Heart Failure

CD4⁺ T Cells Promote the Transition From Hypertrophy to Heart Failure During Chronic Pressure Overload

Fanny Laroumanie, PharmD; Victorine Douin-Echinard, PharmD, PhD; Joffrey Pozzo, MD; Olivier Lairez, MD, PhD; Florence Tortosa, MS; Claire Vinel, MS; Christine Delage, BS; Denis Calise, MS; Marianne Dutaur, MS; Angelo Parini, MD, PhD, Nathalie Pizzinat, PhD

Background—The mechanisms by which the heart adapts to chronic pressure overload, producing compensated hypertrophy and eventually heart failure (HF), are still not well defined. We aimed to investigate the involvement of T cells in the progression to HF using a transverse aortic constriction (TAC) model.

Methods and Results—Chronic HF was associated with accumulation of T lymphocytes and activated/effector CD4⁺ T cells within cardiac tissue. After TAC, enlarged heart mediastinal draining lymph nodes showed a high density of both CD4⁺ and CD8⁺ T-cell subsets. To investigate the role of T cells in HF, TAC was performed on mice deficient for recombination activating gene 2 expression (RAG2KO) lacking B and T lymphocytes. Compared with wild-type TAC mice, RAG2KO mice did not develop cardiac dilatation and showed improved contractile function and blunted adverse remodeling. Reconstitution of the T-cell compartment into RAG2KO mice before TAC enhanced contractile dysfunction, fibrosis, collagen accumulation, and cross-linking. To determine the involvement of a specific T-cell subset, we performed TAC on mice lacking CD4⁺ (MHCIIKO) and CD8⁺ T-cell subsets (CD8KO). In contrast to CD8KO mice, MHCIIKO mice did not develop ventricular dilatation and dysfunction. MHCIIKO mice also displayed very low fibrosis, collagen accumulation, and cross-linking within cardiac tissue. Interestingly, mice with transgenic CD4⁺ T-cell receptor specific for ovalbumin failed to develop HF and adverse remodeling.

Conclusions—These results demonstrate for the first time a crucial role of CD4⁺ T cells and specific antigen recognition in the progression from compensated cardiac hypertrophy to HF. (Circulation. 2014;129:2111-2124.)

Key Words: heart failure • lymphocytes • ventricular remodeling

Heart failure (HF) is a complex syndrome that results from acute injury such as myocardial infarction or more long-standing diseases such as pressure and volume overload. In the case of pressure-overload conditions occurring in aortic stenosis or hypertension, cardiac remodeling evolves progressively from an initial compensatory ventricular hypertrophy to decompensated hypertrophy characterized by cardiac dilatation and contractile dysfunction. Loss of cardiac function is generally associated with fetal gene reprogramming, aberrant calcium handling, loss of cardiomyocytes, and excessive fibrosis. To date, the mechanisms responsible for the progression of cardiac remodeling and for triggering the transition from cardiac hypertrophy to failure remain unknown.

Clinical Perspective on p 2124

Many observations provided strong evidence involving inflammation in the progression of chronic HF. In particular, high circulating levels of proinflammatory mediators (ie, tumor necrosis factor-α and interleukin [IL]-6) correlated with deterioration of cardiac function in patients with HF. The importance of these cytokines was further supported by their biological effects on cardiac contractility and remodeling, which may explain several aspects of the HF syndrome. Recently, the percentage of circulating CD4⁺ T cells expressing inflammatory cytokines was positively correlated with left ventricular dysfunction in patients with HF. However, the significance of these observations and the contribution of T cells to cardiovascular remodeling are still poorly understood. Adaptive cell–mediated immunity plays an important role in the pathogenesis of inflammatory heart diseases such as myocarditis. In experimental autoimmune myocarditis, T lymphocytes and dendritic cells (DCs) have been demonstrated to be obligatory contributors of autoimmune response to tissue damage. DCs possess a strong capacity to ingest external antigens and to present them through the major histocompatibility complex class II (MHCII) complex to induce
CD4$^+$ T-cell responses.$^{10}$ The adoptive transfer of purified T lymphocytes from mice with active myocarditis was sufficient to promote the disease into recipient mice,$^{11,12}$ underlying the importance of these cells in cardiac dysfunction.

More recently, several lines of evidence have involved T lymphocytes in cardiac remodeling after myocardial infarction. Indeed, reports have shown that modulation of immune cell response by ablation of T cells or DCs altered the initial postinfarction healing and remodeling response. By promoting the formation of mature collagen matrix and fibrosis, these immune cells may facilitate early wound healing and improved survival after myocardial infarction.$^{13,14}$ Although immunity may contribute to different hallmarks of HF development, participation of T cells in chronic cardiac remodeling has been poorly addressed in the literature. In the present study, we used complementary mouse models to investigate the role of T lymphocytes in HF progression. Our results showed the key role of CD4$^+$ T cells in ventricular remodeling and the progression from compensated cardiac hypertrophy to HF.

Methods

Detailed methodology is provided in the online-only Data Supplement.

Animals

Wild-type (WT) C57BL/6 mice were obtained from Janvier (Saint Berthevin, France). Mice deficient for recombination activating gene 2 expression (RAG2KO) and mice deficient for MHCI expression (MHCIIKO) as a result of a disruption of the Iaβ gene in C57BL/6 were kindly provided by Dr J.P. van Meerveld.$^{15}$ Mice deficient for CD8α (CD8KO) and mice with transgenic CD4$^+$ T-cell receptor (TCR) specific for ovalbumin (OT-II) mice were from Charles River Laboratories. Twelve-week-old male mice were used in all the experiments and were maintained under specific pathogen-free conditions. In vivo studies were conducted in mice under European laws on the protection of animals (86/609/EEC). Mouse experiments were approved by and performed according to the guidelines of the Ethics and Animal Safety Committee of INSERM Toulouse/ENVT (agreement C3155507).

Statistics

All results are presented as mean±SEM. Two-group comparisons were analyzed by unpaired 2-tailed $t$ tests. In the case of nonnormality, the nonparametric test 2-tailed Mann-Whitney $U$ test was used. Multiple-group comparisons were performed with 1-way ANOVA followed by the Tukey posttest. The Kruskal and Wallis test followed by the Dunn test comparison of pairs was used to analyze data that did not show normal distribution. Values of $P<0.05$ were considered significant. All statistical analyses were performed with GraphPad Prism (version 6.01) software.

Results

Chronic HF Is Associated With Increased Cellularity of Mediastinal Draining Lymph Nodes and Th1 Polarization of T Cells

Draining lymph nodes, the epicenter of the immune responses, are dynamic lymphoid structures. At autopsy, we observed an enlargement of heart mediastinal draining lymph nodes (MLNs) associated with a strong increase in total cell number (Figure 2A and 2B) in mice submitted to TAC. In addition, cells isolated from MLNs of TAC animals and stimulated ex vivo with anti-CD3ε anti-CD28 antibodies secreted a higher level of the T-cell growth factor IL-2 than cells isolated from sham animals (Figure 2C). Moreover, analysis by flow cytometry revealed an increase in CD4$^+$ and CD8$^+$ T cell numbers in MLNs after TAC compared with sham animals (Figure 2D; see the gating strategy in Figure IV in the online-only Data Supplement). These results support the enhanced T-cell proliferation in lymph nodes after TAC. Differences in lymphocyte subsets after TAC were not observed in spleen (data not shown).

