A role for T lymphocytes in mediating cardiac diastolic function

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1Department of Medical Pharmacology, School of Medicine, and 2Sarver Heart Center and Department of Surgery, College of Medicine, University of Arizona; 3Department of Health Promotion Sciences, Mel and Enid Zuckerman College of Public Health; and 4Department of Immunology, College of Medicine, University of Arizona, Tucson, Arizona

Submitted 25 January 2005; accepted in final form 7 April 2005

Yu, Qianli, Ronald R. Watson, John J. Marchalonis, and Douglas F. Larson. A role for T lymphocytes in mediating cardiac diastolic function. Am J Physiol Heart Circ Physiol 289: H643–H651, 2005; doi:10.1152/ajpheart.00073.2005.—The induction of T helper (TH) lymphocytes by distinct TH ligands results in a differentiation to TH1/TH2 subsets based on their unique pattern of cytokine secretion and effector functions. We hypothesized that the relative proportion of TH1/TH2 directly relates to cardiac fibroblast (CF) function and thereby cardiac extracellular matrix (ECM) composition and cardiac diastolic function in the absence of injury or altered wall stress. We compared the effect of selective TH1 with TH2 inducers on cardiac gene expression, ECM composition, and diastolic function in C57BL/6J mice. Twelve weeks after immune modulation, the left ventricular stiffness (β) was significantly increased in the TH1 group and decreased in the TH2 group (P < 0.01). The TH2 group also demonstrated significantly increased end-diastolic and end-systolic volumes (P < 0.01). Cardiac gene expression patterns for pro-matrix metalloproteinase (MMP)-9 and -13 were increased by greater than fivefold in the TH2 group and significantly decreased in the TH1 group (P < 0.05). The total cardiac collagen and cross-linked collagen were significantly increased in the TH1 group and decreased in the TH2 group (P < 0.01). Coculturing lymphocytes harvested from the treated mice with naive primary CF demonstrated a direct control of the lymphocytes on CF pro-collagen, pro-MMP gene expression, and MMP activity. These results suggest that the TH phenotype differentially affects diastolic function through modulating CF pro-collagen and pro-MMP gene expression, MMP activity, and cardiac collagen cross-linking, resulting in altered ECM composition. Thus modulation of TH lymphocyte function could promote adaptive remodeling in heart failure and postmyocardial infarction.

LP-BM5 retrovirus; T-helper cell; cardiac fibroblast; extracellular matrix; collagen types I and III; T helper 1; T helper 2; cross-linking; T-cell receptor peptide

DIASTOLIC HEART FAILURE has recently been reported to have a mortality rate of 23% during a 3.1-yr follow-up study with optimized medical therapy (23). A central contributory factor in diastolic dysfunction is maladaptive remodeling of the extracellular matrix (ECM), whose composition is controlled and maintained by cardiac fibroblasts (1, 7, 13, 16). T lymphocyte infiltration postmyocardial infarction coincides with increased cardiac fibroblast proliferation and fibrotic function (57), which is linked to the notion that lymphocytes are essential in the wound healing process (2, 3). The cooperation of T lymphocytes with fibroblasts in cardiac remodeling secondary to tissue injury is therefore a predictable concept. Correspondingly, we propose that alteration of T lymphocyte function may affect cardiac ECM composition, and thus diastolic function, in the absence of cardiac tissue injury or increased wall stress.

Cardiac fibrillar collagen homeostasis is mediated by cardiac fibroblasts via a balance of synthesis, degradation, and cross-linking (16). The primary forms of synthesized collagen in the heart are collagen type I (Col I), making up 85% of total collagen, followed by 11% collagen type III (Col III). Second, the Col I and Col III degradation pathway is mediated by collagenases, matrix metalloproteinase (MMP)-1, -8, and -13, and gelatinases MMP-2 and -9 (43). Finally, collagen cross-linking has been demonstrated to alter ventricular compliance and may affect collagen degradation (1). Thus any imbalance among collagen biosynthesis, degradation, and cross-linking may contribute to left ventricular diastolic dysfunction (6). Hence, many lines of evidence support that cardiac ECM composition correlates with cardiac mechanical function. However, there is little direct evidence that integrates lymphocyte and cardiac fibroblast functions with ECM composition.

