

CD28/B7 Deficiency Attenuates Systolic Overload-Induced Congestive Heart Failure, Myocardial and Pulmonary Inflammation, and Activated T Cell Accumulation in the Heart and Lungs

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Abstract—The inflammatory response regulates congestive heart failure (CHF) development. T cell activation plays an important role in tissue inflammation. We postulate that CD28 or B7 deficiency inhibits T cell activation and attenuates CHF development by reducing systemic, cardiac, and pulmonary inflammation. We demonstrated that chronic pressure overload-induced end-stage CHF in mice is characterized by profound accumulation of activated effector T cells (CD3⁺CD44^{high} cells) in the lungs and a mild but significant increase of these cells in the heart. In knockout mice lacking either CD28 or B7, there was a dramatic reduction in the accumulation of activated effector T cells in both hearts and lungs of mice under control conditions and after transverse aortic constriction. CD28 or B7 knockout significantly attenuated transverse aortic constriction-induced CHF development, as indicated by less increase of heart and lung weight and less reduction of left ventricle contractility. CD28 or B7 knockout also significantly reduced transverse aortic constriction-induced CD45⁺ leukocyte, T cell, and macrophage infiltration in hearts and lungs, lowered proinflammatory cytokine expression (such as tumor necrosis factor- α and interleukin-1 β) in lungs. Furthermore, CD28/B7 blockade by CTLA4-Ig treatment (250 μ g/mouse every 3 days) attenuated transverse aortic constriction-induced T cell activation, left ventricle hypertrophy, and left ventricle dysfunction. Our data indicate that CD28/B7 deficiency inhibits activated effector T cell accumulation, reduces myocardial and pulmonary inflammation, and attenuates the development of CHF. Our findings suggest that strategies targeting T cell activation may be useful in treating CHF. (*Hypertension*. 2016;68:688-696. DOI: 10.1161/HYPERTENSIONAHA.116.07579.) • [Online Data Supplement](#)

Key Words: congestive heart failure ■ heart ■ inflammation ■ leukocytes ■ lung ■ T-cell activation

The inflammatory response is believed to play an important role in the development of congestive heart failure (CHF). Increased myocardial leukocyte infiltration and elevated systemic and cardiac proinflammatory cytokine levels are commonly associated with CHF,^{1,2} and several of these factors, such as tumor necrosis factor- α and interleukin-1 β , have been demonstrated to accelerate left ventricle (LV) hypertrophy, cardiac fibrosis, and LV dysfunction.³⁻⁵ In addition to inflammation of myocardial tissue, severe CHF results in profound leukocyte infiltration in the lungs of experimental animals and CHF patients,⁶⁻⁹ provoking the development of type-2 pulmonary hypertension that drives the transition from LV failure to right ventricular hypertrophy and failure.

T cell activation plays a central role in inflammation.¹⁰ T cell activation requires at least 2 signals to become fully activated. The first signal occurs after the engagement of T cell

receptor by antigen-major histocompatibility complex proteins present on the surface of antigen-presenting cells. The second important signal is the engagement of costimulatory molecules (like CD28 or ICOS) on the T cell with one of the B7 proteins (B7.1 [CD80] and B7.2 [CD86]), which are also expressed on antigen-presenting cells. CD28 is a potent T-cell costimulator. Inhibition of CD28/B7 signaling with antibodies or genetic ablation of CD28 or B7.1/B7.2 in mice attenuates T-cell activation.^{10,11}

Previous studies have demonstrated that CD4⁺ T cells accumulate in the LV of mice with CHF and contribute to the pathogenesis of CHF.^{12,13} In this study, we found that end-stage CHF is associated with profound infiltration and accumulation of activated effector T cells (CD3⁺CD44^{high} cells) in the heart and lungs, as well as a significant increase in the percentage of effector memory CD4⁺ and CD8⁺ T cells (TEM; CD44^{high}CD62L^{low}

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T cells) in the lungs. To demonstrate a role for CD28 function in CHF development, we tested the hypothesis that transverse aortic constriction (TAC)-induced LV failure and lung remodeling would be attenuated in CD28 or B7 knockout (KO) mice. Our findings indicate that genetic disruption of either CD28 or B7 protein significantly attenuates TAC-induced cardiac and pulmonary inflammation, lung remodeling, as well as LV hypertrophy and dysfunction. The beneficial effect of CD28 or B7 deletion was associated with a dramatic reduction of TAC-induced T cell activation in the heart and lungs.

Materials and Methods

Detailed methods are available in the [online-only Data Supplement](#). Primary antibodies and primers used in this study are listed in Tables S1 and S2 in the [online-only Data Supplement](#).

Mice and TAC Procedure

Animals and Experimental Design

CD28 KO (B6.129S2-Cd28^{tm1Mak/J}; stock No 002666) and B7 KO (B6.129S4-Cd80^{tm1Shr} Cd86^{tm2Shr/J}; stock No. 003610) mice and C57BL/6J wild-type (WT) mice were obtained from Jackson Laboratory. The fusion protein CTLA4-Ig (abatacept, 250 µg), which inhibits CD28/B7 interactions, was administered intraperitoneally every 3 days beginning 1 day before TAC. An isotype control antibody human IgG was used as a vehicle control. Male mice 4 to 5 weeks of age were subjected to a procedure using a 27G needle to create the TAC, a model that mimics clinical systemic hypertension and aortic stenosis, as previously described.² Data were collected 8 weeks after TAC. LV hypertrophy and cardiac function were assessed. Experimental studies were approved by the Institutional Animal Care and Use Committee at the University of Minnesota.

Data Analysis

A normality test (Shapiro–Wilk) provided by SigmaPlot was used to determine whether data were normally distributed. If the data were normally distributed, they are presented as mean±SEM. Student's *t* test was used to test for differences between 2 groups. Two-way ANOVA followed by a Bonferroni correction post hoc test was used to test for differences among >2 groups. If the physiological data were not normally distributed or the sample size in one of the experimental groups was <10, the data are presented as median (quartiles). Nonparametric test (Mann–Whitney or Kruskal–Wallis) followed by the Bonferroni post hoc correction was performed. All pairwise *P* values are 2-sided. The null hypothesis was rejected at *P*<0.05.

Results

End-Stage CHF Caused a Significant Accumulation of Activated T Cells in Lungs

We examined T-cell activation and T-cell accumulation in hearts and lungs of mice with end-stage CHF produced by TAC. As shown in Figure S1A and S1B, ratios of LV weight and lung weight to body weight were increased 2.3-fold and 2.8-fold in CHF mice, respectively. Echocardiography showed that mice with TAC had a significant decrease of LV ejection fraction (EF; 36.0% in CHF versus 77.8% in control mice; Figure S1C), indicating the development of CHF.

