Role of T Lymphocytes in Hypertension-Induced Cardiac Extracellular Matrix Remodeling

Qianli Yu, Katherine Horak, Douglas F. Larson

Abstract—Cardiac remodeling in response to pressure overload involves reorganization of the myocytes and extracellular matrix (ECM). Neurohormonal pathways have been described as effector pathways in left ventricular ECM reorganization in response to pressure overload; we now are assessing the role of the T lymphocyte in this process. Mice with defined differences in T-lymphocyte function (C57BL/6 SCID, C57BL/6 WT, and BALB/c) were treated with 50 mg/L of \textit{N}^6\textendash\textit{nitro-L-arginine methyl ester} in their drinking water for 30 days. The immune function of C57BL/6 WT mice was T-helper type 1 (TH1), BALB/c was TH2, and C57BL/6 SCID was null. The arterial blood pressure increased by 30% in all of the strains of mice. However, ventricular stiffness significantly decreased in the C57 SCID, significantly increased in the BALB/c, and did not change in the C57 WT. The characterization of matrix metalloproteinase induction and activation on day 30 was associated with T-lymphocyte function. The total cardiac fibrillar collagen, percentage of fibrillar collagen cross-linking, and the activity of the cross-linking enzyme lysyl oxidase-like–3 (LOXL-3) significantly decreased in the C57 SCID, significantly increased in the BALB/c, and did not change in the C57 WT. This study revealed that the LOXL-3 pathway, namely, gene expression, enzymatic activities, and LOXL-3-mediated collagen cross-linking, was associated with ventricular stiffness and incongruence with lymphocyte function. These data support the concept that the T lymphocytes may play a fundamental regulatory role in cardiac ECM composition through modulation of collagen synthesis, degradation, and cross-linking. (Hypertension. 2006;48:98-104.)

Key Words: lymphocytes ■ ventricular function ■ collagen

In response to hypertension, the myocardium undergoes a series of changes, including adaptation of the extracellular matrix (ECM) composition. ECM remodeling leading to heart failure is characterized by disproportionate ECM fibrillar collagen synthesis and degradation and collagen cross-linking by the enzyme lysyl oxidase (LOX). These processes are mediated primarily by the cardiac fibroblast (CF), and, therefore, factors that modulate CF function will determine the nature of ECM remodeling in response to increased wall stress. It is understood that the CF function is under local as well as neurohormonal control. We suggest that CF function is also affected by T-lymphocyte function.

T lymphocytes participate in a regulatory role of virtually all immune responses and most nonlymphoid tissues. Several lines of evidence have shown that T lymphocytes are an essential component in the remodeling processes of noncardiac tissues, and others have suggested a role in cardiovascular remodeling and heart failure. The cytokine profile that has been used to describe subtypes of T-helper (CD4\textsuperscript{+}) lymphocytes is namely TH1 and TH2. It is accepted that a pathological increase in neuroendocrine mediators and wall stress induce cardiac remodeling, and we proposed that, in a similar manner, a difference in TH1/TH2 function may contribute to maladaptive cardiac remodeling. We, thus, hypothesized that the genetic differences in the T-helper phenotype of mice will affect cardiac ECM remodeling and function secondary to hypertension. Hypertension was induced in C57BL/6 SCID (C57 SCID), C57BL6 WT (C57 WT), and BABL/c mice by chronic \textit{N}^6\textendash\textit{nitro-L-arginine methyl ester} ([L-NAME]) a nonspecific NO synthase blocker) administration. The left ventricular function, gene expression, selected collagen modifying enzymatic pathways, and ventricular collagen composition were compared among the treatment groups. The results indicate that there are significant differences in mice lacking functional T lymphocytes and also in those genetically predisposed to either TH1 or TH2.