The differentiation process of naive T helper (TH) lymphocytes leads to the generation of effector cells, namely, TH1 and TH2 cells, where TH1 cells are characterized by cytokine interleukin (IL)-2, -12, -15, and -18, interferon (IFN)-γ, and transforming growth factor (TGF)-β expression and TH2 by IL-4, -5, -6, -10, -13, and -17 (39). The lymphocyte has recently been shown to modulate fibroblast collagen formation in noncardiac tissues, namely, hepatic, dermal, pulmonary, and synovial tissues (8, 9, 21, 34). Moreover, decreased cardiac and vascular compliance in preeclampsia, angiotensin II-induced hypertension, and spontaneously hypertensive rats have been associated with TH1 cytokine levels (36, 41, 51). Agents that suppress T-lymphocyte function, such as steroids (10), retinoic acid, sirolimus (18), and cyclosporine A (15), markedly impair wound healing. In liver transplantation patients treated with either cyclosporine A or tacrolimus immunosuppression, there is a significant deterioration of diastolic function without changes in systolic function after 3 mo of immunosuppression (45). Rodent studies support this observation where markedly altered cardiac ECM composition occurred with cyclosporine at clinical immunosuppressive doses (14, 38). Pauschinger et al. (37) demonstrated significantly increased Col I and Col III ratios in dilated cardiomyopathy (DCM) but could not detect the profibrotic cytokines TGF-β1 or TGF-β2 in human DCM heart biopsies (37). This report suggested that other cytokines may be involved in the regulation of cardiac fibroblast collagen production in the heart. Clinical data support this conclusion, as the balance of TH1 and TH2 cytokine gene expression is directly related to the mortality rate of DCM with heart failure (46).
In view of the importance of cytokines in heart disease, the present study determined the effect of selective modulation of lymphocyte function on ventricular function. Susceptible mice, such as the C57BL/6 strain infected with the LP-BM5 variant of murine leukemia virus (MuLV), have a deficient immune response correlated with a decreased TH1-to-TH2 ratio, but TH1 activity that can be restored by the administration of peptides derived from the variable segment of T-cell receptor (TCR)-Vβ chains (56). Our results revealed that the TH2 immune condition significantly alters cardiac ECM and cardiac diastolic function, whereas TH1 leads to profibrotic activity and increased ventricular stiffness. This investigation of lymphocyte-directed cardiac diastolic function is in the absence of cardiac tissue inflammation, cellular infiltration, or injury.

MATERIALS AND METHODS

Mice

Four-week-old female C57BL/J6 mice were obtained from Charles River Laboratories (Wilmington, DE). During the study, the mice were maintained in the facility on an NIH-31-modified mouse sterilized diet (mouse diet no. 7001, Teklad; Madison, WI) and water ad libitum. This study was approved by the animal review committee. The procedures in the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1985) and National Society for Medical Research “Principles of Laboratory Animal Care” were followed in this study. Mice were randomly assigned to one of the following groups: group I, uninfected control mice; group II, mice infected with LP-BM5 retrovirus; group III, uninfected mice given TCR-Vβ peptides 5.2 and 8.1; and group IV, mice infected with retrovirus and vaccinated with TCR-Vβ peptides 5.2 and 8.1.

LP-BM5 Viral Infection

The LP-BM5 retrovirus mixture was administered intraperitoneally to mice in 0.1 ml of minimum essential medium with an esotropic titer of 4.5 log10 plaque-forming units × 10−7/L, which induces TH2 dominant lymphocyte function comparable with that previously published (17, 20, 47, 58). The mice were infected with LP-BM5 2 wk before treatment with the TCR-Vβ peptides, as done previously in earlier studies (27, 40, 49). Hemodynamic studies and tissue acquisition were performed 12 wk postinduction of TH2 immune function with the LP-BM5 mixture.

TCR-Vβ Peptides

The TCR-Vβ peptides corresponding to the CDR1 segments of human Vβ 5.2 and V 8.1 at a dose of 200 pg/mouse and vehicle control were administered intraperitoneally on days 14 and 28 in the noninfected and post-LP-BM5 infection mice. The sequences of these TCR-Vβ peptides were CKPISGHNLSFWRYQT (Vβ 8.1) and CSPKSNGHDTVSWYQQA (Vβ 5.2), and they were manufactured by Minotopes Clayton (Victoria, Australia). Hemodynamic studies and tissue acquisition were performed 12 wk postinduction of TH1 immune function.

Quantification of Left Ventricular Mechanics

The Millar conductance catheter system (CCS) was used, as has been previously described by our laboratory (53–56). All mice were anesthetized with urethane in saline (1.000 mg/kg ip) and α-chloralose in propylene glycol (50 mg/kg ip). This anesthetic technique causes minimal cardiac and vascular depression and inhibits central nervous system catecholamine outflow that may confound data interpretation (11). The mice were ventilated, and the external jugular vein was cannulated for volume administration. The apical portion of the heart and the inferior vena cava were exposed through a substernal-transverse incision, and a Millar conductance catheter (1.4-Fr, Millar; Houston, TX) was inserted into the apex of the left ventricle. Pressure-volume loops were acquired and computed as previously reported (56).