Flow cytometry analysis showed that the percentage of activated effector T cells (CD3⁺CD44^{high} cells that display rapid effector function in inflamed peripheral tissues) was more than doubled in the lungs of CHF mice (27.2±1.7% in control versus 58.1±4.6% in CHF) but not changed in the LV (11.3±0.9% in control versus 11.5±0.6% in CHF; Figure S1D and S1E).

However, total numbers of CD3⁺ T cells were significantly increased in both LV tissues and lungs of CHF mice. Specifically, CD3⁺ T-cell infiltration was 1.4×10⁴ cells per LV and 178×10⁴ cells per lung in CHF mice (Figure S1F). In addition, CD45⁺ leukocyte infiltration was dramatically increased in CHF mice. CD45⁺ cell infiltration was 1.7×10⁵ cells per LV and 106×10⁵ cells per lung in CHF mice (Figure S1G). These data indicate that T-cell activation and accumulation at end-stage CHF are dramatic in the lung, but relatively mild in the LV. Moreover, CHF mice also exhibited a significantly increased spleen weight and an increased percentage of TEM within the CD4⁺ T cell subset in splenocytes and peripheral blood cells (Figures S2 and S3). Flow cytometry gating strategies are presented in Figures S4–S7.

CD28 Deficiency Attenuated TAC-Induced CHF Development

To determine whether T-cell activation affects cardiopulmonary adaptation to LV pressure overload, WT and CD28 KO mice were examined under control conditions and 8 weeks after TAC. LV systolic pressure was similar in WT and CD28 KO mice under control conditions (96.3±9.9 mmHg in WT-control versus 96.5±2.6 mmHg in CD28 KO-control). TAC significantly increased LV systolic pressure after 8 weeks, which was comparable in WT and CD28 KO mice (165.2±9.9 mmHg in WT-TAC versus 175.3±4.7 mmHg in CD28 KO-TAC; Figure S8A and S8B). Under control conditions, CD28 deficiency had no detectable effect on the ratio of LV, left atria, or lung weight to body weight (Figure 1A–1C; Table S3). Echocardiographic measurements showed that CD28 deficiency did not affect LV function under control conditions (Figure 1D–1F). These data indicate that CD28 deficiency has no detectable effect on cardiac structure or function under control conditions.

TAC caused severe LV hypertrophy (3.6±0.2 mg/kg in WT-control versus 7.5±0.3 mg/kg in WT-TAC) and markedly decreased LVEF (78.1±2.6% in WT-control versus 32.1±3.2% in WT-TAC) in WT mice (Figure 1A and 1D). CD28 deficiency significantly attenuated the TAC-induced LV hypertrophy (7.5±0.3 mg/kg in WT-TAC versus 5.6±1.1 mg/kg in CD28 KO-TAC), increase of left atria weight (0.84±0.18 mg/kg in WT-TAC versus 0.17±0.04 mg/kg in CD28 KO-TAC), and increase of lung weight (10.5±1.3 mg/kg in WT-TAC versus 7.1±1.4 mg/kg in CD28 KO-TAC; Figure 1A–1C; Table S3). Echocardiographic measurements showed that CD28 deficiency significantly attenuated the TAC-induced decrease of LVEF (32.1±3.2% in WT-TAC versus 64.5±14.3% in CD28 KO-TAC), increase of LV end-systolic diameter (4.1±0.2 mm in WT-TAC versus 2.5±0.6 mm in CD28 KO-TAC), and increase of LV end-diastolic diameter (4.9±0.1 mm in WT-TAC versus 3.8±0.9 mm in CD28 KO-TAC; Figure 1D–1F). CD28 deficiency also attenuated the TAC-induced increase of LV end-diastolic pressure (18.4±3.3 mmHg in WT-TAC versus 3.9±1.1 mmHg in CD28 KO-TAC), decrease of LV maximum rate of rise of LV systolic pressure (dP/dt_{max} , 5997±827 mmHg/s in WT-TAC versus 8038±1797 mmHg/s in CD28 KO-TAC), and increase of LV minimum rate of decline of LV systolic pressure (dP/dt_{min} , -5943±864 mmHg/s in WT-TAC versus -8930±2005 mmHg/s in CD28 KO-TAC; Figure 1G–1I). Moreover, CD28 deficiency reduced TAC-induced increases of myocardial atrial natriuretic peptide and β-myosin heavy chain