To assess the potential difference in Th1/Th2 polarization, cells isolated from MLNs were stimulated ex vivo for 48 hours by anti-CD3ε anti-CD28 antibodies. MLN cells from the TAC group produced more interferon-γ, the Th1 cytokine, after TCR-dependent activation than cells from sham animals (sham, 13.05±3.01 ng/mL versus TAC, 58.95±10.4 ng/mL; $P<0.01$; Figure 2E). In contrast, the level of the Th2 cytokine IL-4 was very low in sham animals and diminished after TAC (Figure 2E). These results were confirmed by intracellular staining and flow cytometry showing a higher number of interferon-γ–positive CD4$^+$ and CD8$^+$ T cells after TAC (Figure 2F and Figure V in the online-only Data Supplement). The percentage of IL-4–positive cells was negligible (<1%)
Figure 1. Chronic heart failure induced by transverse aortic constriction (TAC) is associated with cardiac T-lymphocyte infiltration. A, Cardiac mRNA expression of chemokines implicated in lymphocyte recruitment: CX3CL1, CXCL16, CXCL10, and CCL17 in sham- and TAC-operated mice (n=6–12 per group). mRNA expression was normalized to GAPDH and represented as fold change to sham. B, Costaining by immunofluorescence of CD3+ (T-cell marker in red) and CD45+ cells (pan-leukocyte marker in green) in cardiac tissue after TAC. Merged image illustrates coexpression, and nuclei were stained by DAPI (blue). Bar, 10 μm. C, Quantification of immunohistochemical CD3-positive cells in cardiac tissue of sham and mice submitted to TAC (n=5). D, Representative flow cytometry dot plots and quantification of T cells (CD3+TCRβ+) and CD4+ (CD3+TCRβ+CD4+) and CD8+ (CD3+TCRβ+CD8+) T-cell subsets isolated from cardiac tissue of sham- and TAC-operated mice (n=5–9). E, Flow cytometry dot plots represented CD44+ and TCRβ+ expression in CD4+ T cells previously gated on CD45+/TCRβ+/CD3+/CD4+. Quantification of CD4+CD44hi cells was expressed as number of cells per heart. Values shown are mean±SEM. TCR indicates T-cell receptor; and WT, wild type. Significance vs sham: *P<0.05, **P<0.01, ***P<0.001 by t test (A, C, and E) or U test (D).
and unaffected by TAC. Taken together, these results suggest a preferential Th1-type polarization of MLN T cells.

Absence of B and T Lymphocytes Attenuates the Transition From Hypertrophy to HF

To determine whether mobilization of lymphocytes in cardiac tissue has an impact on the progression to HF, we submitted RAG2KO mice to TAC. RAG2 encodes the recombination activation gene 2, which catalyzes V(D)J recombination, an essential step in the generation of immunoglobulins and T lymphocyte receptors. As a consequence, RAG2KO mice are completely deficient in mature B and T lymphocytes. According to echocardiography parameters (Table 1), left ventricular dimensions and function were similar in WT and RAG2KO mice under unstressed control conditions. After 6 weeks of TAC, the absence of lymphocyte prevented cardiac dilation but not ventricular hypertrophy and attenuated cardiac contractile dysfunction induced by pressure overload (Table 1).

In addition, mRNA expression of the HF markers atrial and brain natriuretic factors was significantly lower in RAG2KO TAC compared with WT TAC mice (Figure 3A). These results indicate that the absence of B and T lymphocytes prevented cardiac dilation and attenuated cardiac dysfunction induced by TAC, suggesting a role of lymphocytes in the transition from hypertrophy to HF.

Reconstitution of T Lymphocytes Promotes Pressure Overload–Induced HF

To investigate the role of T cells in progression to HF, we transferred purified T cells from donor WT mice into RAG2KO mice. After T-cell reconstitution, RAG2KO+CD3 and RAG2KO mice injected with PBS (RAG2KO+PBS) were submitted to TAC. As expected, a strong infiltration of CD3+ T cells detected by immunofluorescence was retrieved in left ventricular tissue of RAG2KO+CD3 compared with control RAG2KO+PBS mice (Figure 3B). T-cell reconstitution in RAG2KO mice before surgery produced a significant decrease in cardiac contractility as revealed by reduced fractional shortening (FS) in RAG2KO+CD3 compared with control RAG2KO+PBS mice (FS: RAG2KO+PBS, 24.97±3.4% versus RAG2KO+CD3, 16.4±2.0%; P<0.05; Figure 3C and Figure VI in the online-only
Data Supplement). According to deterioration of contractile function, expression of the HF markers atrial and brain natriuretic factors was significantly increased in RAG2KO+CD3 compared with RAG2KO+PBS mice (Figure 3D). Taken together, these results show that T cells contribute to progression of HF after TAC.

T Lymphocytes Promote Fibrosis by Enhancing Collagen Accumulation and Cross-Linking After TAC

Macrophages are known to be involved in inflammatory and fibrotic processes. We observed that TAC-induced HF was associated with macrophage infiltration (identified as CD68+ cells; Figure 4A and 4B) and excessive perivascular and interstitial fibrosis in cardiac tissue of WT mice (Figure 4C and 4D). In the absence of lymphocytes, both macrophage infiltration (WT TAC, 159±20 cells/mm² vs RAG2KO TAC, 97±12 cells/mm²; P<0.05, Figure 4B) and excessive fibrosis (WT TAC, 6.5±1.0% versus RAG2KO TAC, 2.2±0.6%; P<0.01; Figure 4D) were prevented.

After T-cell replenishment, macrophage density (WT TAC, 159±20 cells/mm²; RAG2KO+CD3, 154±13 cells/mm²; Figure 4B) and ventricular fibrosis measured in RAG2KO+CD3 (WT TAC, 6.5±1.0%; RAG2KO+CD3, 6.43%; Figure 4D) were similar to those found in WT mice. These results support a role of T lymphocytes in deleterious cardiac remodeling seen after 6 weeks of TAC in WT animals.

To gain insight into the mechanisms responsible for the ventricular fibrotic response induced by T cells, we determined the levels of collagen deposition in cardiac tissue by picrosirius red staining. As shown in Figure 5A, RAG2KO mice submitted to TAC presented a significant decrease in collagen content compared with WT mice. In RAG2KO mice transferred with T lymphocytes, the amount of collagen was comparable to that observed in WT mice after TAC, supporting the involvement of these cells in promoting cardiac collagen accumulation (Figure 5B).

The mRNA expression of the 2 main procollagens Coll1a1 and Coll3a1 was upregulated after TAC to a similar extent in both the WT and RAG2KO groups (Figure 5C). In addition, T-cell transfer into RAG2KO mice did not significantly increase the expression of Coll1a1 and Coll3a1 mRNAs (Figure 5D). These results indicate that the profibrotic activity of T lymphocytes involves collagen accumulation by a mechanism independent of procollagen gene expression.