RNA Extraction, RT-PCR, and Real-Time PCR

Cardiac tissues were processed according to the methods described by Yu et al. (59). Cardiac and lymphoid tissues were harvested in TRIzol (Invitrogen Life Technologies; Carlsbad, CA). Diluted cDNA was used for reaction with the Quantitect SYBR green PCR kit (Qiagen) at 50 μl and real-time RT-PCR was performed by monitoring the increase of fluorescence of SYBR green using the Rotor-Gene RG-3000 (Corbett Research) in a 72-well rotor. Custom primers were designed using Integrated DNA Technologies. Primer quality (lack of primer-dimer amplification) was confirmed by melting curve analysis. In addition, gel electrophoresis of each primer product was shown to have only a single band. In each experiment, the relative amounts of mRNA for target genes were calculated from the standard curves and 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.

The method of RT-PCR was used to determine the gene expression of pro-Col Iα, and pro-MMP-13 of cultured cardiac fibroblasts cocultured with lymphocytes as described by our previous report (59). The pro-Col Iα(2) (sense) 5′-GGTGATACCTGGATAGGAGAC-3′ and (antisense) 5′-GTCTGACCACTGAGATGGTGTC-3′ primers and pro-MMP-13 (sense) 5′-GATGACCTGTCTGAGGAAGACC-3′ and (antisense) 5′-GGAATGCTGCGAGGAGAGAG-3′ primers were normalized with 18S rRNA QuantumRNA (Ambion; Austin, TX).

Cardiac Fibroblast and Lymphocyte Isolation for Coculture

The isolation protocol provided by Dr. Thomas Borg (University of South Carolina) yields a cardiac fibroblasts purity of >88% using the

Table 1. Real-Time PCR Primers

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Forward</th>
<th>Reverse</th>
</tr>
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<tbody>
<tr>
<td>β-Actin BC040513</td>
<td>5′-TTGTCTGACAGAGTGACAAAG-3′ (204–223 bp)</td>
<td>5′-TGATCGAGATCTGCTGAGAGG-3′ (350–331 bp)</td>
</tr>
<tr>
<td>Pro-Col Iα NM_007743</td>
<td>5′-GCTGTACCTGGATAGGAGAC-3′ (9,929–9,968 bp)</td>
<td>5′-GTCCATGATCTGATGACATGG-3′ (4,080–4,062 bp)</td>
</tr>
<tr>
<td>Pro-Col Iα AK079113</td>
<td>5′-TGATGATCTGATGACACAGGAC-3′ (9,611–9,600 bp)</td>
<td>5′-TGCTGACCACTGAGATGGTGTC-3′ (459–438 bp)</td>
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<tr>
<td>Pro-MMP-13 NM_008607</td>
<td>5′-GCGCACTGGATAGGAGAC-3′ (1,140–1,161 bp)</td>
<td>5′-GATGACCTGTCTGAGGAAGACC-3′ (1,749–1,729 bp)</td>
</tr>
<tr>
<td>Pro-MMP-9 NM_013599</td>
<td>5′-GATGACCTGTCTGAGGAAGACC-3′ (98–119 bp)</td>
<td>5′-GGAATGCTGCGAGGAGAGAG-3′ (534–514 bp)</td>
</tr>
<tr>
<td>IL-4 NM_021283</td>
<td>5′-CCACCAAGATGGATCTGCAAG-3′ (429–447 bp)</td>
<td>5′-GATGACCTGTCTGAGGAAGACC-3′ (1,105–1,086 bp)</td>
</tr>
<tr>
<td>IFN-γ NM_008337</td>
<td>5′-GCTGTACCTGGATAGGAGATGT-3′ (969–988 bp)</td>
<td>5′-GATGACCTGTCTGAGGAAGACC-3′ (1,083–1,102 bp)</td>
</tr>
<tr>
<td>IL-10 NM_015058</td>
<td>5′-CCACCAAGATGGATCGAAAGG-3′ (1,140–1,161 bp)</td>
<td>5′-GATGACCTGTCTGAGGAAGACC-3′ (1,083–1,102 bp)</td>
</tr>
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</table>

Col, collagen; MMP, matrix metalloproteinase; IFN, interferon.

AJP-Heart Circ Physiol • VOL 289 • AUGUST 2005 • www.ajpheart.org
cardiac fibroblast-specific DDR2 antibody with FACS. In brief, ventricular sections were minced and sequentially digested 15 times with 0.54 mg liberase 3 enzyme (Roche Biochemical) in 60 ml of warm sterile Krebs-Henseleit buffer, and the adherent fibroblasts were plated in a T75 with 20 ml of DMEM-HEPES with 10% fetal bovine serum medium. These primary fibroblast cultures were grown to 80% confluence, and the fetal bovine serum was decreased to 1% before the lymphocytes were cocultured.

The spleen was removed with sterile techniques, sliced, and forced through a 30-mesh screen filter. The splenic lymphocytes were isolated with lymphocyte separation medium (Cellgro; Herndon, VA) and washed three times with RPMI culture media. The lymphocytes from the individual treatment groups were added directly to the fibroblast cultures at a concentration of $5 \times 10^5$ lymphocytes/ml. After 48 h, the lymphocytes were removed by washing, the adherent cardiac fibroblasts were removed for RNA extraction and RT-PCR, and the supernatant was used for zymography.