Methods

Mice

Four-week–old female mice with dissimilar immune backgrounds, specifically C57BL/6 SCID (B6.CB17-Prkdc[scid]/SzJ), C57BL/6 WT, and BALB/c, were obtained from Jackson Laboratories. The 3 strains of mice were either randomly selected as controls or administered \textit{L-NAME} (Sigma-Aldrich) at a dose of 50 mg/L in drinking water for 30 days. During the study, the mice were maintained in the facility on an NIH-31–modified mouse sterilized diet (mouse diet 7001, Teklad) and water ad libitum. This study was approved by the animal review committee. The procedures in the Guidelines for the Care and Use of Laboratory Animals and Principles of Laboratory Animal Care (published by the National Society for Medical Re-
mRNA for candidate genes were calculated from the threshold cycle melting curve analysis. In each experiment, the relative amounts of mRNA were normalized to the relative amounts of reference gene RNA (β-actin mRNA), which were obtained from a similar standard curve. Real-time PCR primers are listed in Table 1.

### Determination of MMP Activity
Gelatin zymography assay was according to the method described by Yu et al. Briefly, 10 mg of cardiac tissue was homogenized in 1 mL of zymograph extraction buffer and the extraction supernatants applied to precast 10% polyacrylamide gel Zymogram (Novex). After activation and development, the bands quantified with image analysis software (BIO-RAD GS-800).

### Determination of Hydroxyproline and Collagen Cross-Linking
Hydroxyproline and cross-linking assays were performed as described previously by Yu et al. For measurement of total myocardial hydroxyproline, hydroxyproline levels were quantified by comparison to a standard colorimetric curve of trans-hydroxyproline (Sigma). The data were expressed as micrograms of collagen per milligram of dry heart weight, assuming that collagen contains an average of 13.5% hydroxyproline. Collagen cross-linking was determined using cyanogen bromide digestion. The percentage of cross-linking was determined by comparing the total hydroxyproline to a standard colorimetric curve.

### Determination of LOX Activity
LOX activity was measured by a fluorometric assay modified from the report of Palamakumbura and Trackman. Briefly, ~25 mg of cardiac tissue was homogenized in 300 μL of CelLytic (Sigma) at ~80°C and reconstituted to 1 mL with LOX buffer (1.2 mol/L urea, 25 mg of 1000 mg/kg, IP) and 0.1 mol/L sodium borate [pH 8.2]) then centrifuged at 12,000 g for 10 minutes. After collection of the supernatant, LOX enzymatic activity was detected by the production of H₂O₂ from alkyl monamine substrate and detected with fluorescent resorufin produced by horseradish peroxidase–catalyzed oxidation of N-acetyl-3,7-dihydroxyphenoxazine (Amplex Red) at wavelengths 563 and 587 nm. Parallel unknown samples were prepared by adding 500 μmol/L β-aminopropionitrile fumarate to completely inhibit the activity of LOX. The emission intensity difference can be converted into the amount of hydrogen peroxide produced by the action of LOX when compared with the fluorescence of a nanomoles hydrogen peroxide standard plot.

### RNA Extraction and Real-Time PCR
Cardiac and splenic tissues were processed by methods described by Yu et al. Subsequent to acquisition of pressure-volume loops, cardiac and lymphoid tissues were harvested in TRIzol (Invitrogen Life Technologies). Custom primers were designed using the Primer3 and synthesized by Integrated DNA Technologies. Primer quality was confirmed by melting curve analysis. In each experiment, the relative amounts of mRNA for candidate genes were calculated from the threshold cycle numbers and normalized to the relative amounts of reference gene RNA (β-actin mRNA), which were obtained from a similar standard curve.