Dysregulation of both collagen deposition and assembling in cross-linked fibers, leading to cardiac fibrosis, is a major determinant of cardiac dysfunction. Recently, Satoh et al suggested that T cells might promote collagen fiber formation by stimulating lysyl oxidase (LOX), an enzyme responsible for collagen cross-linking. LOX is produced as an inactive proenzyme (50 kDa) that is cleaved in a shorter active form (32 kDa). In our study, we observed a strong increase in LOX mRNA expression in cardiac tissue of WT mice after TAC (Figure 6A). This phenomenon was associated with an enhanced amount of the pro-LOX and mature form of LOX, as determined by Western blot (Figure 6B). In cardiac tissue of RAG2KO TAC mice, the increase in LOX mRNA was significantly lower than that observed in WT animals after TAC (Figure 6A). In addition, although the amount of the pro-LOX protein was unchanged, the mature LOX was downregulated in RAG2KO after TAC compared with sham (Figure 6C). Reconstitution of T lymphocytes significantly increased LOX mRNA and protein expression in cardiac tissue after TAC, mimicking the changes observed in WT mice (Figure 6A–6D).

To determine whether LOX modification subsequently affected cross-linked collagen density, we examined collagen fibrils by picrosirius red staining under polarized light. Our results showed the presence of abundant birefringent fibers in WT mice after TAC. Collagen fibers, faintly detectable in RAG2KO mice, were strongly visible in T cell–reconstituted mice (RAG2KO+CD3; Figure 6E). Overall, these results indicate that T cells participate in adverse cardiac remodeling after

Table 1. Absence of Lymphocytes in RAG2KO Mice Prevents Cardiac Dilation and Attenuates Cardiac Dysfunction Induced by 6 Weeks of TAC

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<th>WT Sham (n=10)</th>
<th>WT TAC (n=20)</th>
<th>P</th>
<th>RAG2KO Sham (n=17)</th>
<th>RAG2KO TAC (n=17)</th>
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<td>IVSd cm</td>
<td>0.083±0.008</td>
<td>0.109±0.003</td>
<td>†</td>
<td>0.086±0.002</td>
<td>0.107±0.004</td>
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<td>LPVWd cm</td>
<td>0.080±0.008</td>
<td>0.106±0.006</td>
<td>*</td>
<td>0.087±0.002</td>
<td>0.103±0.006</td>
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<td>LVId sympathetic</td>
<td>0.383±0.012</td>
<td>0.460±0.014</td>
<td>†</td>
<td>0.362±0.012</td>
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<tr>
<td>LVId diastolic</td>
<td>0.260±0.029</td>
<td>0.382±0.018</td>
<td>†</td>
<td>0.247±0.003</td>
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<td>EDV, mL</td>
<td>0.148±0.019</td>
<td>0.248±0.020</td>
<td>†</td>
<td>0.121±0.012</td>
<td>0.160±0.010</td>
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<tr>
<td>ESV, mL</td>
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<td>FS, %</td>
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<td>581±29</td>
<td>NS</td>
<td>536±14</td>
<td>543±17</td>
<td>NS</td>
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Echocardiographic parameters of WT and RAG2KO after TAC. Values shown are mean±SEM. EDV indicates end-diastolic volume; ESV, end-systolic volume; FS, fractional shortening; HR, heart rate; IVSd, diastolic interventricular septal wall thickness; LVId, diastolic left ventricular dimension; LVId sympathetic, systolic left ventricular dimension; LVId diastolic, diastolic left ventricular wall thickness; RAG2KO, mice deficient for recombination activating gene 2 expression; TAC, transverse aortic constriction; and WT, wild-type.

Significance vs corresponding sham group:

*P<0.05, †P<0.01, and ‡P<0.001. Significance vs WT TAC: §P<0.05 and ‖P<0.001 by ANOVA.
Lack of Functional CD4+ but Not CD8+ T Cells Prevented Cardiac Remodeling and Failure in Response to Pressure Overload

To define the specific contribution of T cell subsets in pressure overload–induced HF, we submitted mice lacking functional CD4+ T cells (MHCIIKO) and CD8+ T cells (CD8KO) to pressure overload. After TAC, MLNs isolated from MHCIIKO mice did not exhibit a size increase or higher cellularity compared with sham animals (Figure VIIA in the online-only Data Supplement). In addition, MHCIIKO mice did not develop cardiac dilation after TAC (left ventricular internal dimension at diastole: MHCIIKO sham, 0.383±0.014 cm versus MHCIIKO TAC, 0.356±0.008 cm; P=NS; Table 2). Although MHCIIKO mice presented myocardial wall thickening after TAC, cardiac contractile function was totally preserved, as indicated by unchanged FS compared with sham (FS: MHCIIKO sham, 37.61±2.19% versus MHCIIKO TAC, 36.35±2.30%; P=NS; Table 2). The downregulation of SERCA2a and MYH6 isoform genes, generally observed in HF after TAC, was absent in MHCIIKO mice (Figure 7A). In addition, the fact that the expression of atrial and brain natriuretic factors was less increased compared with WT TAC further supports the role of CD4+ T cells in promoting HF (Figure 7A).

The absence of CD4+ T cells also prevented adverse remodeling, as seen by largely reduced fibrosis, collagen content, and macrophage infiltration after 6 weeks of TAC (Figure 7B and 7C and Figure VIII in the online-only Data Supplement). In addition, the lack of CD4+ T cells significantly blunted upregulation of mRNA and protein expression of LOX and the subsequent figure...
collagen fiber formation induced by TAC (Figure 7D–7F).

Taken together, these results highlight that CD4+ T cells are required for the transition from hypertrophy to HF.

Next, we performed TAC in CD8KO mice to determine the role of CD8+ T cells in the progression of HF. At autopsy, TAC mice exhibited swollen MLNs with CD4+ T-cell density very similar to that observed in WT TAC (Figure IXA and IXB in the online-only Data Supplement). Echocardiography showed that cardiac function was altered in CD8KO mice after TAC, as indicated by a dramatic decrease in the FS (FS: CD8KO sham, 37.87±1.53% versus CD8KO TAC, 20.63±2.76%; P<0.001; Figure 8A) and the development of cardiac dilation (left ventricular internal dimension at diastole: CD8KO sham, 0.330±0.006 versus CD8KO TAC, 0.388±0.017; P<0.05; Figure 8A and Figure X in the online-only Data Supplement). TAC also modified the mRNA expression of atrial and brain natriuretic factors and SERCA2a compared with sham mice, confirming the severity of cardiac failure (Figure XI in the online-only Data Supplement). Finally, CD8KO mice subjected to TAC presented excessive fibrosis and collagen

Figure 4. T lymphocytes play a major role in macrophage infiltration and excessive fibrosis after transverse aortic constriction (TAC).

A, Representative staining of macrophages with anti-CD68 antibody (arrows) in cardiac tissue of wild-type (WT) mice, mice deficient for recombination activating gene 2 expression (RAG2KO), RAG2KO mice reconstituted with T lymphocytes (RAG2KO+CD3), and RAG2KO mice injected with PBS (RAG2KO+PBS) after TAC.