Zymography

Gelatin zymography was performed using lymphocyte-fibroblast coculture supernatants. Briefly, culture medium (15 μl) was diluted 1:1 with SDS loading buffer and applied to precast 10% polyacrylamide gel zymograms (Novex; Frankfurt, Germany). After electrophoresis at 100 V at 4°C for 120 min, the gels were washed twice with renaturing buffer at room temperature for 30 min. Zymograms were then transferred into activity buffer and developed at 37°C for 12 h. After fixation and being stained with Coomassie brilliant blue G-250 (0.25%), the zymograms were destained with 10% (vol/vol) acetic acid, and bands were quantified by image analysis (Bio-Rad GS-800).

Determination of Hydroxyproline and Collagen Cross-Linking

Hydroxyproline, an amino acid found exclusively in connective tissues, was used as a means to quantify collagen. Each left ventricle was separated into two samples, dried, and weighed for tissue analysis. One sample was analyzed for total myocardial hydroxyproline after acid hydrolysis according to the methods of Stegeman and Stadler (44). The second sample was extracted and digested with (CNBr) digestion according to Woodiwick et al. (52), which is a modification of that originally described by Mukherjee and Sen (35). The percent cross-linking was determined by comparing the total hydroxyproline with CNBr-soluble hydroxyproline. The data were expressed as micrograms of collagen per milligram of dry heart weight, assuming that collagen contains an average of 13.5% hydroxyproline.

Statistical Analysis

ANOVA with multicomparison procedures was used to test the differences among the defined groups with SPSS version 11.5. Values obtained from treatment groups were compared with control values using Student’s t-test. Comparable non-parametric tests (Kruskal-Wallis and the rank sum test) were substituted when tests for normality and equal variance failed. All data are reported as means ± SE.

RESULTS

Cardiovascular Function Relative to Immune Function

Figure 1 illustrates the effect of immunomodulation on the acquired pressure-volume loops of the four groups. The immunological features of LP-BM5 infection are splenomegaly and a skewing to the T-lymphocyte function to TH2. Table 2 shows the 14-fold increase ($P < 0.001$) in spleen weight-to-body weight ratios in the LP-BM5 groups 12 wk after infection, which is consistent with that previously described by Watson et al. (28, 40, 48). The fundamental TH2 cytokines IL-4 and IL-10 increased by 5-fold ($P = 0.008$) and the TH1 cytokine IFN-γ decreased by 2.1-fold ($P = 0.01$) compared with controls (Table 3). Table 2 shows that left ventricular function of the LP-BM5 treatment group demonstrated a 61% decreased ventricular stiffness ($β$; $P = 0.0009$) and ventricular dilation described by a 28% increase in left ventricular end-diastolic volume ($V_{ed}; P = 0.0009$) and a 43% increase in left ventricular end-systolic volume ($V_{es}; P = 0.00004$) compared with the control group. The preload- and afterload-independent parameters describing isovolumic contraction and relaxation ($dP/dt_{max-Ved}$ and $τ$ Weiss, respectively) were also significantly prolonged ($P < 0.01$) in the infected group compared with the controls. The stroke volume index and cardiac index increased by 43% and 25%, respectively, in the infected mice, which may have been related to a 34% decrease in afterload [arterial elastance ($E_a$); $P = 0.0008$]. Therefore, LP-BM5 treatment induced a TH2 lymphocyte phenotype that coincided with decreased diastolic and systolic function without a reduction in cardiac output.

With selective TH1 stimulation by means of TCR-Vβ 5.2 and 8.1 peptides, β increased without affecting any other parameter compared with the control group. Confirmation of TH1 stimulation is supported by the 12-fold increase in IFN-γ and the 3.5-fold decrease in IL-4 (Table 3). However, in the combined LP-BM5 with TCR peptide treatment, $V_{ed}$, $V_{es}$, β, $dP/dt_{min-Ved}$ and $E_{a}$ did not differ when compared with control group (Table 2). This combination of treatments has been well documented to modulate lymphocyte secretory function (29, 40). Therefore, TH1 is associated with increased left ventricular stiffness and also a reversal of the TH2-mediated decrease in left ventricular stiffness and ventricular dilation.

