Left Ventricular T-Cell Recruitment Contributes to the Pathogenesis of Heart Failure

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Background—Despite the emerging association between heart failure (HF) and inflammation, the role of T cells, major players in chronic inflammation, has only recently begun to be explored. Whether T-cell recruitment to the left ventricle (LV) participates in the development of HF requires further investigation to identify novel mechanisms that may serve for the design of alternative therapeutic interventions.

Methods and Results—Real-time videomicroscopy of T cells from nonischemic HF patients or from mice with HF induced by transverse aortic constriction revealed enhanced adhesion to activated vascular endothelial cells under flow conditions in vitro compared with T cells from healthy subjects or sham mice. T cells in the mediastinal lymph nodes and the intramyocardial endothelium were both activated in response to transverse aortic constriction and the kinetics of LV T-cell infiltration was directly associated with the development of systolic dysfunction. In response to transverse aortic constriction, T cell–deficient mice (T-cell receptor, TCRα−/−) had preserved LV systolic and diastolic function, reduced LV fibrosis, hypertrophy and inflammation, and improved survival compared with wild-type mice. Furthermore, T-cell depletion in wild-type mice after transverse aortic constriction prevented HF.

Conclusions—T cells are major contributors to nonischemic HF. Their activation combined with the activation of the LV endothelium results in LV T-cell infiltration negatively contributing to HF progression through mechanisms involving cytokine release and induction of cardiac fibrosis and hypertrophy. Reduction of T-cell infiltration is thus identified as a novel translational target in HF. (Circ Heart Fail. 2015;8:776-787. DOI: 10.1161/CIRCHEARTFAILURE.115.002225.)

Key Words: cell adhesion ■ heart failure ■ heart ventricles ■ inflammation ■ T lymphocytes ■ ventricular remodeling

Heart failure (HF) is still a leading cause of morbidity and mortality, affecting >24 million people worldwide.1,2 It is a complex syndrome involving the interplay of myocardial factors, inflammation, renal dysfunction, and neurohormonal activation.3,4 Based on the observations that patients with HF have increased circulating proinflammatory cytokines correlating with disease stage and mortality,5,6 clinical trials were launched targeting inflammatory mediators such tumor necrosis factor-α (TNFα) with infliximab and etanercept. These trials were deemed unsuccessful and were terminated prematurely because of lack of both improved survival and hospitalization rate.8,9 The failure of these particular anti-inflammatory agents despite the known activation of the immune system in HF underscores the importance of better understanding the specific inflammatory mechanisms contributing to the development of left ventricular (LV) remodeling and HF progression.

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To date, most of the immune mechanisms studied in HF have focused on the activation of the immune system in response to classical immune triggers, such as infectious or autoimmune myocarditis, that induce an adaptive immune response mediated by CD4+ T lymphocytes, or in response to ischemic heart injury provoked by myocardial infarction.10,11 In humans, recent studies demonstrate a positive correlation between inflammatory cytokines potentially produced by T cells and LV dysfunction in patients with chronic ischemic HF or with idiopathic dilated cardiomyopathy.12 Whether this takes place systemically or as a result of T-cell infiltration in the heart is still unclear, but, at least in the settings of infection, heart transplantation, myocardial infarction causing heart cell death and when self-tolerance to heart antigens is disrupted in autoimmunity, T cells can infiltrate the heart, which is normally devoid of T cells, and negatively affect cardiac function.13-14 However, the role T cells play in the development of LV hypertrophy, remodeling, and dysfunction in response to more common pathologies, such as occurs...
with HF, has only recently begun to be investigated, with recent evidence that CD4+ T cells promote cardiac remodeling in HF in mice. These recent findings raise additional questions including the mechanisms involved in attracting T cells to the heart, the role they play once in the heart, as well as whether T cells can be a novel translational target for potential therapeutic intervention. In addition, the role of T cells in nonischemic HF in humans has not been explored. Therefore, better insights into the kinetics of T-cell recruitment during the progression of HF, the mechanisms mediating T-cell recruitment and the impact they have on cardiac remodeling during HF is warranted. Although systemic inflammation and the T cell–mediated immune response have recently been associated with the prognosis of HF,16,17 whether T cells respond to pressure overload (PO) and develop high affinity for the vascular endothelium as a mechanism that facilitates their infiltration in the heart remains unknown.

In our current investigation, we tested the hypothesis that T cell–mediated immune responses and their recruitment into the heart influence cardiac remodeling and contribute to the pathogenesis of HF. Our study demonstrates that human and mouse T-cell recruitment into the LV negatively contributes to the pathophysiology of HF. It also highlights the potential of depleting T cells from the circulation over the course of HF to ameliorate cardiac fibrosis associated with HF.

