 levels were also increased (24.2±3.2 a.u vs. 12.4±1.3 a.u, respectively, n=15 and 13; P<0.01, Figure 4C).

**Effects of TNF-α on MMPs**

MMPs are involved in atrial structural remodeling and TGF-β appears to alter the ECM gene expression of MMPs. We therefore performed western blots on atrial tissue to determine MMP-2 and MMP-9 levels in the TNF and CON mice. MMP-2 levels were significantly increased in TNF-α treated mice compared with controls (157.3±26.4 a.u vs. 105.8±13.3 a.u, respectively, n=12 in each group; P<0.05, Figure 4E). When normalized to GAPDH, the MMP-2/GAPDH ratios were 1.05±0.13 and 0.66±0.08, respectively (P<0.05, Figure 4F). A sample western blot of MMP-2 and GAPDH in atrial tissue taken from control and TNF-α treated mice is shown in Figure 4G. We found no significant difference in MMP-9 levels between the 2 groups (65.8±2.9 a.u vs. 72.0±2.2 a.u, respectively, n=12 in each group; P=NS) nor in MMP-9/GAPDH ratios (4.21±0.62 and 4.09±0.40, respectively; P=NS).

Figure 5. Summary schematic showing proposed pathways by which tumor necrosis factor-α (TNF-α) induces atrial fibrosis and the formation of an arrhythmogenic substrate for atrial fibrillation.
are also consistent with findings from atrial appendage biopsy samples taken from patients with AF undergoing cardiac surgery (for coronary artery disease or mitral valve disease), which suggest that atrial structural remodeling and fibrosis are related to alterations in MMP expression. In another clinical study of 242 patients undergoing catheter ablation for AF, high pre-ablation plasma concentrations of TGF-α and tissue inhibitor of metalloproteinase 1 were closely related with electrophysiological properties and increased atrial arrhythmogenicity compared with control pulmonary vein cardiomycocytes not exposed to TGF-α. Because AF begets AF, it is plausible that repeated, transient exposure to TNF-α can result in increasing PV arrhythmogenicity and altered atrial substrate, with the eventual result of paroxysmal AF.

Another study limitation relates to fundamental differences between murine and human atria. In this study, we found that a bolus TNF-α injection resulted in decreased connexin-40 expression. Our findings are similar to those reported by other investigators on changes in connexin-40 levels in experimentally induced AF, although data from humans suggests that AF is associated with increased connexin-40 expression. One should therefore be cautious about extrapolating data from animal studies in order to understand a human disease.

Conclusions

TNF-α plays a pathogenic role in the development of atrial fibrosis and altered connexin-40 expression in mice. These effects appear to be mediated via the TGF-β/Smad signaling pathway, conversion of cardiac fibroblasts into myofibroblasts and increased section of MMPs. Collectively, these changes may contribute to the arrhythmogenic substrate and development of AF.

Acknowledgment

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Disclosures

The authors declare that they have no conflicts of interest.

References