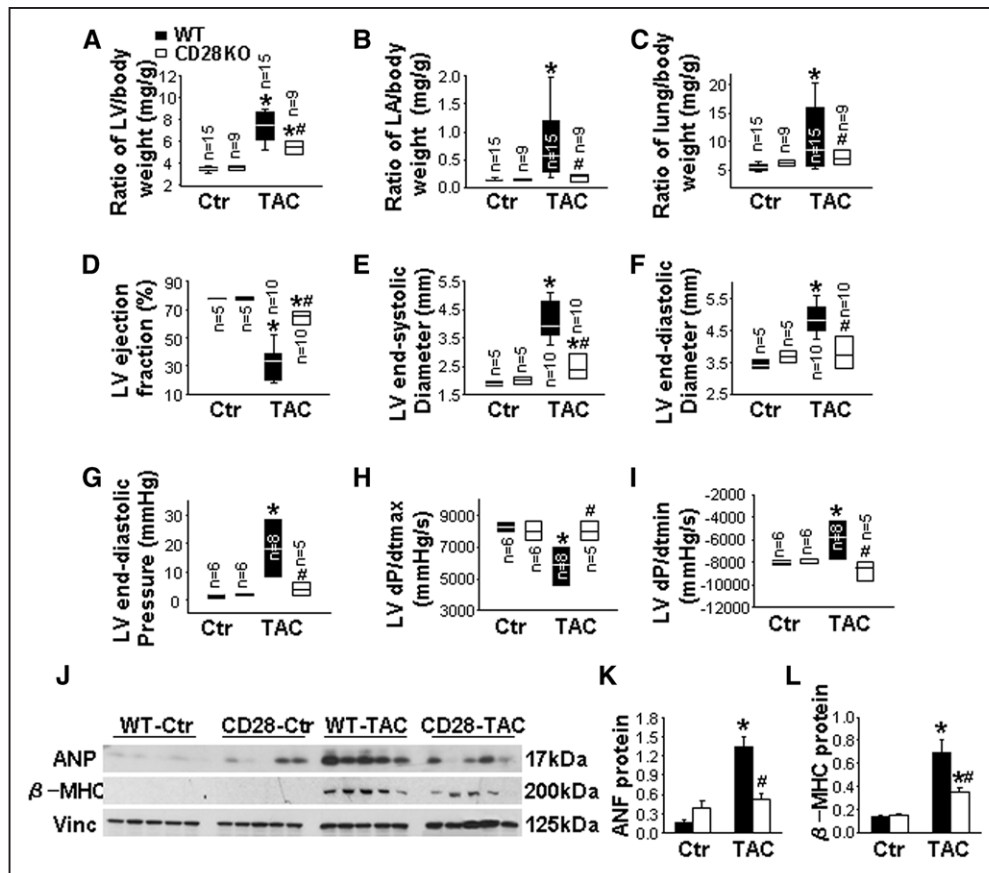


Figure 1. CD28 deficiency attenuates transverse aortic constriction (TAC)-induced cardiac dysfunction and pulmonary congestion. Data were collected from wild-type (WT) and CD28 knockout (CD28 KO) mice under control conditions (Ctr) or 8 weeks after TAC. **A–C,** The ratio of left ventricle (LV), left atria (LA), and lung weight to body weight of mice. **D–F,** Echocardiographic measurements of LV ejection fraction, LV end-systolic diameter, and LV end-diastolic diameter from hearts. **G–I,** Hemodynamics of LV end-diastolic pressure, LV maximum rate of rise of pressure (dP/dt_{max}), and LV maximum rate of decline of pressure (dP/dt_{min}) from hearts. **J–L,** Western blot of atrial natriuretic peptide (ANP), β -myosin heavy chain (β -MHC), and vinculin (Vinc; loading control) in LV lysates of mice. * $P<0.05$ vs control group; # $P<0.05$ vs TAC group of WT mice.

(β -MHC) protein content (Figure 1J–L). Together, these data indicate that CD28 deficiency protects hearts against TAC-induced LV hypertrophy and CHF.

B7 Deficiency Attenuated TAC-Induced CHF Development

Because CD28 deficiency attenuated the TAC-induced LV hypertrophy and dysfunction, we used B7 KO mice to examine the effect of B7 deficiency on TAC-induced CHF. LV systolic pressure was comparable in WT and B7 KO mice under control conditions, as well as 8 weeks after TAC (Figure S8C and S8D). We found that B7 deficiency had no effect on the ratio of LV, left atria, or lung weight to body weight under control conditions (Figure 2A–C; Table S4). Echocardiographic measurements showed that B7 deficiency did not affect LV function under control conditions (Figure 2D–F).

B7 deficiency significantly attenuated the TAC-induced LV hypertrophy (6.9 ± 0.5 mg/kg in WT-TAC versus 5.1 ± 1.0 mg/kg in B7 KO-TAC), increase of left atria weight (0.64 ± 0.16 mg/kg in WT-TAC versus 0.15 ± 0.03 mg/kg in B7 KO-TAC), and increase of lung weight (8.6 ± 1.1 mg/kg in WT-TAC versus 4.9 ± 1.1 mg/kg in B7 KO-TAC; Figure 2A–C; Table S4). Echocardiographic measurements showed that B7

deficiency significantly attenuated the TAC-induced decrease of LVEF ($48.4\pm 6.2\%$ in WT-TAC versus $67.5\pm 11.8\%$ in B7 KO-TAC), increase of LV end-systolic diameter (3.4 ± 0.4 mm in WT-TAC versus 2.4 ± 0.4 mm in B7 KO-TAC), and increase of LV end-diastolic diameter (4.5 ± 0.5 mm in WT-TAC versus 3.8 ± 0.7 mm in B7 KO-TAC; Figure 2D–F). In addition, B7 deficiency also attenuated the TAC-induced increase of LV end-diastolic pressure (21.3 ± 6.1 mmHg in WT-TAC versus 4.7 ± 0.9 mmHg in B7 KO-TAC), decrease of LV dP/dt_{max} (5567 ± 1426 mmHg/s in WT-TAC versus 8730 ± 218 mmHg/s in B7 KO-TAC), and increase of LV dP/dt_{min} (-5579 ± 1491 mmHg/s in WT-TAC versus -8788 ± 1290 mmHg/s in B7-TAC; Figure 2G–I). Moreover, B7 KO significantly reduced the TAC-induced increases of myocardial atrial natriuretic peptide and β -myosin heavy chain protein expression (Figure 2J–L). These data indicate that B7 deficiency protects hearts against TAC-induced LV dysfunction and CHF.

CD28 or B7 Deficiency Inhibited CD3⁺ T-Cell Activation and Accumulation in Hearts and Lungs of Mice After TAC

To determine the effect of CD28/B7 signaling on T-cell activation and inflammation, we assessed changes in the percentage and