**Methods**

**Human Subjects**

Blood samples from nonischemic human subjects with New York Heart Association Class III to IV HF referred for cardiac catheterization as part of their evaluation for advanced HF therapy (n=5) from healthy, non-HF volunteers (males 30–45 years of age). Viable LV free wall tissue control (n=3) from the National Disease Research Interchange (NDRI), and from end-stage HF subjects after LV-assisted device support (n=3). The studies were approved by Tufts University Institutional Review Board and all subjects gave informed consent.

**Mice**

Mice were bred and maintained under pathogen-free conditions. All protocols were approved by the Tufts Medical Center Institutional Animal Care and Use Committee. Mice were euthanized at 10 to 14 weeks of age for tissue collection.

**Mouse Model of Transverse Aortic Constriction**

PO-induced HF was produced by constricting the transverse aorta as previously described.18,19

**In Vivo Transthoracic Echocardiography**

In vivo transthoracic echocardiography assessed in conscious mice as previously described.18 The analysis was performed blinded. M-Mode and 2-dimensional images were obtained from the short-axis view, as described previously.18

**In Vivo Hemodynamics**

LV function was assessed by pressure volume transducing catheter as previously described.19 Absolute volume was calibrated by the saline injection parallel conductance method as described,19 and data were assessed at steady state. Data were digitized and analyzed with custom software (EMKA version 2.1.10).

Flow cytometry was performed to analyze the immune profile present in HF. The data were acquired on a FACSCanto (Becton Dickinson) and analyzed using FlowJo software.

**Histological Analysis**

Heart samples were excised and LV separated from the right ventricle. One third of LV was immediately embedded in optimal cutting temperature and one third fixed in 10% formalin, embedded in paraffin, and cut into 5-μm sections. Hematoxylin and eosin or picrosirius red staining was performed as described.20 Cardiomyocyte cross-sectional area was quantified by tracing the outline of 5 to 12 myocytes in each section.21

**In Vivo T-Cell Depletion**

Wild-type (WT) C57BL/6 mice were treated intraperitoneally with 300 μg/mL of monoclonal oCD3 antibody (BioXcell, West Lebanon, NH) or isotype-matched control monoclonal antibody starting at 48 hours post surgery and then every third day for 4 weeks.

**Real-Time Quantitative Polymerase Chain Reaction**

Total RNA was extracted from mouse heart LV tissues directly using Trizol (Invitrogen). RNA was then reverse transcribed using the ThermoScript RT-PCR system according the manufacturer’s instructions (Invitrogen), and amplified by real-time polymerase chain reaction (PCR) with SYBR green PCR mix (Applied Biosystems). Samples were quantified in triplicates using 40 cycles performed at 94°C for 30 s, 60°C for 45 s, and 72°C for 45 s using an ABI Prism 7900 Sequence Detection System.

**Endothelial Cell Culture**

Human umbilical vein endothelial cells were isolated and cultured as described.22 Confluent human umbilical vein endothelial cell monolayers on fibronectin-coated glass coverslips were stimulated with TNF-α (25 ng/mL) for 4 hours before the adhesion assays. Mouse heart endothelial cells were isolated from hearts of newborn C57/BL6 (WT) animals 7 to 9 days old as described,23 and also plated on fibronectin-coated glass coverslips and stimulated with TNF-α 4 hours before the T-cell adhesion assay.

**Videomicroscopy Image Acquisition and Analysis**

T-cell interactions with mouse heart endothelial cell or human umbilical vein endothelial cells were observed by videomicroscopy under defined laminar flow conditions in a parallel plate apparatus.24,25 T-cell interactions with confluent TNF-α–activated mouse heart endothelial cells or human umbilical vein endothelial cells grown on glass coverslips observed at ×20 magnification. Data were recorded and analyzed using the Nikon Elements Software (NES). Adhesion of T cells on activated endothelial cells was quantified in 6 fields of view per condition.

**Statistics**

Data are expressed as the means±SD unless otherwise indicated. Statistical analyses between 2 groups were performed by Student t test and Mann–Whitney nonparametric test to adjust for nonequal Gaussian distributions among groups. Intergroup comparisons were performed by 2-way ANOVA and Bonferroni post-test to adjust for the multiple comparisons. Kaplan–Meier analysis with log-rank testing was used for survival analysis. Differences were considered statistically significant at P<0.05 and are indicated with an (*). Graph Pad Prism software was used in all analysis.