#### Table 1. Real-Time PCR Primers and Accession Numbers

<table>
<thead>
<tr>
<th>Primers/Accession No.</th>
<th>Forward/Bases</th>
<th>Reverse/Bases</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>5'-TTGTGCAAGGATGGAAG AG-3'</td>
<td>5' -TGA TCC ACA TCT GCT GGA AG-3'</td>
</tr>
<tr>
<td>BC045013</td>
<td>204 to 223</td>
<td>350 to 331</td>
</tr>
<tr>
<td>Pro-Col Ia1</td>
<td>5'-GAAGCGCAGAGACTGGATCG-3'</td>
<td>5' -GTTCGGAGCGATCCAGTACG-3'</td>
</tr>
<tr>
<td>BC003198</td>
<td>1186 to 1205</td>
<td>1327 to 1308</td>
</tr>
<tr>
<td>Pro-Col Ilkα1</td>
<td>5'-TCTGCTTTGCGGTGATGAC-3'</td>
<td>5' -TGCTTGGAATGAGGATCAC-3'</td>
</tr>
<tr>
<td>AK079113</td>
<td>1631 to 1650</td>
<td>1749 to 1729</td>
</tr>
<tr>
<td>LOX</td>
<td>5'-GAGTGGTGGATCCGTGACTCG-3'</td>
<td>5' -GTGGTCGGCACAGATGA-3'</td>
</tr>
<tr>
<td>NM_010728</td>
<td>3056 to 3075</td>
<td>3219 to 3200</td>
</tr>
<tr>
<td>LOXL3</td>
<td>5'-TTCCGCGGAGAAAGAGAT-3'</td>
<td>5' -GGTTGCAATGCGAGAAGA-3'</td>
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<tr>
<td>NM_013568</td>
<td>752 to 771</td>
<td>901 to 882</td>
</tr>
<tr>
<td>Pro-MMP-13</td>
<td>5'-GATGACTGCTGTGAGAAGACC-3'</td>
<td>5' -GTCAATCAGACGACACTGTTGA-3'</td>
</tr>
<tr>
<td>NM_008617</td>
<td>98 to 119</td>
<td>459 to 438</td>
</tr>
<tr>
<td>Pro-MMP-2</td>
<td>5'-AAAGACTGGTGGTGTCTGTA-3'</td>
<td>5' -CAAGAAGCTGGACAGAAGG-3'</td>
</tr>
<tr>
<td>NM_008610</td>
<td>2823 to 2842</td>
<td>2972 to 2953</td>
</tr>
<tr>
<td>Pro-MMP-9</td>
<td>5'-CTTCGACAGCTGCAAGAGATG-3'</td>
<td>5' -CTTGTAAATGGCCCTCCTCTAT-3'</td>
</tr>
<tr>
<td>NM_013599</td>
<td>1140 to 1161</td>
<td>1712 to 1694</td>
</tr>
</tbody>
</table>
Logic differences between the strains (n/H11005 P3) * 0.02.

Figure 1. ELISA cytokine assay of C57 WT mice and BALB/c mice. Cultured splenocytes from C57 WT and BALB/c were assayed for IFN-γ, IL-4, and IL-10 to demonstrate the immunologic differences between the strains (n=3). *P<0.02.

Statistics
ANOVA with multicomparison procedures was used to test the differences among the defined groups or comparable nonparametric tests (Kruskal–Wallis and the rank sum test) were substituted when tests for normality and equal variance failed with SPSS version 11.5. The differences were calculated with a significance level of 0.05 and power of 0.8. All of the data are reported as mean±SEM.

Results
The immune background was compared between C57 WT and BALB/c mice in Figure 1. Figure 1 shows that C57 WT mice are dominant TH1, and BALB/c mice are TH2 dominant. Moreover, the ratio of IFN-γ/IL-4 is 173 in the C57BL WT and 21 in the BALB/c, thus confirming their innate immune polarization. This murine strain difference in immune background has been characterized previously.10