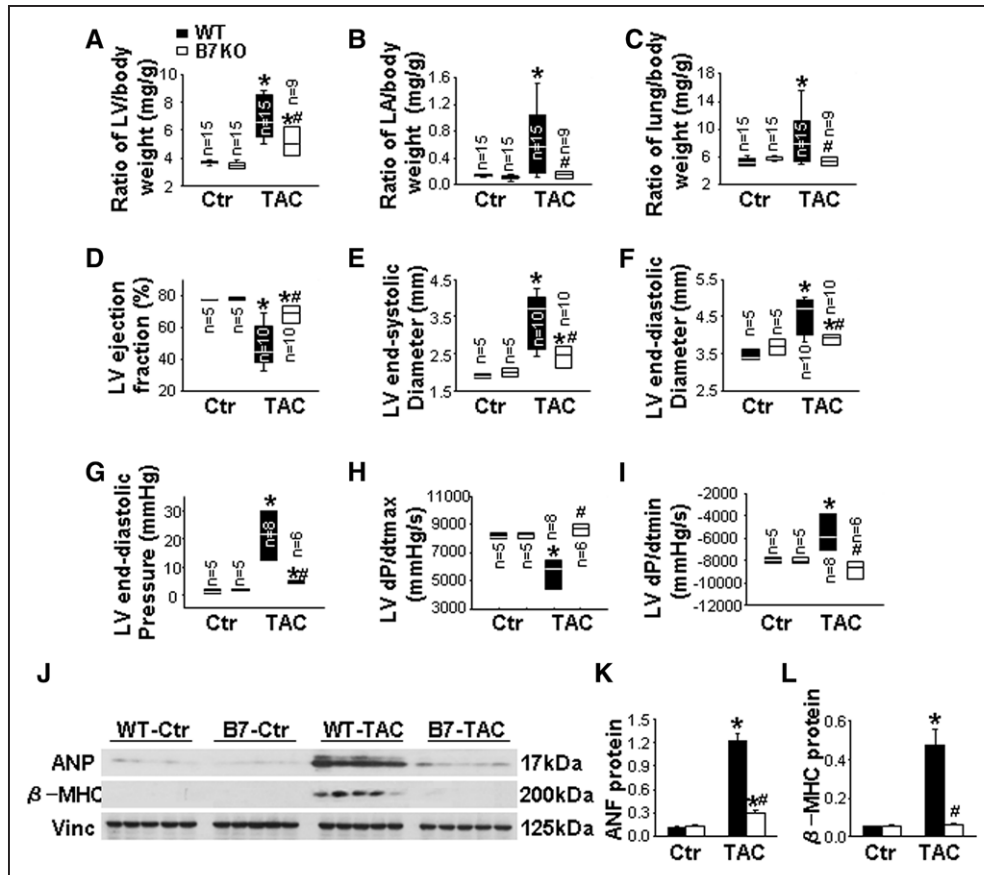


Figure 2. B7 deficiency attenuates transverse aortic constriction (TAC)-induced cardiac dysfunction and pulmonary congestion. Data were collected from wild-type (WT) and B7 knockout (B7 KO) mice under control conditions (Ctr) or 8 weeks after TAC. **A–C,** The ratio of left ventricle (LV), left atria (LA), and lung weight to body weight of mice. **D–F,** Echocardiographic measurements of LV ejection fraction, LV end-systolic diameter, and LV end-diastolic diameter from hearts. **G–I,** Hemodynamics of LV end-diastolic pressure, LV maximum rate of rise of pressure (dP/dt_{max}), and LV maximum rate of decline of pressure (dP/dt_{min}) from hearts. **J–L,** Western blot of atrial natriuretic peptide (ANP), β -myosin heavy chain (β -MHC), and vinculin (Vinc; loading control) in LV lysates of mice. * $P < 0.05$ vs control group; # $P < 0.05$ vs TAC group of WT mice.

number of CD3⁺ T cells, activated effector T cells, and early activated T cells (CD3⁺CD69⁺ cells) in LV tissues and lungs using flow cytometry analysis. The gating strategies are presented in Figures S4 and S5. Under control conditions, CD28 or B7 KO significantly reduced the percentage of activated effector T cells in both LV tissues (11.3 $\pm$ 0.9% in WT-control, 8.7 $\pm$ 0.4% in CD28 KO-control, and 7.4 $\pm$ 0.4% in B7 KO-control) and lungs (28.7 $\pm$ 1.2% in WT-control, 21.0 $\pm$ 1.7% in CD28 KO-control, and 22.3 $\pm$ 1.0% in B7 KO-control). TAC significantly increased the percentage of activated effector T cells in lungs, but did not increase the percentage of these cells in LV tissues. Notably, CD28 or B7 deficiency significantly attenuated the TAC-induced increase of the percentage of activated effector T cells in lungs (54.2 $\pm$ 5.2% in WT-TAC, 22.0 $\pm$ 1.1% in CD28 KO-TAC, and 24.2 $\pm$ 2.1% in B7 KO-TAC) and in LV tissues (11.5 $\pm$ 0.6% in WT-TAC, 8.6 $\pm$ 0.7% in CD28 KO-TAC, and 7.0 $\pm$ 0.6% in B7 KO-TAC) after TAC (Figure 3A–3C). Moreover, CD28 or B7 deficiency considerably reduced TAC-induced accumulation of activated effector T cells in both LV tissues and lungs (Figure 3D). CD28 KO or B7 deficiency also significantly reduced TAC-induced increases of early activated T cells in LV tissues and lungs (Figure S9). In addition, CD28 or B7 deficiency significantly attenuated TAC-induced increases in total CD3⁺ T cells in lungs (10.6 $\pm$ 1.5 $\times 10^5$ in

WT-TAC, 5.9 $\pm$ 0.96 $\times 10^5$ in CD28 KO-TAC, and 6.3 $\pm$ 0.35 $\times 10^5$ in B7 KO-TAC), but did not significantly affect TAC-induced CD3⁺ T-cell accumulation in LV tissues (Figure 3E). Furthermore, CD28 or B7 deficiency had no effect on the total number of CD45⁺ cells in LV tissues (34.0 $\pm$ 1.9 $\times 10^3$ in WT-control, 28.1 $\pm$ 0.7 $\times 10^3$ in CD28 KO-control, and 37.3 $\pm$ 6.0 $\times 10^3$ in B7 KO-control) or lungs (3041 $\pm$ 63 $\times 10^3$ in WT-control, 2901 $\pm$ 77 $\times 10^3$ in CD28 KO-control, and 3130 $\pm$ 111 $\times 10^3$ in B7 KO-control) under control conditions. However, CD28 or B7 deficiency significantly attenuated TAC-induced increases of CD45⁺ cells in LV tissues (18.3 $\pm$ 2.1 $\times 10^4$ in WT-TAC, 9.0 $\pm$ 1.13 $\times 10^4$ in CD28 KO-TAC, and 10.9 $\pm$ 0.66 $\times 10^4$ in B7 KO-TAC) and lungs (683 $\pm$ 85 $\times 10^4$ in WT-TAC, 37.4 $\pm$ 2.47 $\times 10^4$ in CD28 KO-TAC, and 33.20 $\pm$ 1.48 $\times 10^4$ in B7 KO-TAC; Figure 3F). Together, these data demonstrate that pressure overload causes T-cell activation in lungs in mice with end-stage CHF. CD28 or B7 deficiency is effective in attenuating CD3⁺ T-cell activation, as well as CD3⁺ T-cell and CD45⁺ cell accumulation in LV tissues and lungs in mice after TAC.