**Results**

**T Cells From Humans and Mice With HF Have High Affinity for the Activated Vascular Endothelium and Are Recruited Into the Heart’s Left Ventricle**

We used a real-time videomicroscopy approach that mimics physiological shear flow conditions in small capillaries and venules to study the ability of CD3+ T cells from patients.
with Class III to IV nonischemic HF to interact with activated vascular endothelial cells. T cells from HF patients adhered to activated endothelial cells in significantly higher numbers than T cells from non-HF volunteers (Figure 1A and 1B). CD3⁺ T cells also infiltrated the LV of patients with nonischemic end stage HF (Figure 1C), and this was associated with significant cardiac hypertrophy and fibrosis, and the presence of vesiculated pyramids as indicators of pathologically hypertrophied myocytes (Figure 1D–1G). Consistent with the literature, systemic T cells were found to be elevated in patients with HF and were activated based on their ability to differentiate into various T-cell subsets compared with control patients (Figure IA–ID in the Data Supplement). To study this phenomenon further, we evaluated whether T cells responded to PO-induced HF using TAC-induced HF in mice. Consistent with our human data, we observed that CD4⁺ T cells isolated from lymph nodes and spleen 4 weeks after TAC adhered to activated mouse heart endothelial cells in significantly higher numbers than those from sham-operated controls (Figure 2A and 2B). TAC induced LV recruitment of both CD4⁺ and CD8⁺ T cells (Figure 2C–2E), whereas the T-box transcription factor 2 (Tbet) and retinoic acid receptor-related orphan receptors gamma T (RORγT), the signature transcription factors of T helper (Th) type 1 and Th type 17 cells, respectively, were significantly upregulated in the LV, in contrast to forkhead box P3 (Foxp3), the signature transcription factor for T regulatory cells which remained unchanged between sham and TAC mice (Figure 2F). Furthermore, the cytokines TNFα, interleukin-1β and interleukin-6, and the Th1 signature cytokine interferon-γ (IFNγ) were significantly upregulated in TAC versus sham mice.

Figure 1. T-cell activation, adhesion to activated vascular endothelial cells, and infiltration into the left ventricle (LV) in humans. A and B, CD3⁺ T cells isolated from healthy volunteers or from patients with New York Heart Association III to IV heart failure (HF) were drawn in a flow chamber across tumor necrosis factor-α (25 ng/mL) activated human umbilical vein endothelial cell at a shear stress of 0.8 dynes/cm². Data represents mean±SD of 2 independent experiments using 2 different control and 3 different HF samples. Representative images from the videos are shown and arrows point at T cells arrested on the vascular endothelium (B). C, Non-HF and end-stage HF LV tissues were obtained from National Disease Research Interchange and LV-assisted device placements, respectively, and sections were stained for CD3 or isotype control. The arrows point at 2 representative infiltrated CD3⁺ T cells and the quantitative analysis is shown (right). D and E, Representative hematoxylin and eosin staining (D) and cardiac myocyte size quantification (E) of LV sections from non-HF and end-stage HF. Arrows point to vesiculated pyramids, standard histological markers of hypertrophic myocytes. F and G, Representative photomicrographs (F) and quantification (G) of myocardial fibrosis evaluated by picrosirius red staining of LV sections. Data represented as mean±SEM. *P<0.05. Scale bars, 500 μm (C, D, and F).
mice (Figure 2G). In addition, TAC resulted in upregulation of RNA of the endothelial cell adhesion molecules, vascular cell adhesion molecule-1 (VCAM-1), E-selectin, and intercellular adhesion molecule 1 (ICAM-1) (Figure 2H–2J), and significantly enhanced ICAM-1 protein expression in the endothelial cells of the LV intramyocardial vessels (Figure 2K). Taken together, these findings demonstrate that PO in mice and humans activates T cells which become proadhesive and more prone to interact with the activated vascular endothelium in vitro. Moreover, in vivo, TAC promotes upregulation of ICAM-1 and other endothelial cell adhesion molecules that can lead to T-cell recruitment and induces proinflammatory cytokine release that can further promote T-cell survival and subsequent endothelial cell activation.