The effect of chronic L-NAME administration on the left ventricular mechanics was compared among C57 SCID, C57 WT, and BALB/c mice (Table 2). Consistent with the report by Rossi et al,11 chronic L-NAME administration did not induce cardiac hypertrophy. The maximum systolic pressure increased 31% in the C57 SCID mice (P<0.05), 34% (P<0.05) in the C57 WT mice, and 31% (P<0.05) in the BALB/c mice with L-NAME administration compared with their respective controls, which corresponds with the nonanesthetized systolic blood pressures shown in Figure 2. Correspondingly, arteriolar elastance significantly increased in all of the L-NAME–treated groups (P<0.05); however, there were no significant differences among all of the groups related to CO and SV compared with their respective controls. There was a mouse strain separation with the ventricular mechanical parameters. The end-diastolic volumes (Ved) and end-systolic volumes (Ves) were significantly increased in L-NAME–treated C57 SCID mice, decreased in BALB/c mice, and the Ves decreased in the C57 WT mice (P<0.05). The ejection fraction decreased 34% in the C57 SCID mice and increased 17% and 27% in the C57 WT and BALB/c mice, respectively (P<0.05). The response to the increased afterload is supported by the increased maximum rate of pressure increase over time, dP/dtmax, and maximum rate of pressure decrease over time, dP/dtmin, in all of the groups (P<0.05), and

![Graph showing cytokine assay results for C57 WT and BALB/c mice](http://hyper.ahajournals.org/)

**TABLE 2. Effect of L-NAME on Hemodynamics and Ventricular Function**

<table>
<thead>
<tr>
<th>Groups</th>
<th>C57 SCID</th>
<th>C57 WT</th>
<th>BALB/c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>(n=8)</td>
<td>(n=5)</td>
<td>(n=8)</td>
</tr>
<tr>
<td>Hemodynamics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BW</td>
<td>17.4±0.6</td>
<td>17.5±0.6</td>
<td>20.3±0.4</td>
</tr>
<tr>
<td>HW/BW</td>
<td>5.1±0.3</td>
<td>5.0±0.4</td>
<td>4.9±0.1</td>
</tr>
<tr>
<td>HR</td>
<td>442±38</td>
<td>497±20</td>
<td>438±18</td>
</tr>
<tr>
<td>Pmax</td>
<td>83±3</td>
<td>122±10*</td>
<td>86±3</td>
</tr>
<tr>
<td>Ea</td>
<td>5.7±1.2</td>
<td>10.9±1.2*</td>
<td>7.5±0.8</td>
</tr>
<tr>
<td>CO</td>
<td>6.2±0.4</td>
<td>5.6±0.4</td>
<td>5.2±0.3</td>
</tr>
<tr>
<td>SV</td>
<td>14.3±1.0</td>
<td>11.3±0.7</td>
<td>11.4±0.5</td>
</tr>
<tr>
<td>Ventricular function</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ved</td>
<td>18.1±2.0</td>
<td>21.9±1.5*</td>
<td>15.7±1.4</td>
</tr>
<tr>
<td>Ves</td>
<td>5.0±1.6</td>
<td>11.4±1.2*</td>
<td>5.6±0.9</td>
</tr>
<tr>
<td>EF</td>
<td>79±4</td>
<td>52±3*</td>
<td>72±3</td>
</tr>
<tr>
<td>dP/dtmax-Ved</td>
<td>6830±477</td>
<td>9330±1244*</td>
<td>7217±601</td>
</tr>
<tr>
<td>dP/dtmin</td>
<td>49±632±3</td>
<td>6480±572*</td>
<td>5365±335</td>
</tr>
<tr>
<td>SW</td>
<td>950±130</td>
<td>855±64</td>
<td>807±38</td>
</tr>
<tr>
<td>PFRS</td>
<td>91±7</td>
<td>105±5</td>
<td>89±3</td>
</tr>
<tr>
<td>dP/dtmax-Ved</td>
<td>762±250</td>
<td>587±193</td>
<td>678±94</td>
</tr>
<tr>
<td>τ Weiss</td>
<td>8.8±0.6</td>
<td>7.3±0.4</td>
<td>8.6±0.4</td>
</tr>
<tr>
<td>β</td>
<td>0.15±0.01†</td>
<td>0.08±0.03*</td>
<td>0.19±0.01</td>
</tr>
</tbody>
</table>

BW indicates body weight (mg); HW, heart weight (µg); HR, heart rate (beat/min); Pmax, systolic pressure (mm Hg); Ea, elastance of vascular (mm Hg/µL); Ved, end-diastolic volume (µL); Ves, end-systolic volume (µL); SW, stroke work (mm Hg·µL); dP/dtmax-Ved, pressure of end diastole (mm Hg); β, left ventricular stiffness (mm Hg/µL). *(n=8).