CD28 or B7 Deficiency Attenuated TAC-Induced LV Fibrosis

Inflammation causes cardiac fibrosis, which exacerbates LV hypertrophy and CHF development. As shown by Sirius red/

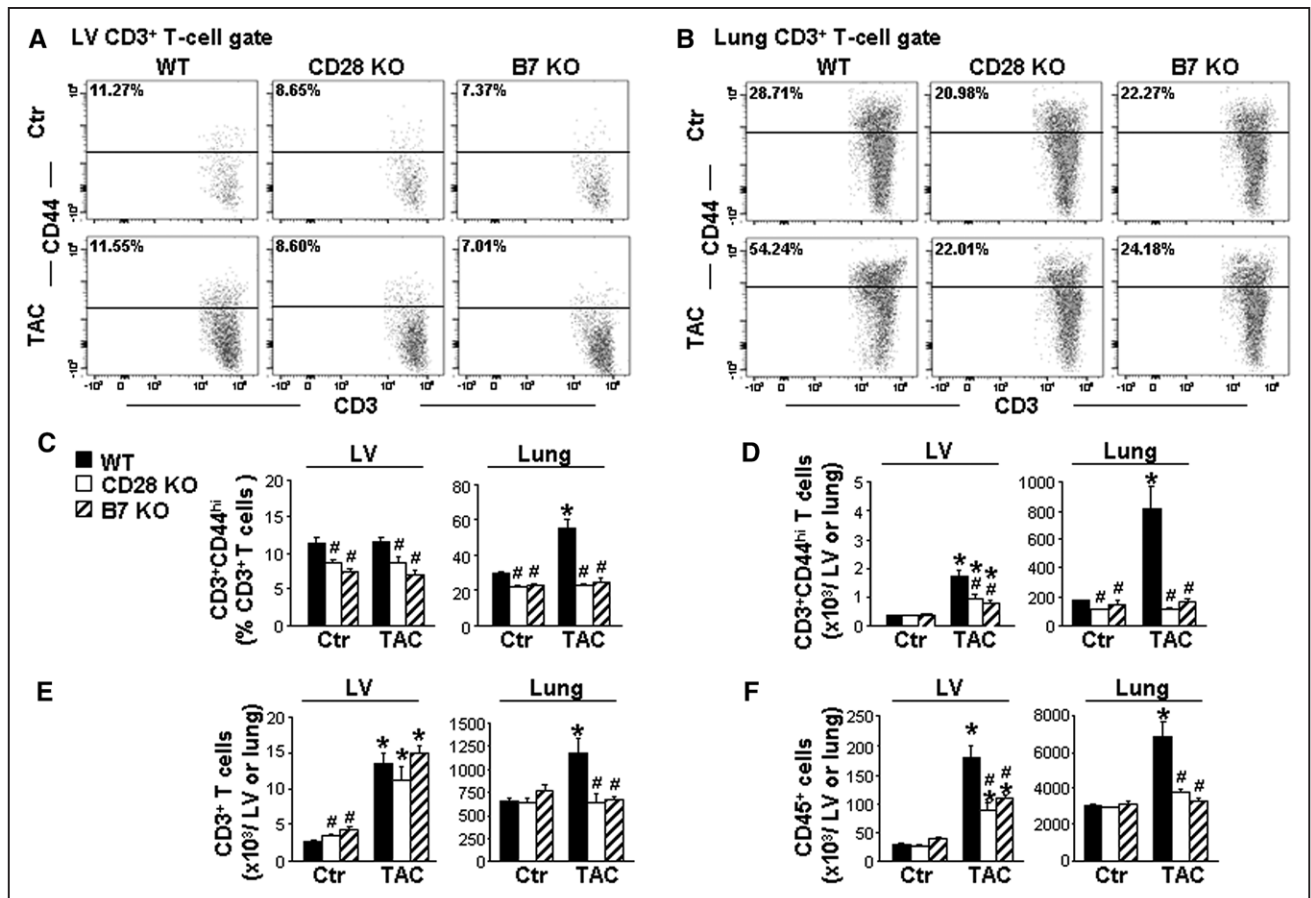


Figure 3. CD28 or B7 deficiency inhibits CD3⁺ T-cell activation and leukocyte accumulation in the left ventricle (LV) and lungs after transverse aortic constriction (TAC). Flow cytometry data were collected from wild-type (WT) and CD28 or B7 knockout (KO) mice under control conditions (Ctr) or 8 weeks after TAC. **A–C**, Flow cytometry plots and quantitative data represent the percentage of CD3⁺CD44^{hi} T cells (activated effector T cells) in LV tissues and lungs. **D–F**, Quantitative data represent total numbers of CD3⁺CD44^{hi} T cells, CD3⁺ T cells, and CD45⁺ cells in LV tissues and lungs. n=5 to 6 per group. *P<0.05 vs control group; #P<0.05 vs TAC group of WT mice.

Fast green staining in Figure S10A and S10B, CD28 or B7 deficiency did not affect LV fibrosis under control conditions. However, CD28 or B7 deficiency significantly attenuated TAC-induced LV fibrosis. CD28 or B7 deficiency also significantly reduced TAC-induced increases in LV mRNA content of transforming growth factor- β , a profibrogenic cytokine associated with fibrosis development (Figure S10C). These data demonstrate that CD28/B7 signaling contributes to pressure overload-induced LV fibrosis.

CD28 or B7 Deficiency Inhibited TAC-Induced T-Cell Activation and Accumulation in Both CD4⁺ and CD8⁺ Subsets in Lungs

Because CD28 or B7 deficiency significantly attenuated the TAC-induced CD3⁺ T-cell activation and accumulation in lungs, we further determined the effect of CD28 or B7 deficiency on the activation and accumulation of CD4⁺ and CD8⁺ T cells. As shown in Figure 4A–4C and 4E, under control conditions, CD28 or B7 deficiency resulted in an $\approx$ 50% decrease in the percentage of TEM within the CD4⁺ or CD8⁺ T-cell subset. TAC significantly increased the percentage of CD4⁺ and CD8⁺ TEM (2.2- and 1.8-fold, respectively). CD28 or B7 deficiency totally abolished the TAC-induced increases in the percentage of CD4⁺ TEM and largely reduced the increase in

the percentage of CD8⁺ and CD3⁺ TEM (Figure 4A–4C and 4E; Figure S11A). In addition, CD28 or B7 deficiency markedly attenuated TAC-induced increases in CD4⁺, CD8⁺, and CD3⁺ TEM in lungs of mice (Figure 4D and 4F; Figure S11B). Moreover, CD28 or B7 deficiency abolished the TAC-induced increase in the percentage of early activated T cells in their corresponding CD4⁺ and CD8⁺ T-cell subset and their total numbers in lungs (Figure S12). CD28 or B7 deficiency also significantly reduced the TAC-induced increases in CD4⁺ and CD8⁺ activated effector T cells (Figure S13). These data indicate that CD28 or B7 deficiency is highly effective in inhibiting pressure overload-induced T-cell activation in both CD4⁺ and CD8⁺ subsets in lungs.

