

ORIGINAL RESEARCH ARTICLE

Engineered Regulatory T Lymphocytes Promote Infarcted Heart Repair

Min Zhang, MD, PhD*; Yongying Qin^{ID}, MD, PhD*; Ting Zhou^{ID}, MD, PhD; Meilin Liu, MD, PhD; Tingting Tang^{ID}, MD, PhD; Ni Xia, MD, PhD; Shaofang Nie^{ID}, MD, PhD; Bingjie Lv, MD, PhD; Zhengfeng Zhu, MD, PhD; Jiao Jiao, MD, PhD; Muyang Gu, MD, PhD; Jingyong Li^{ID}, MD, PhD; Chen Chen^{ID}, MD, PhD; Desheng Hu^{ID}, MD, PhD; Weimin Wang^{ID}, MD, PhD; Li Zhang^{ID}, MD, PhD; Chaolong Wang^{ID}, MD, PhD; Zhilei Shan^{ID}, MD, PhD; Xiang Cheng^{ID}, MD, PhD

BACKGROUND: Myocardial infarction (MI) initiates a dysregulated healing process characterized by excessive fibrosis and unresolved inflammation, resulting in suboptimal cardiac repair in clinical settings. Regulatory T lymphocytes (Tregs) naturally orchestrate cardiac repair after MI, but their therapeutic potential is limited by inefficient homing to ischemic myocardium. We hypothesize that FAP (fibroblast activation protein)-specific CAR (chimeric antigen receptor) engineering overcomes this barrier by enabling precise delivery of Tregs to FAP⁺-enriched infarct zones, thereby focally amplifying reparative activity within injured myocardium.

METHODS: In murine MI and ischemia–reperfusion models, C57BL/6J mice were injected with lentivirus-engineered FAP CAR Tregs (FCTRs) or mock Tregs derived from wild-type, IL-10 (interleukin-10) knockout (*IL-10*^{-/-}) or Areg (amphiregulin) knockout (*Areg*^{-/-}) donors after infarction. The cardiac outcomes and underlying mechanisms mediated by FCTRs were thoroughly analyzed. Systemic toxicity was evaluated to ensure safety.

RESULTS: Intravenous injections of FCTRs on day 3 after injury led to targeted engraftment in the damaged cardiac tissue. Compared with controls treated with vehicle or mock Tregs, mice receiving FCTRs exhibited remarkable cardiac functional recovery in both MI and ischemia–reperfusion models by day 14, accompanied by reduced fibrosis and decreased inflammation, all achieved without compromising the integrity of cardiac tissue. Absence of IL-10 in the engineered CAR Tregs abrogated their therapeutic efficacy, whereas the ablation of Areg showed no functional impairment. We further demonstrated that the beneficial effects of FCTRs depended on IL-10 production, which inhibited pathogenic myofibroblast differentiation by suppressing Smad2/3-dependent signaling. In addition, IL-10 secretion by these engineered Tregs promoted the polarization of inflammatory monocytes into reparative M2 macrophages and resolved excessive inflammatory responses. No treatment-related adverse effects were observed.

CONCLUSIONS: We pioneered FAP-targeted CAR Tregs as a dual-action precision therapy resolving post-MI fibrosis and inflammation through IL-10-dependent mechanisms. By spatiotemporally suppressing myofibroblast differentiation and remodeling immune niches, this strategy prevents maladaptive remodeling while accelerating functional recovery, establishing a translational platform for fibrotic diseases across organ systems.

Key Words: interleukin-10 ■ myocardial infarction ■ receptors, chimeric antigen ■ T-lymphocytes, regulatory

Editorial, see p XXX

Myocardial infarction (MI) remains one of the leading causes of death worldwide and presents a considerable challenge to cardiac recovery.¹ The post-MI healing process becomes dysregulated,

marked by excessive fibrosis and uncontrolled inflammation, which compromises cardiac repair in clinical settings.^{2,3} As a consequence, survivors of MI often face a risk of developing chronic heart failure (HF) because of

Correspondence to: Xiang Cheng, MD, PhD, Department of Cardiology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1277 Jiefang Rd, Jianghan District, Wuhan 430022, Hubei, China. Email nathancx@hust.edu.cn

*M. Zhang and Y. Qin contributed equally.

Supplemental Material is available at <https://www.ahajournals.org/doi/suppl/10.1161/CIRCULATIONAHA.125.076321>.

For Sources of Funding and Disclosures, see page XXX.

© 2026 American Heart Association, Inc.