*P<0.05 vs controls; †P<0.05 vs C57 control.
stroke work (SW) and preload recruitable SW (the slope of SW plotted against $V_{ed}$ (PRSW) increased only in the C57 WT and BALB/c mice ($P<0.05$). The parameters for isovolumic contraction and relaxation, $dP/dV_{ed}$-Ved and Tau Weiss, respectively, did not change with L-NAME administration. Most striking was the passive diastolic function parameter, $\beta$, which with the control C57 SCID and BALB/c mice was markedly less compared with the C57 WT ($P<0.05$). Additionally, L-NAME administration decreased $\beta$ in C57 SCID mice, from 0.15 to 0.08 mm Hg/μL ($P<0.05$), did not change in the C57 WT mice, and significantly increased, from 0.12 to 0.17 mm Hg/μL ($P<0.05$), in the BALB/c mice, when compared with their respective controls. In summary, those hemodynamic parameters related to the cardiac ECM were most affected by the mouse strain differences with L-NAME administration, because there were no differences in isovolumic contraction ($dP/dV_{ed}$-Ved) and isovolumic relaxation ($\tau$).

The comparison of the gene expression in cardiac tissues among the 6 groups with real-time PCR is shown in Table 3. Data were reported as the normalized threshold cycle number, which implies that if the cycles decrease the expression is increased. Data were represented by real-time PCR cycle numbers normalized by respective $\beta$-actin and expressed as mean±SE (n=4).

**TABLE 3. Effect of L-NAME on Cardiac Gene Expression**

<table>
<thead>
<tr>
<th>Groups</th>
<th>C57 SCID</th>
<th>C57 WT</th>
<th>BALB/c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td>Control</td>
</tr>
<tr>
<td>Pro-Col Ix1</td>
<td>2.43±0.06†</td>
<td>2.69±0.14</td>
<td>3.00±0.17</td>
</tr>
<tr>
<td>Pro-Col Ilx1</td>
<td>11.54±0.18</td>
<td>10.63±0.94</td>
<td>11.02±0.48</td>
</tr>
<tr>
<td>Pro-MMP-2</td>
<td>3.57±0.27</td>
<td>3.12±0.45</td>
<td>3.25±0.08</td>
</tr>
<tr>
<td>Pro-MMP-9</td>
<td>8.44±0.31†</td>
<td>8.05±0.43</td>
<td>7.65±0.21</td>
</tr>
<tr>
<td>Pro-MMP-13</td>
<td>9.96±0.31</td>
<td>8.84±0.39*</td>
<td>9.17±0.13</td>
</tr>
<tr>
<td>LOX</td>
<td>9.67±0.34</td>
<td>9.65±0.16</td>
<td>9.13±0.34</td>
</tr>
<tr>
<td>LOXL3</td>
<td>8.89±0.17†</td>
<td>9.77±0.13*</td>
<td>9.24±0.19</td>
</tr>
</tbody>
</table>

These data are expressed as threshold cycle number, which implies that if the cycles decrease the expression is increased. Data were represented by real-time PCR cycle numbers normalized by respective $\beta$-actin and expressed as mean±SE (n=4).

* $P<0.05$ vs respective controls; † $P<0.05$ vs C57 WT control.
Figure 4 compares the LOX enzymatic activity in the study groups. L-NAME administration decreased LOX activity in C57 SCID mice ($P \leq 0.03$) while increasing in BALB/c mice ($P \leq 0.02$) and not changing in C57 WT mice ($P$ value was not significant). Moreover, the correlative relationship between collagen cross-linking and ventricular stiffness ($\beta$) demonstrated $R^2$ at 0.5985 ($P < 0.01$), as shown in Figure 5. This relationship reveals that matured and stabilized collagen is a determinant of the diastolic function.