CD28 or B7 Deficiency Attenuated TAC-Induced Lung Macrophage Infiltration and Proinflammatory Cytokine Expression

Because activated T cells regulate macrophage function, we further determined whether CD28 or B7 deficiency affected the number of lung macrophages expressing the macrophage marker F4/80 (Figure S5). Under control conditions, the percentage and total number of macrophages were not altered in either CD28 KO or B7 KO mice (Figure 5A and 5B). TAC significantly increased the percentage and total number of

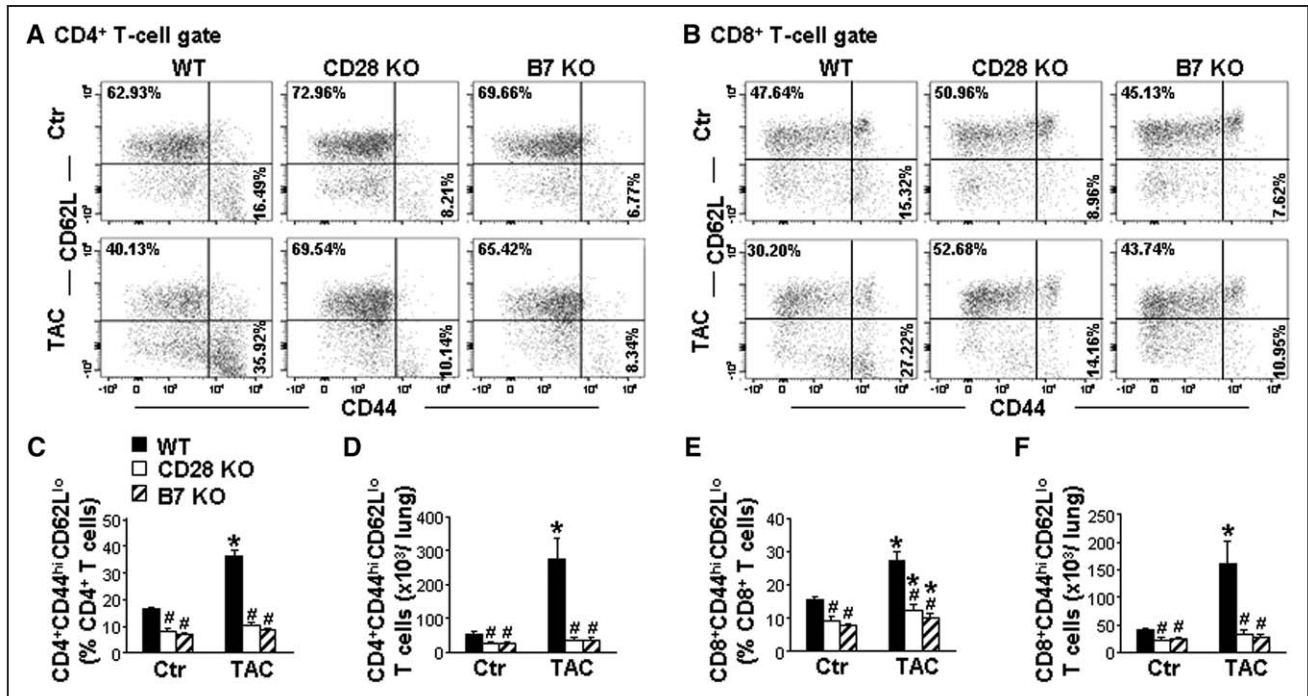


Figure 4. CD28 or B7 deficiency inhibits transverse aortic constriction (TAC)-induced CD4⁺ and CD8⁺ T-cell activation and accumulation in the lung. Flow cytometry data of lungs were collected from wild-type (WT) and CD28 or B7 knockout (KO) mice under control conditions (Ctr) or 8 weeks after TAC. **A** and **C**, Flow cytometry plots and quantitative data represent the percentage of CD44^{hi}CD62L^{lo} T cells (effector memory T cells; TEM) within the CD4⁺ population of lungs. **D**, Total numbers of CD4⁺CD44^{hi}CD62L^{lo} T cells in lungs. **B** and **E**, Flow cytometry plots and quantitative data represent the percentage of CD44^{hi}CD62L^{lo} T cells within the CD8⁺ population of lungs. **F**, Total numbers of CD8⁺CD44^{hi}CD62L^{lo} T cells in lungs. n=5 to 6 per group. **P*<0.05 vs control group; #*P*<0.05 vs TAC group of WT mice.

macrophages in the lung, which was notably reduced in CD28- and B7-deficient mice (Figure 5A and 5B). Furthermore, CD28 or B7 deficiency also significantly suppressed TAC-induced increases of lung mRNA levels of interleukin-1 β , tumor necrosis factor- α , and monocyte chemoattractant protein-1 (Figure 5C–5E). These data demonstrate that CD28 or B7 deficiency is effective in attenuating TAC-induced lung macrophage infiltration and proinflammatory cytokine expression.

CTLA4-Ig Attenuated TAC-Induced T-Cell Activation and LV Dysfunction

To further determine whether inhibition of T-cell activation can attenuate CHF development, the fusion protein CTLA4-Ig, which attenuates CD28/B7 signaling, was administered to the mice before TAC. TAC markedly increased LV systolic pressure after 8 weeks, which was comparable in CTLA4-Ig-treated and vehicle-treated mice (Figure S14A). CTLA4-Ig treatment significantly reduced the TAC-induced increases in the percentage of CD4⁺ TEM (17.1 $\pm$ 0.9% in control, 22.7 $\pm$ 1.4% in TAC-vehicle, and 14.2 $\pm$ 1.7% in TAC-CTLA4-Ig; Figure S14B), as well as the percentage of CD4⁺CD69⁺ T cells in lungs (Figure S14C), indicating CTLA4-Ig treatment inhibited T-cell activation in our experimental setting.