Alterations of Cardiac Pro-Collagen and Pro-MMP mRNA With T Lymphocyte Polarization

To compare cardiac mRNA expression, real-time PCR analysis was carried out using total RNA isolated from the midportion of the left ventricle. Important in the context of procollagen and pro-MMP gene expression, cardiac angiotensin-converting enzyme (ACE) and TGF-β1 gene expressions were not different from the control group in these treatment groups (data not shown). However, Table 3 demonstrates that the LP-BM5 group had a sixfold decrease in cardiac procollagen cross-linking and upregulation of pro-MMP-9 and -13 gene expressions.
expressions. Especially striking was the fivefold reduction of pro-MMP-9 and -13 gene expression in cardiac tissues with TCR peptide stimulation of TH1 function. The combined treatments resulted in reduced pro-Coll Iα2 expression that was not different from the LP-BM5 group and a similar suppression of the MMPs as seen in the TCR peptide group. These data suggest that there is a separation in the immune regulation of collagen synthetic from degradative genes, namely, TH1 immune function relates to MMP gene expression and TH2 to collagen gene expression.

Cardiac Collagen Content and Cross-Linking

We sought to determine whether the difference in β relative to the immune condition was associated with the total collagen and/or the level of cross-linking. Total collagen (Fig. 2), as determined with the hydroxyproline method, decreased by 23% (P = 0.005) in the LP-BM5 group and increased by 34% (P = 0.03) in the TCR peptide group compared with the control group. The correlation of total cardiac collagen versus β was found to be R² = 0.409 (P = 0.01).

To further evaluate the involvement of collagen cross-linking related to immunomodulation and β, we compared the measured collagen cross-linking in response to immune mod-

Table 2. Effect of immune modulation on left ventricular hemodynamic function

<table>
<thead>
<tr>
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<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
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</thead>
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<tr>
<td><strong>n</strong></td>
<td>20</td>
<td>9</td>
<td>20</td>
<td>4</td>
</tr>
<tr>
<td><strong>Age, mo</strong></td>
<td>3.5 ± 1.3</td>
<td>3.0 ± 0.8</td>
<td>3.4 ± 1.6</td>
<td>27.0 ± 8.2</td>
</tr>
<tr>
<td><strong>Body weight, g</strong></td>
<td>4.2 ± 0.6</td>
<td>6.18 ± 2.8</td>
<td>4.2 ± 0.6</td>
<td>5.78 ± 7.2</td>
</tr>
</tbody>
</table>

Left ventricular and aortic functional parameters

- HR, beats/min: 552 ± 11 vs. 483 ± 18 (P < 0.05-compared with control; §P < 0.01 compared with LP-BM5)
- SVI, µL/g: 0.37 ± 0.02 vs. 0.53 ± 0.07 (P < 0.005-compared with control; *P < 0.05 compared with LP-BM5)
- EF, %: 55.8 ± 2.6 vs. 64.4 ± 3.1 (P < 0.01 compared with control; †P < 0.05 compared with LP-BM5)
- CI, l/min -1 • g -1: 204 ± 11 vs. 254 ± 36 (P < 0.01 compared with control; §P < 0.01 compared with LP-BM5)
- SVI, mmHg μL -1 • g -1: 28.1 ± 1.6 vs. 31.9 ± 4.6 (P < 0.01 compared with control; †P < 0.05 compared with LP-BM5)
- β, mmHg/µL: 0.18 ± 0.02 vs. 0.07 ± 0.02 (P < 0.01 compared with control; †P < 0.05 compared with LP-BM5)
- Vmax, µL: 23.8 ± 1.0 vs. 33.1 ± 2.9 (P < 0.01 compared with control; †P < 0.05 compared with LP-BM5)
- Vmax, µL: 11.0 ± 0.7 vs. 14.9 ± 1.5 (P < 0.01 compared with control; †P < 0.05 compared with LP-BM5)
- dP/dmax, mmHg/s: 9.095 ± 454 vs. 8.649 ± 827 (P < 0.01 compared with control; †P < 0.05 compared with LP-BM5)
- dP/dmin, mmHg/s: −6.711 ± 369 vs. −5.024 ± 606 (P < 0.01 compared with control; †P < 0.05 compared with LP-BM5)
- PRSW, mmHg: 123 ± 3 vs. 327 ± 33 (P < 0.01 compared with control; †P < 0.05 compared with LP-BM5)
- dP/dmax • Vmax, mmHg/µL: 565 ± 26 vs. 327 ± 33 (P < 0.01 compared with control; †P < 0.05 compared with LP-BM5)
- τ, Weiss, ms: 6.0 ± 0.3 vs. 8.1 ± 0.6 (P < 0.01 compared with control; †P < 0.05 compared with LP-BM5)
- Eo, mmHg/µL: 6.8 ± 0.3 vs. 4.5 ± 0.5 (P < 0.01 compared with control; †P < 0.05 compared with LP-BM5)