**Discussion**

The present study hypothesized that mice with dissimilar T-helper immune backgrounds will remodel differently in response to chronic hypertension. Accordingly, the goal of this study was to examine the effects of chronic hypertension induced by L-NAME in mice that differ in T-lymphocyte function, namely C57 SCID, C57 WT, and BALB/c. The C57 SCID mice are devoid of functional T lymphocytes, and we demonstrated that the C57 WT are a predominant TH1 and the BALB/c mice are TH2, which is consistent with other reports. The important findings of this study are as follows. The cardiac output did not differ compared with the respective controls; however, there was a major separation among the 3 strains of mice related to cardiac ECM remodeling and ventricular mechanics affected by the ECM structure. The amount of ventricular collagen and the extent of cross-linking markedly differed when comparing the 3 groups of mice. Considerable differences in fibrillar cardiac collagen composition were noted among the different immune control groups. L-NAME administration did not alter cardiac ECM in the C57 WT; however, differences were evident the C57 SCID and BALB/c compared with the controls. Generally, the heart dilated in the C57 SCID phenotype in response to increased afterload was because of a diminution of collagen and cross-linking. C57 WT (TH1) demonstrated minimal structural changes, and, conversely, the BALB/c phenotype (TH2) demonstrated a decrease in ventricular volumes and increased ventricular collagen and cross-linking with increased afterload.

This study shows that the immune background of the mouse may affect the remodeling processes that occur in response to increased chronic afterload. Because the C57 SCID mice are of a C57 WT background, they are most comparable with the C57 WT except for lacking functional T lymphocytes. It is implicit that C57 WT mice are genetically different from the BALB/c. This difference, however, is a classical immunologic model to define host phenotype resistance or susceptibility, which are dependent on T-lymphocyte function.

<table>
<thead>
<tr>
<th>TABLE 4. Effect of L-NAME on Ventricular Total Collagen Content and Cross-Linked Collagen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groups</td>
</tr>
<tr>
<td>--------------</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Soluble</td>
</tr>
<tr>
<td>Crosslinked</td>
</tr>
<tr>
<td>% Cross-linked</td>
</tr>
</tbody>
</table>

Total cardiac fibrillar collagen and cross-linked collagen was assayed by determination of the hydroxyproline concentrations. The data were expressed as microgram of collagen per milligram of dry heart weight, assuming that collagen contains an average of 13.5% hydroxyproline ($n=4$).
* $P < 0.05$ versus respective controls; † $P < 0.05$ versus C57 WT control.
cardiac hypertrophy. Various authors have reported that this model has been shown by us and others not to result in

CCL4-induced liver injury compared with the BALB/c with a C57BL/6 developed significantly less hepatic fibrosis after respective SCID phenotypes. They demonstrated that fibrosis when comparing C57BL/6 and BALB/c with their respective T-helper subsets accounted for the extent of hepatic cytokines. Consistent with our findings, Shi et al found that T-helper subsets accounted for the extent of hepatic fibrosis when comparing C57BL/6 and BALB/c with their respective SCID phenotypes. They demonstrated that C57BL/6 developed significantly less hepatic fibrosis after CCL4-induced liver injury compared with the BALB/c with a similar degree of cellular necrosis. Given the lack of functional T and B lymphocytes in the SCID mice, our data and that of Shi et al strongly support the role of the lymphocyte in modulation of fibrosis. This concept is firmly supported by our recent observation in a SCID mouse model that failed to remodel subsequent to myocardial infarction; however, placement of a fibroblast patch graft on the infarcted site induced restorative remodeling and normal hemodynamic LV function.

NOS inhibition with chronic administration of L-NAME induces a dose-dependent increase in systolic blood pressure that leads to cardiac and vascular remodeling. However, this model has been shown by us and others not to result in cardiac hypertrophy. Various authors have reported that chronic NO synthesis inhibition results in cardiac fibrosis, which corresponds with our cardiac collagen determinations. The chronic hypertension induced by L-NAME may induce myocardial ischemia or, alternatively, change the mitochondrial function directly because of inhibition of NO relating to fibrosis. However, we have demonstrated that there is a marked difference in cardiac collagen in mice with varied immune backgrounds that appears not to be related to that suggested above. Therefore, this model provides a good opportunity to investigate the regulatory role of T lymphocytes in the wall stress.