**T-Cell Recruitment Into the LV Is Associated With the Progression of HF in Response to TAC**

We next investigated the time course of LV T-cell infiltration after TAC using quantitative flow cytometry to determine whether it was directly associated with the progression of HF. Forty-eight hours after TAC, we did not detect significant T-cell infiltration in the LV, and at this time point, LV weight and LV systolic function remained similar between TAC and sham controls. Infiltration of T cells to the LV occurred during...
the development of cardiac remodeling and systolic dysfunction 2 and 4 weeks after TAC (Figure 3A–3C), peaking at 4 weeks. Further analysis revealed that Gr1+ neutrophils and F4/80+ macrophages did not follow the same dynamics as T cells (Figure 3D, 3E, and 3I). Four weeks after TAC, there was a notable increase in infiltrated CD45.2+ leukocytes (Figure 3F), and these included T cells and CD11b+ myeloid cells (Figure 3G, 3H, and 3I). From our immunohistochemistry studies, these T cells included CD4+ and CD8+, possibly IFNγ producers given the increased LV levels of IFNγ in response to TAC observed in Figure 2. Moreover, we isolated the mediastinal lymph nodes of both sham and TAC mice and found that it not only was significantly enlarged in TAC mice when compared with sham (data not shown), but it indeed had more activated T cells that had therefore lost the expression of L-selectin (Figure 3J). Importantly, we were able to determine the levels of circulating T cells and Th-cell subsets and found that these were increased in response to TAC (Figure II in the Data Supplement), similarly to what we observed in patients with HF (Figure I in the Data Supplement). Taken together, these data support the idea that T cells respond to cardiac-specific damage in the mediastinal lymph nodes become activated and are recruited to the LV. This recruitment correlates with the development of pathological cardiac remodeling and worsening of systolic function.

**T Cells Are Major Contributors to the Pathogenesis of HF**

Based on our findings that T-cell recruitment into the LV correlates with adverse remodeling, we next assessed the functional necessity of T cells to promote HF. We specifically tested the response to LV PO in mice deficient in T cells harboring a genetic deletion of the α-chain of the T-cell receptor (TCRα−/−). Echocardiography analysis 4 weeks post TAC showed that in contrast to WT mice, TCRα−/− mice had normal LV dimensions (Figure 4A), preserved systolic function, measured by fractional shortening (Figure 4B), and preserved LV end diastolic diameter (Table). This was consistent with invasive hemodynamic measurements revealing that despite similar aortic pressure gradients generated in response to 4 weeks TAC in WT and TCRα−/− mice (Figure 4C), the stroke volume, end diastolic pressure, and systolic and diastolic functions were all preserved in TCRα−/− mice (Figure 4D–4G; Table). Intriguingly, of all the mice that underwent TAC surgery, 30% of the WT TAC mice died by 4 weeks, whereas no deaths occurred in TCRα−/− mice (Figure 4H). As expected, the LV of TCRα−/− mice was free of recruited T cells. Moreover, Gr1+ neutrophils were found recruited in the LV in similar numbers in sham and TAC mice in both WT and TCRα−/− mice (Figure 4I). In addition, TAC did not induce significant upregulation of endothelial cell adhesion
molecules, including ICAM-1, or upregulation of proinflammatory cytokines in TCRα−/− mice (Figure III in the Data Supplement). RORγT and Tbet were also significantly decreased when compared with WT, suggesting that most of the Tbet expression observed in WT mice (Figure 2; Figure IIII and IIIJ in the Data Supplement) may be attributed to Th1 cells. Taken together, our results support a role for T-cell recruitment to the LV being critical for the observed cardiac dysfunction in response to TAC. The absence of T cells results in a suppressed proinflammatory state within the LV, and as such, the deleterious actions of cytokines TNFα and IFNγ on cardiac-resident cells and endothelial cell activation are prevented. Thus, subsequent immune cell recruitment and LV function is protected from any negative impact mediated by T cells. This could potentially explain why TCRα−/− do not develop HF.

**T Cells Regulate Cardiac Hypertrophy and Fibrosis in Response to PO**

To study the T cell–dependent mechanisms leading to improved cardiac function and survival in TCRα−/− mice, we initially evaluated cardiac hypertrophy in WT and
TCRα−/− mice in response to TAC. As expected, the gross LV weight normalized to tibia length was increased in WT mice in response to 4 weeks TAC. However, the LV weight and size of TCRα−/− mice were similar between sham and TAC mice, and significantly decreased in TCRα−/− mice versus WT mice in response to TAC (Figure 5A and 5B). We further analyzed by hematoxylin and eosin the cardiomyocyte cross-sectional area and found that while cardiac myocytes from WT mice have increased sectional area in response to TAC, those from TCRα−/− mice remain similar in area to the cardiac myocytes from TCRα−/− sham controls (Figure 5C and 5D). Several studies have demonstrated that atrial natriuretic peptide and brain natriuretic peptide are elevated in serum of HF patients; these 2 molecules are considered serum markers of HF in response to pressure and volume overload, and their increased transcription in LV tissue represents a phenotype of pathological, rather than physiological, hypertrophy. Therefore, we evaluated the levels of the natriuretic peptides by real-time quantitative PCR using LV tissues and revealed that while cardiac myocytes from WT mice have increased sectional area in response to TAC, those from TCRα−/− mice remain similar in area to the cardiac myocytes from TCRα−/− sham controls (Figure 5C and 5D).