B, Quantification of CD68-positive cells per 1 mm² in WT (n=10), RAG2KO (n=10), RAG2KO+PBS (n=7), and RAG2KO+CD3 (n=6). C, Representative transverse sections of whole mouse heart subjected to TAC for 6 weeks and stained with Masson trichrome. Bottom, Enlarged portion of heart section. Scale bar, 1 mm.

D, Quantification of fibrosis in slides as C (n=10 for WT and RAG2KO, n=7 for RAG2KO+PBS, n=6 for RAG2KO+CD3). Values shown are mean±SEM. Significance vs WT sham: ***P<0.001. Significance vs WT TAC: †P<0.01, ††P<0.01. Significance vs RAG2KO+PBS: #P<0.05, ANOVA.
content as observed in WT mice after TAC (Figure 8B and Figures XII and XIII in the online-only Data Supplement). Our results clearly showed that, unlike CD4+ T cells, CD8+ T cells are not required for developing chronic HF.

To further assess the implication of antigen recognition by the CD4+ T cells, we performed experiments on OT-II mice. After TAC, these mice did not develop cardiac dilation (left ventricular internal dimension at diastole: OT-II sham, 0.347±0.013 cm versus OT-II TAC, 0.360±0.019 cm; P=NS; Figure 8C and Figure IV in the online-only Data Supplement) and showed preserved cardiac function (FS: OT-II sham, 42.80±3.36% versus OT-II TAC, 35.25±3.77%; P=NS; Figure 8C and Figure IV in the online-only Data Supplement) and a reduced ventricular fibrosis (OT-II sham, 0.6±0.6% versus OT-II TAC, 1.8±0.4% P=NS; Figure 8D). These findings suggest that deleterious effects of CD4+ T cells depend on antigen recognition.

Discussion

Using combined approaches in different mouse models, we demonstrated here the requirement of CD4+ T cells in the transition from compensated hypertrophy to HF.

Our results showed that end-stage HF was characterized by an accumulation of both CD4+ and CD8+ T cells in cardiac ventricles. In agreement with these results, overexpression of chemokines such as CX3CL1, CCL17, CXCL10, and CXCL16 involved in the recruitment and homing of T cells in injured tissues16–18,22 has also been observed during HF. The upregulation of CCL17, expressed mainly by activated DCs in various nonlymphoid organs,19 was concomitant with higher cardiac infiltration of DCs in failing heart.

HF was also correlated with robust expansion of heart MLNs, an increased number of CD4+ and CD8+ T cells, and elevated secretion of the T-cell growth factor IL-2 after ex vivo TCR stimulation. These data support T-cell proliferation

[Figure 5. T lymphocytes increase collagen content but do not modify procollagens expression after transverse aortic constriction (TAC). Collagen deposition in cardiac tissue of wild-type mice (WT) and mice deficient for recombination activating gene 2 expression (RAG2KO; A) and RAG2KO mice reconstituted with T lymphocytes (RAG2KO+CD3) and RAG2KO mice injected with PBS (RAG2KO+PBS; B) submitted to TAC based on picrosirius red staining viewed under white light. Quantification of total collagen deposition in cardiac tissue of WT and RAG2KO and of RAG2KO+PBS and RAG2KO+CD3 mice, C, Cardiac mRNA expression of type I and III procollagens (Coll1a1 and Coll3a1) in WT and RAG2KO mice (n=10) and (D) in RAG2KO+PBS (n=7) and RAG2KO+CD3 (n=6). Data were normalized to GAPDH and calibrated to the average of WT sham (B) or RAG2KO+PBS (C). Values shown are mean±SEM. Significance vs sham or WT TAC: *P<0.05, **P<0.01. Significance vs RAG2KO+PBS: ###P<0.01 by t test (A, B, and D) or ANOVA (C).]
Figure 6. T lymphocytes enhance upregulation of collagen cross-linking enzyme lysyl oxidase (LOX) induced by transverse aortic constriction (TAC).

A. Cardiac mRNA expression of LOX in wild-type mice (WT) and mice deficient for recombination activating gene 2 expression (RAG2KO; n=10) and in RAG2KO mice injected with PBS (RAG2KO+PBS; n=7) and RAG2KO mice reconstituted with T lymphocytes (RAG2KO+CD3; n=6) after TAC. Data were normalized to GAPDH and calibrated to the average of WT sham.

B. Representative immunoblots and quantitative analysis of pro-LOX protein (50 kDa) and mature LOX protein (32 kDa) expression in WT, RAG2KO, and RAG2KO mice reconstituted with T lymphocytes (RAG2KO+CD3; n=4 per group). Results were normalized to GAPDH and expressed as fold over corresponding controls.

C. Collagen fibers observed after picrosirius red staining under polarized light in cardiac tissue of WT, RAG2KO+PBS, and RAG2KO+CD3 mice after TAC (×200). Values shown are mean±SEM. Significance vs sham: *P<0.05, **P<0.01, ***P<0.001. Significance vs WT TAC: †P<0.05. Significance vs RAG2KO+PBS: #P<0.05 by ANOVA (A), U test (B and D), or t test (C).
Interestingly, CD8KO and WT animals had a high density in the online-only Data Supplement). In addition, activated/
tissue of MHCIIKO mice after TAC further argues against a
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phocytes, but not B lymphocytes (F.L., unpublished data),
transfer of T lymphocytes in cardiac remodeling and in transition from
has been poorly defined.

dysfunction in patients with HF.6,23 To date, the relationship
tion between T-cell polarization to Th1 and left ventricular
tions made in clinical studies describing a positive correla-
production. These results are in agreement with observa-
participation of both CD4+ and CD8+ T cells in interferon-
ɛ/anti-CD28 stimulation revealed
in MLNs during chronic HF. Cytokines production by MLN T
cells assessed after anti-CD3/anti-CD28 stimulation revealed a significant increase in the Th1-type cytokine (interferon-γ) and a decrease in the Th2 type cytokine (IL-4) production in mice subjected to TAC. Intracellular staining uncovered the participation of both CD4+ and CD8+ T cells in interferon-γ production. These results are in agreement with observations made in clinical studies describing a positive correlation between T-cell polarization to Th1 and left ventricular dysfunction in patients with HF.6,23 To date, the relationship between T-cell activation and the development of chronic HF has been poorly defined.

In our present study, we evaluated the participation of T lymphocytes in cardiac remodeling and in transition from hypertrophy to HF by using lymphocyte-deficient mice as models. After TAC, hearts of RAG2KO mice did not undergo ventricular dilation, exhibited preserved contractile function, and showed a drastic reduction in fibrosis. Transfer of T lymphocytes, but not B lymphocytes (F.L., unpublished data), enhanced contractile dysfunction and promoted adverse ventricular remodeling in RAG2KO after TAC. Although infiltration of both CD4+ and CD8+ T cells was observed in failing hearts of WT animals, only the CD4+ T-cell subset plays a crucial role in cardiac dysfunction. Indeed, MHCIIKO mice lacking mature CD4+ T cells with normal CD8+ T-cell compartment24 were preserved from ventricular dilation and contractile dysfunction and exhibited blunted ventricular fibrosis after TAC. In contrast, CD8KO mice developed cardiac failure and displayed adverse remodeling. Moreover, the fact that a higher number of CD8+ T cells was observed within cardiac tissue of MHCIIKO mice after TAC further argues against a role of this subset in the pathology (Figure VIIA and VIIIB in the online-only Data Supplement). In addition, activated/effector CD4+ T-cell infiltration within the failing heart of WT mice emphasized a specific participation of this T-cell subset in cardiac dysfunction. As mentioned, HF was associated with both CD4+ and CD8+ T cell augmentation in MLNs. Interestingly, CD8KO and WT animals had a high density of CD4+ T cells in their MLNs, whereas no modification of CD8+ T-cell number was retrieved in MHCIIKO mice after TAC (Figure VIIA in the online-only Data Supplement). This observation suggests that CD4+ T cells may contribute to the CD8+ T-cell expansion seen in MLNs of WT animals with HF. Taken together, these results show a major role of the CD4+ T-cell subset in hyperplasia of MLNs associated with HF and in the deterioration of cardiac function during chronic pressure overload.