CTLA4-Ig treatment significantly attenuated the TAC-induced LV hypertrophy (3.25 $\pm$ 0.05 mg/kg in control, 5.66 $\pm$ 0.21 mg/kg in TAC-vehicle, and 4.72 $\pm$ 0.25 mg/kg in TAC+CTLA4-Ig; Figure 6A). Moreover, echocardiographic measurements showed that CTLA4-Ig treatment also attenuated the TAC-induced decrease of LVEF (74.6 $\pm$ 1.2% in control, 52.4 $\pm$ 5.3% in TAC-vehicle, and 70.9 $\pm$ 1.5% in TAC-CTLA4-Ig)

and increase of LV end-systolic diameter (2.2 $\pm$ 0.1 mm in control, 3.0 $\pm$ 0.3 mm in TAC-vehicle, and 2.2 $\pm$ 0.1 mm in TAC-CTLA4-Ig; Figure 6B and 6C). LV end-diastolic diameter was not altered (Figure 6D). These data indicate that inhibition of CD28/B7 signaling by CTLA4-Ig treatment protects hearts against TAC-induced LV hypertrophy and dysfunction.

Discussion

This study demonstrated that LV failure produced by chronic pressure overload caused an increase in total number of activated effector T cells and TEM in the LV and lungs. Using CD28/B7 KO mice or CTLA4-Ig to block CD28/B7 interactions, we also demonstrated that intact CD28/B7 signaling contributed significantly to TAC-induced LV hypertrophy and dysfunction, lung remodeling, and right ventricular hypertrophy. CD28/B7 interaction strongly enhanced myocardial and pulmonary infiltration of activated T cells and CD45⁺ leukocytes and resulted in increased fibrosis and cytokine expression in these tissues. These findings suggest that T-cell activation contributes to CHF development and that inhibition of T-cell activation may be useful in attenuating CHF development and progression by suppressing inflammatory responses.

The increased T-cell accumulation in LV and lung tissues in CHF mice seems to be a result of increased antigen-specific T-cell recruitment as well as nonspecific T-cell recruitment secondary to the proinflammatory environment in these tissues. The increased adhesion molecules (such as E-selectin and P-selectin) and chemokines on the resident cells and the selectin ligands and chemokine receptors on T cells contribute to the T-cell accumulation in these tissues. Increased selectin ligands

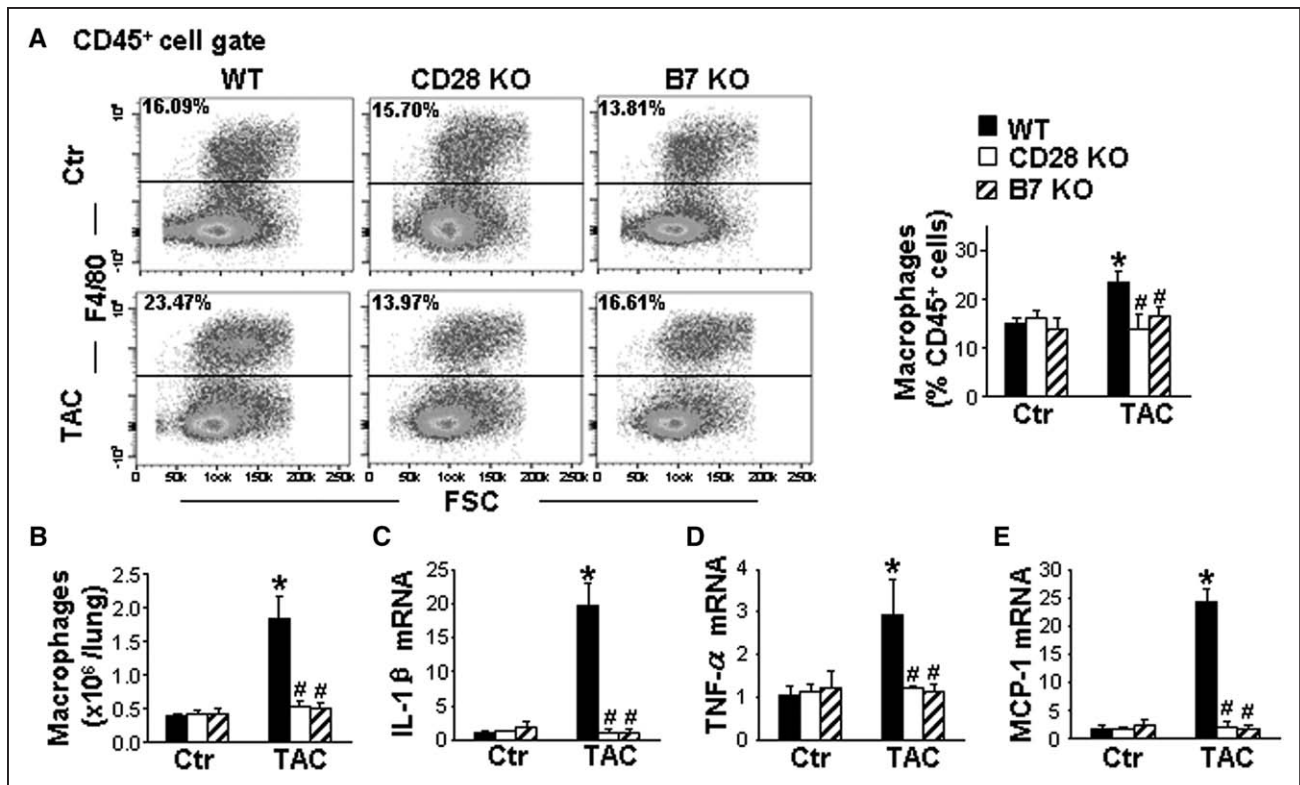


Figure 5. CD28 or B7 deficiency attenuates transverse aortic constriction (TAC)-induced lung macrophage infiltration and proinflammatory cytokine expression. Data were collected from lungs of wild-type (WT) and CD28 or B7 knockout (KO) mice under control conditions (Ctr) or 8 weeks after TAC. **A**, Flow cytometry plots and quantitative data represent the percentage of macrophages (F4/80⁺) in the lung. **B**, Quantitative data of flow cytometry represent the total number of macrophages in the lung. **C–E**, Quantitative reverse transcription polymerase chain reaction (RT-PCR) results of interleukin (IL)-1 β , tumor necrosis factor- α (TNF- α), and monocyte chemoattractant protein-1 (MCP-1) mRNA levels in lung lysates. $n=5$ to 6 per group. * $P<0.05$ vs control group; # $P<0.05$ vs TAC group of WT mice.

and chemokine receptors during T-cell differentiation¹⁴ are known to promote T-cell recruitment into the inflamed sites.¹⁵ In addition, antigen-presenting cells (such as dendritic cells and macrophages) might also enhance antigen-specific T-cell recruitment by presenting resident cell debris or through other signaling mechanisms.¹⁶ Recent studies from Dr Harrison and his associates have demonstrated that oxidized self-antigen(s) contributes to Angiotensin II-induced T-cell activation and hypertension in mice.^{17,18} In the context that oxidative stress is increased during CHF development, oxidized proteins in heart and lungs are likely the contributors to the TAC-induced T-cell activation. However, it is not clear what endogenous antigen(s) mediates T-cell activation in CHF.