### Table. Characterization of Heart Failure in WT and TCRα−/− Mice

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sham</th>
<th>TAC (4 wk)</th>
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<tbody>
<tr>
<td>Total body weight, g/tibia length, mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT (n=sham 11, TAC 16)</td>
<td>1.457±0.022</td>
<td>1.437±0.021</td>
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<tr>
<td>TCRα−/− (n=sham 5, TAC 8)</td>
<td>1.455±0.032</td>
<td>1.491±0.044</td>
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<tr>
<td>Total lung weight, mg/tibia length, mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT (n=sham 11, TAC 16)</td>
<td>0.009±0.000</td>
<td>0.0123±0.001*</td>
</tr>
<tr>
<td>TCRα−/− (n=sham 5, TAC 8)</td>
<td>0.009±0.000</td>
<td>0.009±0.000†</td>
</tr>
<tr>
<td>Heart rate, beats per min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT (n=sham 3, TAC 7)</td>
<td>535±45</td>
<td>553±40*</td>
</tr>
<tr>
<td>TCRα−/− (n=sham 3, TAC 7)</td>
<td>494.7±49.30</td>
<td>525.2±42.18</td>
</tr>
<tr>
<td>LV end-systolic pressure, mmHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT (n=sham 3, TAC 7)</td>
<td>114±12</td>
<td>130±18*</td>
</tr>
<tr>
<td>TCRα−/− (n=sham 3, TAC 7)</td>
<td>99.95±8.79</td>
<td>142.3±9.29*</td>
</tr>
<tr>
<td>LV end-diastolic diameter, mm</td>
<td></td>
<td></td>
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<tr>
<td>WT (n=sham 3, TAC 5)</td>
<td>3.674±0.121</td>
<td>4.007±0.115*</td>
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<tr>
<td>TCRα−/− (n=sham 3, TAC 5)</td>
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<td>3.753±0.277†</td>
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<td>Posterior wall thickness, mm</td>
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<tr>
<td>WT (n=sham 3, TAC 5)</td>
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<td>1.4±0.3*</td>
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<tr>
<td>TCRα−/− (n=sham 3, TAC 5)</td>
<td>1.091±0.149</td>
<td>0.952±0.133†</td>
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<tr>
<td>Maximum pressure, mmHg</td>
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<td></td>
</tr>
<tr>
<td>WT (n=sham 3, TAC 7)</td>
<td>93.46±7.689</td>
<td>136.91±8.003*</td>
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<tr>
<td>TCRα−/− (n=sham 3, TAC 7)</td>
<td>94.90±8.698</td>
<td>144.8±9.063*</td>
</tr>
</tbody>
</table>

Values are means±SD. LV indicates left ventricle; TAC, thoracic aortic constriction; and WT, wild-type.

*P<0.05, 4 wk of TAC vs sham.
†P<0.05, TAC TCRα−/− vs TAC WT.

### T-Cell Depletion After TAC Prevents Cardiac Fibrosis and LV Dysfunction

Because of the above observations that genetic depletion of T cells inhibits pathological remodeling and the HF phenotype, we hypothesized that depletion of T cells in the onset of LV PO prevents pathological cardiac remodeling and may represent a potential novel therapeutic target. We tested whether administration of anti-CD3 antibody could effectively reduce T cells in the setting of LV PO. We therefore depleted WT mice of T cells beginning 48 hours post TAC surgery, when we demonstrate that the mice are responding to TAC but T cells have not yet infiltrated the LV (as shown in Figures 3 and 7A). Approximately 95% of CD3+ T cells were depleted compared with the isotype control IgG–treated animals in both sham and TAC mice after 4 weeks (Figure 7B and 7C). Likewise, CD4+ T cells were also depleted using the anti-CD3 antibody compared with the control-treated mice (Figure 7D and 7E). Although IgG antibody control treated mice had CD4+ T cells infiltrated in the heart in response to TAC, anti-CD3 treatment resulted in absent LV T-cell infiltration (Figure 7F). TAC-treated mice depleted of CD3+ T cells also demonstrated absence of cardiac fibrosis, compared with isotype control IgG–treated mice (Figure 7G and 7H). Furthermore, TAC anti-CD3–treated mice had increased % fractional shortening in contrast to isotype antibody control TAC treated mice (Figure 7I). Although the fibrosis and echocardiography data mimicked the phenotype observed for TCRα−/− mice, WT mice depleted of T cells still developed cardiac hypertrophy (Figure 7J–7L). These data demonstrate the feasibility of reducing T cells in the setting of TAC and suggest a robust antifibrotic effect of T-cell depletion in the LV that results in improved cardiac function despite the increase in LV hypertrophy.