We suggest that cardiac collagen content and collagen cross-linking are associated with T-lymphocyte function. The fact that the C57 SCID mice exhibited a markedly differed response to the induced wall stress compared with C57 WT emphasizes that lymphocyte-derived cytokines play a role in cardiac collagen homeostasis. The gene expression after 30 days of L-NAME administration for pro-collagen I and III did not differ compared with controls. The MMP-9 and -13 gene expression and activities did not parallel the cardiac collagen content, which implies that the MMP activities are temporally altered during the course of cardiac remodeling and also do not represent the entire portfolio of cardiac MMPs. Moreover, the enzyme that mediates collagen cross-linking, LOX, also appeared to be affected by the differences in immune background. There are 2 LOX enzymes described in the cardiac tissue, LOX and LOXL3, of which LOX is the only one that has been shown to have collagen as a substrate. Although LOXL3 has been shown recently to be highly expressed in cardiovascular tissues, its substrate has yet to be proven (K. Csizsar, personal communication, July 2005). However, our data demonstrated that the LOX enzymatic function (Figure 3) parallels LOXL3 and not LOX gene expression (Table 3) and fibrillar collagen cross-linking (Table 4) in the 3 control and treatment groups. The increased cardiac collagen and collagen cross-linking paralleled the ventricular stiffness as also described by Badenhorst et al and, in addition our immunologically dissimilar mice, suggests that the ventricular stiffness is related to T-lymphocyte function.

Other reports have demonstrated that T-lymphocyte function affects cardiac diastolic function. We have shown previously with a TH2 murine model of HIV that there is significant diastolic dysfunction. It is known that immunosuppressed transplanted cardiac and liver patients develop diastolic dysfunction. Rodent studies exhibited markedly altered ECM composition with cyclosporine at clinical doses without other confounding factors, such as hypertension or renal dysfunction. Conversely, cardiac hypertrophy and fibrosis associated with hypertension have been related with altered immune function. Activated T cells of both TH1 and TH2 subtypes can regulate fibroblasts activities by secreting IFN-γ and IL-4, respectively. Most importantly, the role of TH1 cytokines in fibrotic conditions has been suggested to occur during the initiation of fibrosis and the TH2 cytokines during later stages. We propose, therefore, that the TH1 immune responses may parallel the development of early compensatory fibrotic responses, whereas TH2 may constitute a latter phase of fibrotic remodeling in the heart.

Alternate T-lymphocyte subsets that may support our findings include regulatory T cells nTreg, Tr1, and TH3. Regulatory T cells (nTreg) CD4+CD25+ are functionally mature from the thymus, Tr1 are induced by IFN-γ from naïve T-cells and secrete IL-10, and TH3 lymphocytes are
dominant sources of transforming growth factor-β1. The immunosuppressive activity of TH3 depends on the production of transforming growth factor-β1, which suppresses both TH1 and TH2 lymphocyte function.17 Mast cells also regulate activities of fibroblasts through IL-4 secretion,28 as do basophils29 and eosinophils.30 These cells, however, are under the direct control of TH1 and TH2 cytokines. The macrophages have also been described as mediators of cardiac fibrosis.31 In our model, however, SCID mice have functional phages have also been described as mediators of cardiac remodeling processes. The suggested role of the T lymphocyte in the ventricular remodeling processes in response to chronic increased afterload provides a new and novel insight into the molecular pathways with potential therapeutic implications for heart failure. The differential responses of T lymphocytes as a consequence to increased ventricular wall stress in the human, as shown with mice, may be related to genotypic variation in addition to age, gender, and exposure to infectious processes. Further studies are necessary to determine the interactions among immune function, products of the stressed heart, and neurohormonal pathways that appear to be integrated in these cardiac remodeling processes.

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Disclosures

None.

References


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