Table 3. Effect of immune modulation on splenic and cardiac gene expression

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
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<tbody>
<tr>
<td><strong>Spleen tissue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-4</td>
<td>1</td>
<td>5.1†</td>
<td>−3.5*</td>
<td>4.2‡</td>
</tr>
<tr>
<td>IL-10</td>
<td>1</td>
<td>5.3†</td>
<td>ND</td>
<td>1.8§</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>1</td>
<td>−2.1†</td>
<td>12.6‡§</td>
<td>2.0§</td>
</tr>
<tr>
<td><strong>Heart tissue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Actin</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pro-Col Iα2</td>
<td>1</td>
<td>−6.4†</td>
<td>−0.6§</td>
<td>−6.2†</td>
</tr>
<tr>
<td>Pro-Col IIIα1</td>
<td>1</td>
<td>2.1</td>
<td>−2.3†‡</td>
<td>−0.5‡</td>
</tr>
<tr>
<td>Pro-MMP-9</td>
<td>1</td>
<td>6.7†</td>
<td>−5.2*</td>
<td>−0.5§</td>
</tr>
<tr>
<td>Pro-MMP-13</td>
<td>1</td>
<td>2.9*</td>
<td>−5.4†‡</td>
<td>−4.7*‡</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. Real-time PCR analysis of splenic tissue 3 mo after treatment with LP-BM5 and TCR peptide shows the relative induction of T-helper cell (TH1) or TH2 cytokine gene expression. The level of candidate cardiac genes for collagen and MMPs were expressed as ratios to control group I gene expression using real-time PCR. *P < 0.05 compared with control; †P < 0.01 compared with control; ‡P < 0.05 compared with LP-BM5 infection; §P < 0.01 compared with LP-BM5.

Fig. 2. Effect of immune modulation on total cardiac collagen. The apical portion of the left ventricle was assayed for hydroxyproline (HPRO) concentrations and normalized against the dry weights of each sample. HPRO concentrations were converted to relative collagen levels using the assumption that collagen contains an average of 13.5% HPRO. Data are expressed as means ± SE. *P < 0.05 compared with control; †P < 0.01 compared with control; ‡P < 0.01 compared with LP-BM5.
compared with control; c cardiac fibroblasts. Figure 5 shows that pro-Col I related to the treatment groups and coincubated with primary LP-BM5, and LP-BM5 plus TCR peptide groups were pooled untreated C57BL/J6 mice. Lymphocytes from the control, mice to primary cardiac fibroblast cultures harvested from phenotype, we applied lymphocytes isolated from the treated expression were a direct or indirect effect of the lymphocyte contained an average of 13.5% HPRO. Data are expressed as means ± SE. *P < 0.05 compared with control; **P < 0.01 compared with control; †P < 0.05 compared with LP-BM5 infection; ‡P < 0.01 compared with LP-BM5. The LP-BM5 group revealed a markedly decreased collagen cross-linking by 4.8-fold (P = 0.001), and TCR peptide treatment resulted in a 67% (P = 0.03) increased collagen cross-linking (Fig. 3). A realistic interpretation is that, because MMP expression was significantly altered with the treatments, these cross-linking results represent a cumulative effect due to the 3-mo treatment period. Most importantly, the relationship between collagen cross-linking and β (Fig. 4), with $R^2 = 0.616$ (P = 0.005), reveals that this collagen maturation step is an essential determinant of the filling phase of diastole.

Coculturing of Lymphocytes With Primary Cardiac Fibroblasts

To determine whether the observed changes in cardiac gene expression were a direct or indirect effect of the lymphocyte phenotype, we applied lymphocytes isolated from the treated mice to primary cardiac fibroblast cultures harvested from untreated C57BL/J6 mice. Lymphocytes from the control, LP-BM5, and LP-BM5 plus TCR peptide groups were pooled related to the treatment groups and coincubated with primary cardiac fibroblasts. Figure 5 shows that pro-Col Iε and pro-MMP-13 cardiac fibroblast gene expressions are directly related to the induced lymphocyte function. The LP-BM5 treatment alone caused a 30% decrease in pro-Col Iε and a 13-fold increase in pro-MMP-13 cardiac fibroblast gene expression. The addition of TCR peptide treatment to the LP-BM5 group resulted in a sixfold increase in pro-Col Iε and a twofold decrease in pro-MMP-13 gene expression. Figure 6 shows the zymography results of the supernatant taken from the coculture at 48 h. TCR peptide treatment induced a decrease in the MMP-9 active form (P = 0.04), whereas LP-BM5 treatment significantly increased the MMP-9 latent form (P = 0.02). Even though the in vitro effects were after 48 h of coculture, whereas the in vivo results were after 3 mo, this coculture model provides supportive evidence for the in vivo experiments, suggesting that the lymphocyte phenotype directly corresponds to cardiac fibroblast function.