### Discussion

Our findings in this study reveal several aspects identifying T-cell recruitment into the LV as a negative contributor to pathological cardiac remodeling in HF. Our observations using human T cells and LV tissue from nonischemic end-stage HF patients indicate systemic T-cell activation, enhanced T-cell adhesion to activated vascular endothelial cells under physiological flow conditions in vitro, and T-cell LV infiltration, directly correlating with LV myocyte hypertrophy and fibrosis. We obtained similar results using WT mice undergoing PO-induced HF, established for the first time the kinetics of LV T-cell infiltration, and further characterized T-cell activation, LV endothelial cell activation, and LV T-cell infiltration. Our studies using TCRα−/− mice together with the successful pharmacological depletion of T cells in the setting of LV PO are in support of a role for T...
cells in PO-induced HF as both T-cell depletion approaches resulted in significantly attenuated LV fibrosis and improved LV function and HF. Our findings support a model in which LV recruitment of activated T cells in the setting of PO contributes to myocardial dysfunction, remodeling, and ultimately HF. Our findings also suggest that pharmacological downregulation of T-cell recruitment in the LV may represent a novel strategy for the prevention and potential treatment of HF in humans.

**Combination of T Cell and Intramyocardial Endothelial Cell Activation in Response to PO Lead to LV T-Cell Recruitment and HF**

There is recent growing awareness that humans with non-ischemic and noninfectious induced HF present chronic systemic inflammation potentially mediated by T cells.26-27 T cells are also found infiltrated in the heart in both humans and experimental mouse models in response to classical triggers of the T cell–mediated immune response.28 Intriguingly, the
mechanisms of action of the T cells and whether they play a role systemically, in the heart or in both, are poorly understood to date. We demonstrate for the first time that human T cells from nonischemic HF are activated and predisposed to interact with activated vascular endothelial cells in vitro and infiltrate the human LV in vivo. Our studies in WT mice responding to PO support this concept because naïve non–activated T cells do not have the properly modified adhesion ligands to roll and arrest on the heart vascular endothelium, as we and others have previously described.\textsuperscript{23,29,30} Furthermore, we demonstrate that T-cell activation occurs in the mediastinal lymph nodes in response to cardiac antigens, as the lymph node size was significantly increased in response to TAC (data not shown) and a high percent of the T cells present had lost the expression of L-selectin. Moreover, both mice and humans with nonischemic HF had higher numbers of blood circulating

Figure 7. T-cell depletion in wild-type (WT) mice post transverse aortic constriction (TAC) surgery results in preserved cardiac fibrosis and improved systolic function. A, Diagram of experimental protocol for T-cell depletion with αCD3 Ab in WT sham and TAC mice. IgG Ab was used as control. B and D, Representative fluorescence-activated cell sorting dot plots (C and E) and quantification of CD3\(^+\) T cells (B and G) and CD4\(^+\) T cells (D and E) in lymph nodes of 4-week WT sham or TAC mice treated as in A. F, Representative immunohistochemical staining of CD4\(^+\) T cells and quantification (right) of left ventricular (LV) T cells in the indicated groups 4 week post TAC. G, Representative photomicrographs and (H) quantification of LV fibrosis evaluated by picrosirius red staining of LV sections in the indicated groups. I, Echocardiography analysis in sham and TAC WT mice treated with control IgG Ab or depleted of T cells with αCD3 Ab after sham or TAC surgery. J, LV weight, normalized to tibia length. K, Representative hematoxylin and eosin staining and (L) quantification of cardiac myocyte area of LV sections among different groups. Data represent means±SD. *P<0.05. Scale bars, 500 \(\mu\)m (n=3–4 IgG and n=4–6 αCD3).
T cells and effector T-cell subsets, although the nature of the antigens inducing this T-cell response remains unknown. The possibility that peptide antigens resulting from cardiac cell death trigger this response because it has been suggested in response to myocardial infarction is unlikely, given that TAC does not induce cardiac apoptosis significantly until 8 weeks post banding, and T cells infiltrate the heart as early as 2 weeks post TAC. We speculate that PO disrupts the immune cell tolerance taking place in the heart allowing self-cardiac antigens to induce a T-cell immune response because it has been described in autoimmune myocarditis. These antigens may include α-myosin, which is not expressed in the thymus and therefore can affect immune tolerance in 2 ways: not being negatively selected against by clonal deletion and not being able to positively select α-myosin–specific natural T regulatory cells. However, this requires further investigation.