Our results also showed a striking effect of T cells in fibrotic response induced by pressure overload. Indeed, the significant increase in ventricular fibrosis and collagen deposition observed in WT mice after TAC was not retrieved in RAG2KO mice, which lack T cells. However, T-cell transfer induced a fibrotic response similar to that found in WT mice after TAC. This fibrotic process was not affected by the absence of CD8+ T cells but was prevented in mice lacking the CD4+ T-cell subset. The profibrotic activity of T lymphocytes appeared independent of procollagen gene expression because all groups of mice (WT, RAG2KO, RAG2KO+CD3, CD8KO, and MHCIIKO mice) exhibited similarly increased procollagen I and III mRNA after TAC.

Interestingly, the increase in fibrosis and collagen accumulation was concomitant with enhanced collagen fiber density observed in WT mice after TAC. The assembling into final collagen fibers is a process mediated by LOX enzymes. Moreover, the induction of LOX seems to be a general feature observed in a variety of fibrotic processes in different organs and interestingly has been reported in the fibrotic myocardium of patients with chronic HF.25,26 In WT mice, LOX expression and ventricular collagen fiber density were increased after TAC, whereas these effects were strongly reduced in MHCIIKO and RAG2KO mice. In addition, cardiac contractile dysfunction induced by the transfer of T cells into RAG2KO mice was accompanied by the upregulation of LOX expression and accumulation of collagen fibers in heart tissue. These results reveal a new contribution of T lymphocytes in fibrosis and collagen accumulation in chronic pressure overload. T

### Table 2. Absence of CD4+ T Cells in MHCIIKO Mice Prevents Cardiac Dilation and Dysfunction Induced by 6 Weeks of TAC

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Echocardiographic parameters of WT and MHCIIKO mice after TAC. Values shown are mean±SEM. EDV indicates end-diastolic volume; ESV, end-systolic volume; FS, fractional shortening; HR, heart rate; IVSd, diastolic interventricular septal wall thickness; LVIDd, diastolic left ventricular dimension; LVIDs, systolic left ventricular dimension; LVPWd, diastolic left posterior wall thickness; MHCIIKO, mice lacking CD4+; TAC, transverse aortic constriction; and WT, wild-type.

Significance vs corresponding sham group: *P<0.05, †P<0.01, and ‡P<0.001. Significance vs WT TAC: §P<0.001 by ANOVA.
Figure 7. Absence of CD4+ T cell (MHCIIKO mice) prevents fetal gene activation and adverse cardiac remodeling induced by transverse aortic constriction (TAC). A, Cardiac mRNA expression of atrial (ANF) and brain (BNF) natriuretic peptides, \(\alpha\)- and \(\beta\)-myosin heavy chains (MYH6, MYH7), and sarco(endo)plasmic reticulum Ca\(^{2+}\)ATPase (SERCA2a) in wild-type (WT) and MHCIIKO mice (sham, \(n=6\); TAC, \(n=13\)). B, Quantification of fibrotic areas after Masson trichrome staining in cardiac tissue of WT and MHCIIKO mice after TAC (\(n=8\)). C, Quantification of total collagen deposition in cardiac tissue of WT and MHCIIKO mice submitted to TAC based on picrosirius red staining viewed under white light (\(n=8\)). D, Cardiac mRNA expression of lysyl oxidase (LOX; \(n=6–13\)). Data were normalized to GAPDH and calibrated to the average of WT sham. E, Representative immunoblot for LOX in cardiac tissue of MHCIIKO mice and quantitative analysis of pro-LOX protein (50 kDa) and mature LOX protein (32 kDa) expression (\(n=4\) per group). Results were expressed as fold over corresponding sham. F, Picrosirius red-stained sections (observed in C) viewed under polarized light in cardiac tissue of WT and MHCIIKO mice after TAC (\(\times 200\)). Values shown are means±SEM. Significance vs sham: *\(P<0.05\), **\(P<0.01\), ***\(P<0.001\). Significance vs WT TAC: †\(P<0.05\), ††\(P<0.01\), †††\(P<0.001\) by ANOVA (A, B, and D) or U test (C and E).
cells also participate in cardiac collagen cross-linking through the induction of LOX expression.

It is noteworthy that, in the case of acute cardiac injury, T lymphocytes seem to have a positive effect by improving wound healing of the myocardium and collagen maturation. These results, along with ours, underline the relevance of T lymphocytes in fibrotic response that could be positive during cardiac repair after acute ischemia and negative during chronic pressure overload. Recently, numerous studies delineated strong implication of IL-17 and IL-1 T cell–secreted cytokines in cardiac fibrosis observed in autoimmune heart diseases. The participation of these cytokines in pressure overload-induced HF deserves further investigation.

Figure 8. Contrary to mice lacking CD8 T-cell subsets (CD8KO), T-cell receptor transgenic mice specific for ovalbumin (OT-II) were prevented from having chronic heart failure. A, Left ventricular internal dimension at diastole (LVIDd) and fractional shortening (FS) were evaluated in wild-type (WT; n=7–13) and CD8KO (n=5–8) mice after 6 weeks of transverse aortic constriction (TAC). B, Quantification of fibrotic areas after Masson trichrome staining in cardiac tissue of WT and CD8KO mice after TAC (n=4–8). C, LVIDd and FS were evaluated in WT (n=8–9) and OT-II (n=4–8) mice after TAC. D, Quantification of fibrotic areas in cardiac tissue of WT and OT-II mice after TAC. Values shown are mean±SEM. Significance vs sham: *P<0.05, **P<0.01, ***P<0.001. Significance vs WT TAC: ††P<0.01: †††P<0.001 by ANOVA.
The participation of immune system in cardiac remodeling is supported by previous reports describing the beneficial effect of immunomodulation in hypertensive remodeling. Indeed, sustained angiotensin II infusion in mice induced hypertrophic cardiac remodeling and increased the number of activated circulating CD4+ T subsets. Immunosuppressive treatment of mice by adoptive transfer of regulatory T cells has been shown to attenuate both angiotensin II–induced cardiac fibrosis and hypertrophy. Regulatory T cells are a lineage of T cells with anti-inflammatory properties and suppressive effects on immune responses. They also strongly reduced ventricular fibrosis with only a small effect on left ventricular hypertrophy in a mouse model of ventricular pressure overload.