CHF dramatically increased the total number and the percentage of activated effector T cells in the lungs. To our surprise, end-stage CHF caused only a mild increase in the total number of activated effector T cells in LV tissues and did not affect the percentage of these cells. The accumulation of activated effector T cells in LV tissues and lungs in mice with CHF indicate that these tissues emit TAC-induced signals that promote T-cell infiltration or promote tissue-specific T-cell priming during CHF development. The profound increase of leukocytes, macrophages, T cells, and activated T cells in the present study is consistent with our previous observation using the same CHF model.^{13,19} The significant increase of activated effector T cells in peripheral blood and spleen in CHF mice indicates that T-cell

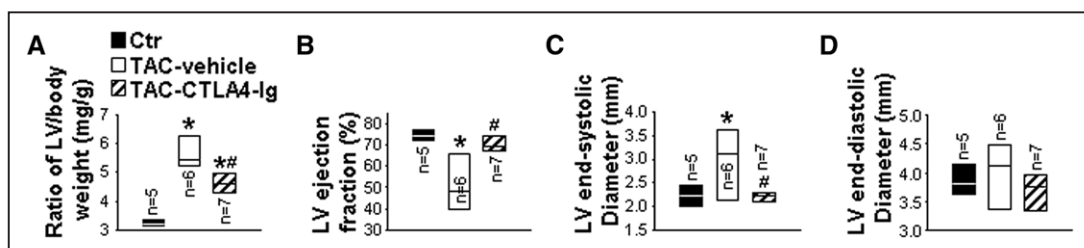


Figure 6. CD28/B7 blockade attenuates transverse aortic constriction (TAC)-induced cardiac hypertrophy and dysfunction. Data were collected from mice under basal conditions (Ctr) or treated with human-Ig (vehicle) or CTLA4-Ig under TAC conditions. **A**, The ratio of left ventricle (LV) and body weight of mice. **B–D**, Echocardiographic measurements of LV ejection fraction, LV end-systolic diameter, and LV end-diastolic diameter from hearts. * $P<0.05$ vs control group; # $P<0.05$ vs TAC group of vehicle mice.

activation is a systemic response during CHF development. Our findings that CHF caused a significant increase in the percentage of activated effector T cells in the lungs, but unchanged percentage of these cells in the LV, suggest that T-cell activation has a greater impact on pulmonary inflammation than on LV inflammation in animals with end-stage CHF.

Because previous studies demonstrated that T cells contribute to the pathogenesis of CHF and that CD4⁺ T cells promote the transition from LV hypertrophy to heart failure,^{12,13} the dramatic increase in CD4⁺ TEM might promote TAC-induced CHF progression by modulating lung inflammatory responses. The reduced accumulation of TEM in LV tissues and lungs by CD28/B7 deficiency or CD28/B7 blockade after TAC is in agreement with the reports that T-cell activation contributes to tissue inflammation in other disease models, such as inflammatory responses in skin diseases,²⁰ organ transplant-induced inflammation and rejection,^{21–24} and cardiovascular diseases, including hypertension and atherosclerosis.^{25,26} Our finding supports an important role of T-cell activation in CHF development and progression and a critical role for CD28/B7 signaling in regulating T-cell activation during CHF development.

Although CD28 or B7 deficiency completely abolished the TAC-induced increases in CD4⁺ TEM, we observed only a partial attenuation of the TAC-induced increases in CD8⁺ TEM in CD28 KO or B7 KO mice. The finding that CD28 or B7 deficiency led to a stronger inhibition of T-cell activation in CD4⁺ T cells than in CD8⁺ T cells is likely related to the constitutive expression of CD28 on CD4⁺ T cells and a relatively high abundance of CD28 expression on CD4⁺ T cells as compared with that on CD8⁺ T cells in mice.²⁷ The finding that CD28 or B7 deficiency only partially inhibited the TAC-induced tissue inflammatory responses in LV tissues and lungs suggests that additional proinflammatory signaling pathways independent of CD4⁺ and CD8⁺ T-cell activation may also play important roles in CHF-associated inflammatory responses and CHF development.

The present study has several limitations. First, both CD28 and B7 deficiency significantly attenuated TAC-induced LV hypertrophy and dysfunction, as well as tissue inflammation. The dramatically reduced TAC-induced lung inflammation and remodeling in CD28- and B7-deficient mice is likely to be a collective effect of the reduced cardiac, pulmonary, and systemic T-cell activation in these mice. However, we are unable to determine the relative contribution of cardiac, pulmonary, and systemic T-cell activation in the CHF progression and lung remodeling. In addition, because global CD28 KO and B7 KO mouse strains were used for our study, some of the observed phenotypes may be a result of chronic adaptation to the global gene deletion. Furthermore, the present study could not determine whether the cardioprotective effects in CD28 KO and B7 KO mice are because of CD28 and B7 gene deletion in leukocytes or in other cell types.

In summary, we demonstrate that CD28/B7 signaling contributes significantly to chronic pressure overload-induced cardiac and pulmonary inflammation and the development of CHF. Our study suggests that strategies that target CD28/B7 signaling may be useful in treating CHF.

Perspectives

Congestive heart failure (CHF) is accompanied by increased leukocyte accumulation and increased inflammatory cytokine expression in hearts and lungs, which exacerbates the development and progression of CHF. The interaction of the CD28 coreceptor with B7 proteins regulates T-cell activation. In this article, we demonstrate that CD28 or B7 deficiency attenuates systolic overload-induced CHF development by reducing cardiac and pulmonary inflammation and remodeling. Our study provides direct evidence that strategies targeting T-cell activation may be useful in treating CHF.

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Disclosures

None.

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Novelty and Significance

What Is New?

- CD28/B7 deficiency inhibits effector T-cell accumulation, reduces myocardial and pulmonary inflammation, and attenuates the development of congestive heart failure.

What Is Relevant?

- Congestive heart failure development is associated with T-cell activation-involved inflammatory responses. Thus, inhibition of T-cell activation by blocking CD28/B7 signaling may be a promising strategy in treating congestive heart failure.

Summary

CD28/B7 signaling contributes to chronic pressure overload-induced cardiac and pulmonary inflammation and the development of congestive heart failure, suggesting that strategies targeting T-cell activation may be useful for congestive heart failure treatment.