Our data indicate that in addition to T cells, CD11b+ myeloid cells infiltrate the LV after 4 weeks of TAC. This is in line with other models of PO that demonstrate macrophage infiltration in the heart, however, the similar infiltration of F480+ macrophages observed in sham and TAC in our studies suggest that these macrophages are not activated. Because endothelial ICAM-1 is significantly upregulated in response to PO, it is possible that T cells and CD11b+ cells use this pathway to infiltrate the LV. How endothelial ICAM-1 becomes upregulated in response to PO remains under investigation, but one likely possibility is that PO induces cytokine production by cardiac-resident cells and these activate the endothelial cells and promote subsequent T-cell recruitment. We interestingly identified significant upregulation of the Th1 signature cytokine, IFNγ, and transcription factor, Tbet. Although these are not exclusively expressed on Th1 cells, the fact that these are not upregulated in T cell–deficient mice (TCRα−/−) suggests that Th1 cells are a major subset of T cells infiltrated in the LV and can alter cardiac myocyte and fibroblast function via IFNγ. Further studies will be needed to establish a definitive role for specific T-cell subsets in the LV and whether once infiltrated in the LV can further activate heart endothelial cells in vivo, similarly to what we previously described in vitro. Collectively, our results support a mechanism in which PO acts 2 ways: by exposing self-cardiac antigens that result in T-cell activation, and by inducing cytokine release in cardiac-resident cells that lead to endothelial activation. These are both required for optimal T-cell recruitment to the LV, where T-cell cytokine secretion of TNFα and IFNγ could contribute to cardiac dysfunction.

Functional Necessity of T Cells in Cardiac Remodeling and HF

Whether such a small number of activated T cells infiltrated in the LV can have an impact in cardiac function was evaluated and confirmed in our studies with TCRα−/− mice, which showed preserved adverse cardiac remodeling, including cardiac hypertrophy, cardiac fibrosis, and cardiac function in response to TAC. Our data are in agreement with recent data published during the preparation of our article using a different genetic depletion of T cells approach that included the use of recombination activating gene 2 (Rag2−/−) mice and the major histocompatibility complex II (MHCII−/−) mice, both showing blunted adverse cardiac remodeling and preserved ventricular function in response to 6-week TAC. The authors demonstrated that systemic activation of CD4+ T cells in the draining mediastinal lymph nodes is critical for the T-cell immune response observed in response to TAC.

Our present study using a different strain of T cell–deficient mice, TCRγ−/−, and 4-week TAC not only confirms a role for T cells in adverse remodeling and HF, but also demonstrates for the first time that T-cell infiltration specifically in the LV of the heart occurs in WT mice as early as 2 weeks post TAC, suggesting a much earlier activation of T cells contributing to the progression of HF. Unlike the previous study, we have also investigated invasive LV hemodynamics in TCRγ−/− mice and importantly implicated T cells in diastolic HF, a condition for which there are no current treatments available. Moreover, the protective systolic and diastolic HF phenotype resulted in 100% survival in TCRγ−/−. Together with the lack of an LV proinflammatory environment observed in these mice, these studies support a mechanism in which cytokine production by infiltrated activated T cells have a negative impact in LV function and survival to TAC.

Genetic Versus Pharmacological T-Cell Depletion Effects on Cardiac Fibrosis and Hypertrophy

Genetic depletion of T cells using different approaches coincide with the better outcome for HF induced by PO therefore placing T cells as central regulators of pathological cardiac remodeling. It is noteworthy that genetic depletion of T cells can somehow affect other immune subsets during development, as T cells are required by other cells in the immune system to perform their functions effectively. Also, in these genetically modified mice strains, T-cell depletion occurs before the onset of PO and resultant ventricular dysfunction and is thus less directly therapeutically applicable to patients with pre-existing HF. To circumvent these limitations, we, for the first time, successfully achieved T-cell depletion selectively in the setting of TAC. Our data strongly support a role for T cells in cardiac fibrosis and function induced by TAC that is independent of any whole body genetic modification. Furthermore, it strengthens the idea that targeting T cells after the stimulus for HF has been induced is beneficial in HF prognosis potentially by preventing T-cell LV infiltration. While using the genetic and pharmacological depletion approach, we recapitulate the finding that T cells can regulate fibrosis in a variety of tissues, more intriguing is the striking observation that the lack of T cells in TCRγ−/− abrogates the hypertrophic response. This phenomenon has been observed in other deficient mice, including T cell–deficient mice, but it is not observed when we pharmacologically deplete T cells in the onset of HF. One could speculate that the remaining T cells in WT mice after depletion can secrete prohypertrophic mediators, such as angiotensin II, and possibly mediate physiological hypertrophy mechanisms. These mechanisms require further investigation.