DISCUSSION

The present study demonstrates that skewing the immune response to either TH1 or TH2 markedly affects systolic and diastolic ventricular function. This immunological modification of ventricular function coincides with MMP and collagen gene expression, myocardial collagen content, and collagen cross-linking. The selective induction of TH1 lymphocytes, accomplished by using TCR-Vβ 5.2 and 8.1, increased β, perhaps through decreased MMP activity and increased collagen synthesis and cross-linking. Conversely, enhancement of TH2 with LP-BM5 retrovirus resulted in ventricular dilation and decreased β through enhanced MMP activity and decreased collagen synthesis and cross-linking. The TH2-induced diastolic dysfunction was reversed by concurrent enhancement of the TH1 immune response. However, the selected lymphocyte effects of the LP-BM5 retrovirus on TH2 induction was not reversed by TH1 TCR peptide treatment, as evidenced by splenic weights and IL-4 and IFN-γ gene expression. Therefore, there appears to be a simultaneous induction of the TH1 and TH2 lymphocyte populations in the combined treatment group. Our results suggest, therefore, that genes affected by TH2 remain elevated together with those affected by TH1. Essentially, this parallel induction of TH1 and TH2 lymphocyte functions results in the normalization of cardiac function.

LP-BM5 and TCR Peptides

The TCR-Vβ peptides 5.2 and 8.1 are specific to the T lymphocyte, do not directly affect fibroblast function or other organ systems, and have been demonstrated to significantly induce TH1 lymphocyte cytokines (29, 31, 32, 40). Support for using TCR peptides in this investigation is described by Kessels et al. (25), who proposed TCR peptides as a viable therapeutic for the induction of virus or tumor-specific immunity due to their specificity in redirecting T-cell immunity. Similarly, the LP-BM5 retrovirus infection of lymphocytes is selective due to the administered virus mixture containing a
MuLV complex of BM5def and BM5eco viruses. The BM5def virus is the causative agent for the induction of immunodeficiency, and the BM5eco virus is necessary for the virus propagation and thus functions as a helper virus. The dependency of BM5def on BM5eco for lymphocyte infection and viral replication results in only one round of viral replication. To exclude the possibility of retrovirus infection of cardiac fibroblasts, we coincubated LP-BM5 with primary cardiac fibroblast cultures. We found no significant increase of LP-BM5 copy numbers as detected by cPCR over time (data not shown). LP-BM5-induced TH2 lymphocyte function is rapid and sustained until the death of the mouse (17, 20, 28, 47, 58). We have reported that ventricular dilation and decreased $\beta$ occurs in the absence of cardiac lymphocytic infiltrates and inflammatory mediators (4, 40). Moreover, we have not been able to demonstrate inducible nitric oxide synthase or TNF-$\alpha$ overexpression in cardiac tissues of LP-BM5-infected mice (4). We have previously demonstrated an increased intracellular space that may account for the ventricular dilation that has been hypothesized to result from the increased production of hydrophilic glycosaminoglycans (4, 50). Therefore, it can be concluded that TCR peptides and the LP-BM5 murine model do not induce obvious myocardial pathology, infection, or inflammation. This, therefore, supports the conclusion that TCR peptide- and LP-BM5-induced TH1- and TH2-related function in lymphocytes are the mediators of the reported hemodynamic and ECM changes.

Fig. 5. Effect of coculturing lymphocytes with cardiac fibroblasts. Lymphocytes were harvested from control, LP-BM5 only, and combined LP-BM5 + TCR mice 12 wk after treatment and cocultured with naive primary cardiac fibroblasts. Lymphocytes from 3 mice in each group were pooled and added to the cardiac fibroblast cultures at a concentration of $5 \times 10^5$ lymphocytes/ml. After 48 h of coincubation, lymphocytes were removed, and cardiac fibroblast RNA was analyzed for pro-collagen Ia2 and pro-matrix metalloproteinase (MMP)-13 with RT-PCR. Graphed data are ratios of candidate genes versus 18S RNA.

Fig. 6. MMP activity of the supernatant from coculturing lymphocytes with cardiac fibroblasts. Lymphocytes were harvested from control, TCR, LP-BM5, and combined LP-BM5 plus TCR mice 12 wk after treatment and were cocultured with naive primary cardiac fibroblasts. A: MMP activity of the coculture supernatants was assayed with zymography for MMP-2 and -9 to demonstrate activity. B: TH1 reduced MMP activity in vitro and TH2 enhanced MMP activity. $^aP < 0.05$ compared with control.
T-LYMPHOCYTE DIASTOLIC FUNCTION

ECM

The extracellular factors affecting passive ventricular tension include collagen characteristics attributable to content, type, and cross-linking in addition to collagen coupling to the adhesion molecules via the Z band (5). When there is a significant change in the myocardial collagen fraction, the diastolic function is compromised, which is supported by our correlation of total collagen with β. Cardiac fibroblasts are ubiquitous cardiac cells providing mechanical strength to the tissue by secreting ECM components that define the composition and function of the cardiac ECM (7, 22, 50). A reduction in the number of fibrillar collagen tethers allows for myocardial dilation and slippage of myocytes (50), which may account for the observed significantly increased \( V_{ed} \) and \( V_{es} \) in the LP-BM5 treatment group.