Circulation is available at www.ahajournals.org/journal/circ

Clinical Perspective

What Is New?

- This study pioneers the development of FAP (fibroblast activation protein)–targeting CAR (chimeric antigen receptor) regulatory T lymphocytes engineered to precisely home to the post-myocardial infarction microenvironment.
- This approach spatiotemporally reduces fibrosis and inflammation, mitigating adverse remodeling and improving cardiac function through targeted immunomodulation.
- The therapeutic efficacy of these cells is mediated by IL-10 (interleukin-10) secretion, which orchestrates 3 synergistic mechanisms: suppressing pathogenic myofibroblast differentiation, reprogramming proinflammatory monocytes into tissue-restorative M2 macrophages, and resolving maladaptive inflammation.

What Are the Clinical Implications?

- The engineered regulatory T lymphocytes represent a breakthrough therapeutic strategy to enhance cardiac repair and prevent heart failure after myocardial infarction, addressing a major unmet need in cardiovascular therapy.
- By exerting dual antifibrotic and anti-inflammatory effects through targeted immunomodulation, this approach offers a transformative strategy to promote postinjury cardiac recovery while addressing the limitations of current nonspecific therapies in fibrosis and inflammation management.
- This precision-based cell delivery strategy offers a safer and more effective alternative to conventional therapies by enhancing therapeutic efficacy, minimizing off-target effects, and avoiding systemic immunosuppression, holding great promise for rapid clinical translation.

inadequate myocardial repair.⁴ To address this issue, it is essential to identify and develop therapeutic interventions that can enhance cardiac repair.

Regulatory T lymphocytes (Tregs) have emerged as a key player in immune regulation and have shown promise in cardiac repair after MI.^{5–7} Experimental studies have demonstrated that increasing the number of Tregs can improve cardiac function, whereas their depletion leads to larger infarcts and impaired heart healing.^{6,8} Transfer experiments using MYHCA_{614–629}-specific cluster of differentiation (CD) 4⁺ T cells have revealed that these cells can transform into Tregs within the heart, improving ventricular function and accelerating collagen deposition during the post-MI healing process.⁹ Moreover, our previous study has shown that thymus-derived Tregs infiltrate the infarcted myocardium and promote cardiac repair through the expression of SPARC.¹⁰ The crucial role of Tregs in regulating fibrosis and modulating inflammation

Nonstandard Abbreviations and Acronyms

Areg	amphiregulin
αSMA	α-smooth muscle actin
CAR	chimeric antigen receptor
CD	cluster of differentiation
CD39	cluster of differentiation 39
CF	cardiac fibroblast
CTLA4	cytotoxic T-lymphocyte-associated protein 4
EBI3	Epstein-Barr virus–induced gene
EF	ejection fraction
FAP	fibroblast activation protein
FCTR	FAP CAR Treg
Foxp3	forkhead box P3
FS	fractional shortening
GARP	glycoprotein A repetitions predominant
GFP	green fluorescent protein
GZMB	granzyme B
HF	heart failure
IFNγ	interferon-γ
IL-1β	interleukin-1β
IL-6	interleukin-6
IL-10	interleukin-10
IL-13	interleukin-13
LAG3	lymphocyte-activation gene 3
LAP	latency-associated peptide
LV	left ventricular
MHC	major histocompatibility complex
MI	myocardial infarction
MKTR	mock Treg
PD1	programmed cell death protein 1
Postn	periostin
PRF	perforin
SPARC	secreted protein acidic and rich in cysteine
TGFβ	transforming growth factor-β
TNFα	tumor necrosis factor-α
Treg	regulatory T lymphocyte

highlights the potential for Treg-based cell therapy to enhance cardiac healing after MI.^{8,11} Nevertheless, challenges, including limited cell availability, undetermined frequencies of alloreactive Tregs, and risks of off-target immunosuppression, require resolution. Inefficient Treg homing to ischemic myocardium represents a key barrier, undermining therapeutic efficacy and demanding innovative targeting strategies.^{8,10,12,13}

The CAR (chimeric antigen receptor) strategy combines the benefits of adoptive T cell immunotherapy with the precision of antibodies, enabling the recognition of antigens regardless of major histocompatibility complex