In addition to these previous studies, our results showed that CD4+ T cells are required for adverse remodeling and HF. The preserved cardiac function of OT-II mice after TAC suggests that the deleterious effect of CD4+ T cells is mediated by an inappropriate recognition of antigen. Putative antigens triggering T-cell activation have been proposed in cardiac disease. At present, the identity of antigens incriminated in the detrimental effects during pressure overload induced chronic HF remains to be defined.

Conclusions

Our results support the concept that T cells, and more specifically the CD4+ T-cell subset, play a crucial role in pressure overload–induced cardiac remodeling leading to HF. These cells aggravate tissue remodeling and trigger the transition from compensated hypertrophy to HF. Intervention that modulates CD4+ T-cell activity might represent a novel therapeutic target for the treatment of HF.

Acknowledgments

We thank Genome and Transcriptome–Génopole Toulouse facility and I.J. Maoret for excellent technical assistance with the polymerase chain reaction; Y. Nicaise, F. Capilla, and Dr C. Guilleau-Frugier for histology help; the flow cytometry facility (IPBS-Toulouse); and S. Guerder for helpful discussions.

Sources of Funding

This project was supported by grants from INSERM and Région Midi-Pyrénées. Dr Laroumanie is supported by grants from the Région Midi-Pyrénées and Groupe de Réflexion sur la Recherche Cardiovasculaire.

Disclosures

None.

References

Cardiac remodeling induced by pressure overload is a common precursor to heart failure in humans. Despite the widespread use of therapeutics, mortality and morbidity remain elevated in patients with heart failure, suggesting that pathological mechanisms are insufficiently affected by current treatments. In this study, we showed for the first time the critical role of the adaptive immune system in the onset of chronic heart failure. Indeed, using different models of immunodeficient mice, we demonstrated that the absence of T lymphocytes prevents the transition from cardiac hypertrophy to heart failure after ventricular pressure overload. In addition, we identified the CD4+ T-cell subsets as the major actor of the adverse ventricular remodeling. This maladaptive effect of CD4+ T cells involves a profibrotic process associated with collagen accumulation and maturation, leading to cardiac dysfunction. These data underline that adverse remodeling and evolution to heart failure are mediated by an inappropriate activation of immune system, probably triggered by neoantigen generation or by self-tolerance breakdown. These studies identify a previously undefined role for CD4+ T cells in pressure overload–induced cardiac remodeling and in the transition to heart failure. Prevention of deleterious T-cell activation may represent a new therapeutic approach to improve outcomes during pressure overload–induced heart failure in humans.

CLINICAL PERSPECTIVE
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CD4$^+$ T Cells Promote the Transition From Hypertrophy to Heart Failure During Chronic Pressure Overload

Fanny Laroumanie, Victorine Douin-Echinard, Joffrey Pozzo, Olivier Lairez, Florence Tortosa, Claire Vinel, Christine Delage, Denis Calise, Marianne Dutaur, Angelo Parini and Nathalie Pizzinat

_Circulation._ 2014;129:2111-2124; originally published online March 21, 2014;
doi: 10.1161/CIRCULATIONAHA.113.007101

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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Data Supplement (unedited) at:
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SUPPLEMENTAL MATERIAL
Supplemental Methods

Transverse aortic constriction

Mice were anesthetized with intraperitoneal ketamine (60 mg/kg) and xylazine (6 mg/kg). Anesthesia was maintained during chirurgical procedure with isoflurane (1.5%). Transverse aortic constriction (TAC) was performed as previously described. TAC was induced by ligation of the transverse aorta using a 7-0 Prolene suture. For this purpose, two knots of a 1.5 mm distance (26-gauge needle circumference) were made on the thread which was carefully introduced around the aorta. Both knots were tight together to perform constriction. Sham operated mice underwent the same surgery without constriction. Mice were sacrificed after 6 weeks of TAC. A single operator performed surgical procedures for all the experiments.

Echocardiography and hemodynamic measurements

Animals were anesthetized with 2% isoflurane and examined by trans-thoracic echocardiography (echocardiograph Vivid 7 ultrasound, GE) 6 weeks after surgery. Cardiac ventricular dimensions were measured on M-mode images as previously described. The echocardiography operator was blinded to genotype and mice chirurgical procedure.

Real time PCR analysis

RNA was extracted using the Qiagen RNaseasy Mini Kit from cardiac tissue according to the manufacturer’s instructions. DNase treatment was systematically performed. Quality and quantification of extracted RNA were assessed by Experion analysis. The cDNA was synthesized using Superscript II First-Strand system (Invitrogen). The absence of contaminants was checked by RT-PCR assays of negative control samples in which the Superscript II was omitted. mRNA was analyzed by real-time PCR using PowerSYBR green (Life Technology) probe method and the primers listed in Supplemental Methods. Melting curve analysis was performed to ensure purity of the PCR products and relative quantification was determined using the comparative CT method with data normalized to GAPDH and calibrated to the average of control group (Sham WT unless otherwise notified).
### Western Blotting

Mouse tissues were lysated using lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton ×100) supplemented with complete protease inhibitor cocktail (Sigma). A 30-µg sample of total protein were loaded on a 12.5% polyacrylamide gel and transferred to PVDF membrane. The membrane was blocked with 1% BSA in TBS-Tween 20 (0.1%) for 1h. A specific rabbit polyclonal antibody against lysyl oxidase (Thermo Scientific) was used (1:500). Bands were detected by peroxidase conjugated secondary antibodies (Cell Signaling) and visualized with the ECL chemiluminescence system (GE Healthcare). Proteins were detected using an automatic densitometer (Chemidoc, Bio-Rad). The blots were also probed with a monoclonal GAPDH antibody (Cell Signaling) as a control for loading.

### Immunohistology

To wash out blood cells from cardiac coronary vessels, we injected 5 ml of PBS in cardiac ventricles before removing heart from anesthetized animals. Cardiac tissues were frozen in OCT tissue embedding compound (Tissue Tek, EMS) at -80°C. For immunohistology staining, cryosections of 5µm were fixed with cold acetone. Endogenous biotin and background peroxidase were blocked (Avidin/Biotin Blocking kit from Vector Labs and peroxidase blocking buffers from Dako). Sections were stained using Vectastain Elite ABC Kit (Vector) and colorimetric detection was visualized using
DAB substrate. Finally, sections were counterstained with hematoxylin, dehydrated, mounted and digitized with a Hamamatsu NanoZoomer.

The antibodies used for histology staining were as follows: anti-CD68 (clone FA-11, AbD serotec) and anti-CD3ε (clone 145-2C11, AbD serotec).

**Immunofluorescence**

Cryosections of 5µm were blocked using the Avidin/Biotin blocking kit (Vector Labs) followed by 2% normal goat serum (Sigma-Aldrich) and 1% BSA in PBS. Sections were stained overnight with rat anti-mouse CD45 (clone 30-F11, BD Pharmingen), CD4 (clone GK 1.5, eBioscience) or CD8α (clone 53-6.7, eBioscience) and hamster anti-mouse CD3 followed by staining with secondary antibody: anti-rat DL488 and anti-hamster-Biotin/Streptavidin DL549. Nuclei were stained by DAPI.