The accepted regulatory factors of cardiac ECM collagen include angiotensin II, TGF-β1, connective tissue growth factor, osteopontin, and endothelin-1 [reviewed in Dostal (12)]. In summary, we found no change in gene expression for ACE and TGF-β1. However, the pro-Col \( \text{I}_\text{OA} \) and pro-MMP genes were remarkably affected by immunomodulation, with the pro-MMP genes being oppositely altered by TH1 and TH2 conditions and the pro-Col \( \text{I}_\text{OA} \) gene with only TH2.

Collagen Cross-Linking

The level of collagen cross-linking detected with immunomodulation parallels that of diastolic stiffness. Human cardiac tissues demonstrate left ventricular dilation with normal and increased total cardiac collagen concentrations (19). Collagen cross-linking has, therefore, been shown to be the primary determinant of myocardial stiffness and dilation (1, 52). Kato et al. (24) demonstrated that selective inhibition of cardiac collagen cross-linking with 6 wk of treatment with \( \beta \)-aminopropionitrile markedly decreased \( \beta \) and increased \( V_{ed} \) (24). We found that the relative abundance of ventricular collagen cross-linking also is associated with \( \beta \) and, additionally, that cross-linking is increased with TH1 treatment but decreased with TH2 immune function. MMP gene expression was particularly high in TH2 mice; it is therefore likely that the collagen turnover was correspondingly high and thereby possibly affecting the collagen maturation process related to cross-linking, as suggested by Gunja-Smith et al. (19). These observations collectively suggest that passive diastolic function is related to cardiac collagen cross-linking and cross-linking is affected by immune function.

MMP and Immune Function

Consistent with our report, IL-4 has been shown to induce MMP-9 in the absence of IFN-γ (42). Cardiac fibroblasts are the primary cells involved in cardiac remodeling, including synthesizing ECM collagen and MMPs (30). They are also a major source of TGF-β1 and angiotensin II, which are the primary stimulatory factors for cardiac fibroblast function related to collagen and MMP synthesis. However, we report that TGF-β1 and ACE were not overexpressed in our model of immunomodulation. This does not exclude noncardiac sources for these secretory factors. Given that our model did not incorporate myocardial injury, the role of proinflammatory cytokines is not expected to contribute to the induction of MMPs, especially because our previous studies failed to identify inducible nitric oxide synthase and TNF-α in cardiac tissues after LP-BM5 treatment (4). Our data also support those of others suggesting that the chronic loss of interstitial collagen is associated with both systolic and diastolic function (26). It can be concluded that TH2 cytokines induced MMPs in our cardiac tissues that were significantly inhibited by TH1 cytokines with a parallel change in ventricular function.

Integration

The challenging aspect in defining the role of the lymphocyte TH1/TH2 paradigm in remodeling of the cardiovascular ECM is the integration of this system with the neurohormonal pathways that are well characterized as regulators of cardiovascular remodeling. It is known that inhibition of TH1 function prevents cardiac remodeling in angiotensin II-treated mice (33), whereas angiotensin II enhances TH1 via angiotensin type 1 receptors (41). Accordingly, our in vivo findings do not exclude neurohormonal and wall stress contributions to cardiovascular ECM remodeling but suggest that the lymphocyte is also a major contributor in the overall process. However, our in vitro studies provide persuasive evidence that there is a direct and differential effect of polarized TH1 and TH2 lymphocytes on cardiac fibroblast gene expression and function. It is accepted that a pathological increase in neurohormonal or wall stress induces remodeling, and we propose that, in a similar manner, an imbalance in TH1/TH2 function may contribute and direct the overall cardiovascular remodeling process. Therefore, we selected a model that minimizes neurohormonal and wall stress components, namely, LP-BM5 and TCR peptides. The study contained herein suggests that altering T-lymphocyte function may induce changes in cardiac fibroblast function that could provide a means to manipulate the remodeling processes resulting in optimized cardiac function. We provided support for this concept through selective inducers of lymphocyte TH1/TH2 function. Therefore, immunomodulation of T-lymphocyte function for treatment of cardiovascular disease is a concept that may have promising clinical significance. In the future, we plan to demonstrate that modulation of the immune function may have a significant therapeutic effects on pathological remodeling caused by neurohormonal and/or wall stress pathways.

GRANTS

This study was supported by American Heart Association Grant 0455575Z, the Steinbrenn Heart Failure Research Award, and by Arizona Disease Control Research Commission Grant 5018.

REFERENCES


14. Fletcher F, Ain M, and Jacobs R. Hugina AW and Wirth S. 


49. Watson RR, Wang JY, Dehghanpisheh K, Huang DS, Wood S, Ardestani SK, Liang B, and Marchalonis JJ. T cell receptor V beta complementarity-determining region 1 peptide administration moder-