Conclusions and Limitations

Our combined observations in humans and mice provide evidence that T cells and their recruitment to the LV are critical regulators of pathological cardiac remodeling in HF in the setting of PO. Our observations are in line with the recently proposed changing paradigm suggesting that certain types of HF occur in novel cell compartments, such as the intramyocardial vascular endothelium and the recruited immune cells. One limitation
of the human studies is that most nonischemic HF patients take β-adrenergic signal blockers which can affect T-cell activation,6,10 also affected by hypertension.6,10 However, our mouse data corroborate the human data, and the TCRα−/− mice have similar basal systolic pressure as WT, as assessed by aortic blood pressures in sham mice. Collectively, our data contribute to a broader understanding of the novel role T cells play in cardiac remodeling and highlight that pharmacological T cells depletion prevents HF and may serve as a potential strategy to improve the structural, functional, and molecular deficits of the failing heart.

Disclosures

None.

References

23. McMullen JR, Jennings GL. Differences between pathological and physiological cardiac hypertrophy: novel therapeutic strategies to treat
Clinical Perspective

Systemic inflammation represents a pivotal contributor to the pathogenesis of cardiac remodeling and heart failure (HF). However, clinical trials involving inflammatory cytokine blockade have failed to date to improve outcomes for HF and have therefore raised questions about the specific inflammatory mechanisms influencing cardiac remodeling in HF. We observed that subjects with nonischemic end-stage HF had significant T-cell infiltration into the left ventricle, which correlated with increased hypertrophy and fibrosis. Further analysis using a murine model of pressure overload recapitulated these findings and supported a T-cell–mediated immune response taking place systemically that also resulted in left ventricle T-cell infiltration associated with pathological cardiac remodeling. We studied mice deficient in T cells to test the requirement of T cells in the pathogenesis of cardiac remodeling and HF. Strikingly, mice deficient in T cells were protected from left ventricle pressure overload–induced cardiac dysfunction, including diastolic dysfunction, for which there are currently no treatments available. Furthermore, pharmacological depletion of T cells after the onset of HF was associated with improved cardiac function and survival. Our work provides important new insights into specific inflammatory mechanisms taking place in HF, placing T cells and their infiltration in the heart as central regulators of pathological cardiac remodeling. Targeting T cells in cardiac remodeling may serve as a potential strategy for the treatment of HF, and additionally high levels of circulating T-cell subsets may act as predictors of the progression of HF.
Left Ventricular T-Cell Recruitment Contributes to the Pathogenesis of Heart Failure

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Supplemental Figure 1

A. 
- Isotype: 0.008%
- Non-HF: 24.2%
- HF: 72%

B. 
- CD4+ T cells (%)
- Non-HF: 20
- HF: 80

C. 
- Th17: 0.02%
- Th1: 0.055%
- Treg: 1.7%

D. 
- % T cells
- Th1: Non-HF: 1, HF: 2
- Th17: Non-HF: 3, HF: 4
- Treg: Non-HF: 5, HF: 6
Supplemental Figure 2

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Supplemental Figure 3.
Supplemental Figure Legend

**Supplemental Figure 1: Systemic T cells in HF Patients (A)** Representative FACS dot plots and (B) quantification of CD4⁺ T cells from peripheral blood mononuclear cells (PBMCs) in normal and class III-IV heart failure patients. (C) Representative FACS dot plots and (D) quantification of systemic CD4⁺ T cell subsets, Th17, Th1 and Tregs in normal and class III-IV heart failure patients. n=2 non-HF and n=3-4 HF. Treg plot shows highly regulatory T cells (Foxp3+CD25hi) labeled as 1, also increased in HF as compared to control.

**Supplemental Figure 2: Increased circulating CD4+ T cells and T cell subsets in PO induced heart failure in WT mice. (A)** Representative FACs dot plot and (B) quantification of CD4 expression in blood after 4 weeks sham and TAC surgery in WT mice. (C) Representative FACS dot plots and (D) quantification of systemic CD4⁺ T cell subsets, Th1 and Th17 in WT mice 4 weeks after sham and TAC surgery. N=2 sham and n=3 TACs. Data represent mean ± SD; *p<0.05.

**Supplemental Figure 3: (A-C)** RNA expression for adhesion molecules VCAM-1, Esel, and ICAM-1 respectively in WT and TCRα⁻/⁻ mice 4 weeks post sham and TAC surgery (n=4-6 shams and 7-10 TACs per group). (D) Representative immunohistochemical staining of ICAM-1 in the vessels of WT and TCRα⁻/⁻ mice 4 weeks post sham and TAC surgery. (E-H) Inflammatory cytokines and (I,J) T cell transcription factors in WT and TCRα⁻/⁻ mice after 4 weeks of sham and TAC surgery (n=4-6 shams and 7-10 TACs per group). Data represent mean ± SD; *p<0.05.