**Histological Staining Methods**

Cryosections of 10µm were stained with Masson’s trichrome and picrosirius red in order to assess cardiac fibrosis and collagen content. Three sections per heart were stained and digitized with a Hamamatsu NanoZoomer. Finally, stained areas and total surface from each section were determined using color-based thresholding and quantified by ImageJ software. To assess collagen fibers, picrosirius red stained sections were studied under polarized light.

**Reconstitution of T cell populations into RAG2KO recipient mice before TAC**

Spleens of control WT mice were flushed with cold PBS and erythrocytes were eliminated using ACK lysis buffer. Splenic CD3+ T cells were isolated by immunomagnetic selection (EasySep CD3-negative selection kit, StemCell Technologies). T cell purity was routinely greater than 90%. RAG2KO recipient mice were injected with T cells (2x10⁷ cells in a volume of 250 µl) or PBS into the left external jugular vein. Two weeks after injection, RAG2KO+CD3 and control RAG2KO+PBS mice underwent TAC.

**Ex vivo re-stimulation of T cells**

For cytokine production measurements, 5×10⁵ mediastinal lymph nodes cells were stimulated in 500µl RPMI supplemented with 5% FBS, non essential amino acid and 50µM β-mercaptoethanol per well in 48-well plates. T cells were stimulated with 1.5 µg/ml of anti-CD3 and anti-CD28 mAb (BioLegend).
Levels of IL-2 were evaluated after 12h and levels of IL-4 and IFNγ after 48h, using enzyme-linked immunosorbent assay (ELISA, eBioscience) kits according to the manufacturer's instructions. The absorbance was measured at 450 and 570 nm.

**Intracellular cytokine assay**

Mediastinal lymph nodes cells (2x10^6 /ml) were resuspended in RPMI supplemented with 5% FBS, non essential amino acid and 50μM β-mercaptoethanol per well in 24-well low-adherence plates. Cells were stimulated with phorbol ester (PMA 50 ng/ml) and calcium ionophore (ionomycin 1 μg/ml) for 4 hours. Brefeldin A was added during the last 2 hours (10 μg/ml). Cells were stained for surface expression, fixed, and permeabilized using Cytofix/Cytoperm kit (BD Biosciences) and stained for intracellular IFNγ and IL-4 before analysis on LSRII.

**Isolation of cardiac immune cells**

The hearts were cut into pieces and digested in RPMI containing 0.12 mg/ml of Liberase TM (Roche) for 10 min at 37°C with vigorous stirring. The supernatant was then added to 10 ml of ice-cold RPMI supplemented with 10% of heart inactivated fetal bovine serum (FBS). Two milliliters of fresh digesting solution was added to the remaining tissue fragments. Cell suspensions were pooled and erythrocytes were lysed by using ACK lysis buffer. Cell suspensions were stained for flow cytometry analysis.

**Flow cytometry**

Spleens were flushed and crushed in cold PBS and erythrocytes were removed by using ACK lysis buffer. Then, spleen, lymph node and cardiac cell suspensions were passed through a 40µm strainer. Fc receptors were blocked with 5 μg/ml of anti-mouse CD16/CD32 in PBS containing 4% FBS and 2mM EDTA for 20 min at 4°C. Cells were stained with mixtures of antibodies for 30 min at 4°C then incubated with Live/dead Yellow fluorescent reactive dye (Invitrogen) dead staining kit accordingly to the manufacturer's instruction before fixation with PFA (1%). Cardiac cell numbers were quantified using CountBright absolute counting beads (Life Technologies). Prior to acquisition, cells were resuspended in PBS/FBS/EDTA solution. For cardiac cell suspensions, 52 000 beads were added in each cardiac cell samples before acquisition. Flow cytometric multiparameter acquisition was
performed on a LSR II (Digital LSR II; BD Biosciences) and data were analyzed with FlowJo 7.6.3 software (Tree Star).

Cells were stained with directly conjugated Abs against: CD4 APC/Cy7 (clone RM4-5), CD8α PB (clone 53-6.7), IFNγ PE (clone XMG1.2), IL-4 AF647 (clone 11B11), CD45 PE/Cy7 (clone 30-F11), CD3ε FITC (clone 145-2C11), B220 APC (clone RA3-6B2), TCRβ PerCP-Cy5.5 (clone H57-597), CD44 PE (clone IM7), CD11b PE (clone M1/70), CD11c PECy7 (clone N418), MHCII PerCPCy5.5 (clone M5/114.15.2) and matching isotype controls from eBioscience and BioLegend. CD16/CD32 for Fc blocking was from eBioscience.
(Data Supplement Figure 4)

MLN gating strategy

Live cells gate

Leukocytes gate

T cells gate

T cell subsets gate

(Data Supplement Figure 5)

Gated on live cells

CD4+ T cells

CD8+ T cells
(Data Supplement Figure 6)

Repopulation of T cells in RAG2KO mice

A

B

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(Data Supplement Figure 7)

A

B

(Data Supplement Figure 8)

A

B
(Data Supplement Figure 9)

A

Number oftrial cells/MLIL (n=3/cells)

Sham CDK0 TAC CDK0

B

Positive cell/MLIL (ng/cells)

Sham CDK0 TAC CDK0

(Data Supplement Figure 10)

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(Data Supplement Figure 11)

Cardiac mRNA/GAPDH

ANF BNF MYH7

Cardiac mRNA/GAPDH

MYH6 SERCA2a
(Data Supplement Figure 12)

![Graph showing mRNA expression GAPDH](image)

(Data Supplement Figure 13)

A

![Bar graph showing % Collagen](image)

B

![Image of CD8KO TAC](image)

(Data Supplement Figure 14)

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Figure Legends

Data Supplement Figure 1. Flow cytometry gating strategy to quantify T cells within cardiac tissue. Cells are first gated (G1) on a forward scatter/side scatter (FSC-A/SSC-A) dot plot and live cells are selected (G2). Cells from G2 are further characterized by the expression of CD45 (leukocytes, G3). Finally, T cells are first identified in TCR_β positive cells (G4) and further analysed for CD3_ε and CD4 or CD8 expression. Countbright beads are identified by their FSC-A/SSC-A parameters (G0) and are used to normalize cell acquisition numbers.

Data Supplement Figure 2. Accumulation of CD4+ and CD8+ T cells within cardiac tissue of wildtype mice after 6 weeks of TAC. (A) Quantification and representative immunofluorescence of CD4+ T cells and (B) CD8+ T cells in cardiac tissue (red color) of Sham and mice submitted to TAC (n=5). Nuclei were stained by DAPI (blue color). Values shown are mean±SEM. Significance vs Sham: *P<0.05, **P<0.01 by U test.

Data Supplement Figure 3. Accumulation of dendritic cells into cardiac tissue of mice after 6 weeks of TAC. Quantification by flow cytometry analysis of the number of dendritic cells within cardiac tissue of Sham and TAC animals (n=4). Dendritic cells were defined in CD45+ B220− population as cells expressing CD11b and high level of CD11c and MHCII molecules. Results were expressed in number of cells per heart. Values shown are mean±SEM. Significance vs Sham: *P<0.05 by U test.

Data Supplement Figure 4. Flow cytometry gating strategy to quantify T cell subsets within mediastinal draining lymph nodes (MLNs). Cells are first gated (G1) on a forward scatter/side scatter (FSC-A/SSC-A) dot plot and live cells are selected (G2). Cells from G2 are further characterized by the expression of CD45 (leukocytes, G3). Finally, T cells are gated on CD3_ε+ TCR_β+ double positive cells (G4) and T cell subsets are defined by CD4 (G5) or CD8 (G6) expression.
Data Supplement Figure 5. Flow cytometry gating strategy to quantify T cells expressing IFNγ or IL-4 after ex vivo stimulation. After gating on live cells, T cells are gated on CD3+ TCRβ+ double positive cells and T cell subsets are defined on CD4 (G1) or CD8 (G2) expression. IFNγ positive CD4+ T cells were identified in G3 and IFNγ positive CD8+ T cells in G4.

Data Supplement Figure 6. Transfer of T cells into RAG2KO recipient mice reestablishes the presence of T cells in spleen with similar level to WT after TAC. Echocardiographic parameters after reconstitution of T cell compartment. (A) Percentage of CD3+TCR-β+ cells in spleen of WT and RAG2KO+CD3 mice after TAC was expressed as percentage of CD45+ cells (n=6-7). (B) Echocardiographic parameters of RAG2KO+PBS and RAG2KO+CD3 mice after TAC. IVSd indicates diastolic interventricular septal wall thickness; LVPWd, diastolic left posterior wall thickness; LVIDd, diastolic left ventricular dimension; LVIDs, systolic left ventricular dimension; EDV, end diastolic volume; ESV, end systolic volume; FS, fractional shortening and HR, heart rate. Values shown are mean±SEM. Significance vs RAG2KO+PBS: *P<0.05 by t test. NS for not significant.

Data Supplement Figure 7. No changes in cellularity of MLNs isolated from mice lacking CD4+ T cells (MHCIIKO mice) after transverse aortic constriction (TAC). Accumulation of CD8+ T cells within cardiac tissue of MHCIIKO mice after TAC. (A) Quantification of total cellular density (left panel), T cells and CD8+ T cell subsets densities (right panel) in MLNs expressed as cell number per MLNs isolated from MHCIIKO Sham and TAC operated mice (n=6). (B) Quantification by flow cytometry analysis of CD8+ T cells within heart of Sham and TAC MHCIIKO mice (n=6). CD8+ T cells were expressed as number of cell per heart. Values shown are mean±SEM. Significance vs Sham: *P<0.05 by U test.

Data Supplement Figure 8. Mice lacking CD4+ T cells (MHCIIKO) exhibit less macrophage accumulation and no modification of procollagen genes expression as compared to WT after 6 weeks of transverse aortic constriction (TAC). (A) Quantification of CD68 positive cells per mm² in WT and MHCIIKO mice (n=8). (B) Cardiac mRNA expression of type I and III procollagens (Coll1a1 and Coll3a1) in WT and MHCIIKO mice (n=6-13 per group). Data were normalized to GAPDH and
calibrated to the average of WT Sham. Values shown are mean±SEM. Significance vs Sham: *P<0.05, **P<0.01. Significance vs WT TAC: †P<0.01 by ANOVA.

Data Supplement Figure 9. MLNs isolated from mice lacking CD8⁺ T cells (CD8KO) exhibit higher density of CD4⁺ T lymphocytes. (A) Quantification of cellular density of MLNs expressed as cell number per MLNs isolated from Sham and TAC operated mice (n=4-6). (B) Quantification by flow cytometry analysis of CD3⁺TCRβ⁺ double positive cells (defined T cells) and CD4⁺ T cells. Results were expressed in number of cells per MLNs. Values shown are mean±SEM. Significance vs Sham CD8KO: **P<0.01 by U test.

Data Supplement Figure 10. After 6 weeks of transverse aortic constriction (TAC), mice lacking CD8⁺ T cells (CD8KO) develop cardiac dilation and dysfunction compared to Sham. Echocardiographic parameters of WT Sham, WT TAC, CD8KO Sham and CD8KO TAC. IVSd indicates diastolic interventricular septal wall thickness; LVPWd, diastolic left posterior wall thickness; LVIDd, diastolic left ventricular dimension; LVIDs, systolic left ventricular dimension; EDV, end diastolic volume; ESV, end systolic volume; FS, fractional shortening and HR, heart rate. Values shown are mean±SEM. Significance vs Sham: *P<0.05, **P<0.01, ***P<0.001, Significance vs WT TAC: †P<0.05, ††P<0.01 by ANOVA. NS for not significant.

Data Supplement Figure 11. Absence of CD8⁺ T cell (CD8KO mice) prevents fetal genes activation induced by TAC. Cardiac mRNA expression of the atrial and brain natriuretic peptides (ANF, BNF) as well as the α and β myosin heavy chain (MYH6, MYH7) and the sarco(endo)plasmic reticulum Ca²⁺-ATPase (SERCA2a) in WT and CD8KO mice (Sham, n=4; TAC, n=8) mRNA expression was normalized to GAPDH and represented as fold change to WT Sham. Values shown are mean±SEM. Significance vs Sham: *P<0.05, **P<0.01, ***P<0.001; significance vs WT TAC: †P<0.05, ††P<0.01. Significance vs Sham WT: #P<0.05 by ANOVA.

Data Supplement Figure 12. Mice lacking CD8⁺ T cells (CD8KO) exhibit no modification of procollagen genes expression compared to WT after transverse aortic constriction (TAC). Cardiac mRNA expression of type I and III procollagens (Coll1a1 and Coll3a1) in WT and CD8KO
mice (n=4-8 per group). Data were normalized to GAPDH and calibrated to the average of WT Sham. Values shown are mean±SEM. Significance vs Sham: *P<0.05, **P<0.01 by ANOVA.

Data Supplement Figure 13. Mice lacking CD8+ T cells (CD8KO) exhibit no modification of collagen content and cross-linking after transverse aortic constriction (TAC) compared to WT TAC. (A) Quantification of total collagen deposition in cardiac tissue of WT and CD8KO mice submitted to TAC based on picrosirius red staining viewed under white light (n=4-8). (B) Representative cardiac tissue of CD8KO mice submitted to TAC, stained with picrosirius red staining and viewed under white (left) and polarized (right) light (x200). Values shown are mean±SEM.

Data Supplement Figure 14. After 6 weeks of transverse aortic constriction (TAC), OTII mice did not develop cardiac dilation and dysfunction compared to Sham. Echocardiographic parameters of WT Sham, WT TAC, OTII Sham and OTII TAC. IVSd indicates diastolic interventricular septal wall thickness; LVPWd, diastolic left posterior wall thickness; LVIDd, diastolic left ventricular dimension; LVIDs, systolic left ventricular dimension; EDV, end diastolic volume; ESV, end systolic volume; FS, fractional shortening and HR, heart rate. Values shown are mean±SEM. Significance vs Sham: *P<0.05, **P<0.01, ***P<0.001, Significance vs WT TAC: ††P<0.01, †††P<0.001 by ANOVA. NS for not significant.
Supplemental References