(MHC) compatibility.^{13,14} In recent years, CARs have not only achieved considerable success in treating hematological malignancies but also have emerged as a promising approach for redirecting Treg cells in preclinical murine and humanized mouse models for adoptive cell therapy in Alzheimer disease, type 1 diabetes, inflammatory bowel disease, vitiligo, and other conditions.^{13,15,16} FAP (fibroblast activation protein), a cell surface glycoprotein involved in tissue remodeling and cancerous growths, is found at higher levels in failing hearts, both ischemic and nonischemic, compared with its minimal or absent expression in normal murine and human hearts.^{17–22} Recent studies indicate that using FAP-targeting CAR T cells, through both adoptive transfer and in vivo induction, can effectively target damaged hearts and prevent cardiac remodeling in mice with hypertensive injury.^{17,19,23} This emerging evidence positions FAP as a highly attractive antigen for directing therapeutic immune cells to the damaged areas of infarcted hearts, offering a novel and promising approach for post-MI intervention.^{17,19,21} Therefore, we hypothesized that FAP-CAR engineering addresses this barrier by facilitating precise delivery of Tregs to FAP⁺-enriched infarct zones, thereby focally amplifying reparative activity within injured myocardium.

METHODS

The corresponding author will provide supporting data upon request. Detailed Methods describing our research process are provided in the Supplemental Material.

Animals

Adult male C57BL/6J mice (10–12 weeks old; weight, 23–26 g) were purchased from Beijing Vital River Laboratory Animal Technology. Additional genetically modified mouse strains with a C57BL/6J background were used, including IL-10 (interleukin-10) global knockout mice (*IL-10^{-/-}*), Ai9/Rosa26-tdTomato reporter mice, Areg (amphiregulin) global knockout mice (*Areg^{-/-}*), and tamoxifen-inducible *Postn-MerCreMer* (*Postn^{MCM}*) mice. These mouse lines were obtained from the Jackson Laboratory. For specific tdTomato labeling of activated cardiac fibroblasts (CFs) after MI, a dual transgenic model was generated by crossing *Postn^{MCM}* with Ai9 mice. Cre recombination was induced by 5-day intraperitoneal tamoxifen injections (20 mg/mL in corn oil).²⁴

All mice were maintained under specific pathogen-free conditions, adhering to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication 86-23, revised 1985). Animal experimental protocols were reviewed and approved by the Animal Care and Utilization Committee of Huazhong University of Science and Technology, ensuring alignment with international standards for ethical research practices.

Statistical Analysis

Data are expressed as mean±SEM. Normality was assessed using the Shapiro-Wilk test, with $P\geq 0.05$ considered indicative

of a normal distribution. Comparisons between 2 groups were conducted using the unpaired Student *t* test when data followed a normal distribution, or the Mann-Whitney *U* test when data deviated from normality. Comparisons among multiple groups were performed using 1-way or 2-way ANOVA with appropriate post hoc testing. Survival outcomes were estimated using Kaplan-Meier curves, and differences among multiple groups were evaluated using the log-rank test. The cardiac rupture rate was assessed using the Fisher exact test. Significance levels were defined as * $P<0.05$, ** $P<0.01$, *** $P<0.001$, or not significant. All statistical analyses were performed using GraphPad Prism 10.0.

RESULTS

FAP-Specific Responsiveness of Treg Cells Expressing FAP CAR

We engineered a primary lentiviral CAR construct that includes an anti-FAP single-chain variable fragment for precise FAP binding, along with intracellular CD28 and CD3ζ signaling domains to activate immune cells upon FAP engagement.^{14,17,19,25} The CAR construct featured GFP (green fluorescent protein) as a robust reporter, and an empty vector expressing only GFP (mock) served as a control. Activated mouse Treg cells were transduced with lentiviral vectors (Figure 1A), resulting in ≈60% expression of GFP or FAP CAR in *Foxp3⁺* (forkhead box P3⁺) Treg cells (Figure 1B and 1C). Both mock Tregs (MKTRs) and FAP CAR-transduced Tregs exhibited consistent phenotypes similar to primary Tregs that had not undergone lentivirus transfection. They maintained similar expression levels of essential cell surface markers, such as CD4 and CD25, along with key regulatory factors, such as *Foxp3* and the negative regulatory receptor CTLA4 (cytotoxic T-lymphocyte-associated protein 4; Figure 1D).

To confirm FAP CAR-mediated Treg activation, both MKTRs and FAP CAR Tregs (FCTR) were restimulated with FAP. Evaluation of Treg activation markers, such as surface CD69, GARP (glycoprotein A repetitions predominant), and LAP (latency-associated peptide), revealed that only GFP⁺ FAP CAR-transduced Tregs showed increased expression of these markers after FAP stimulation, demonstrating specific responses compared with MKTRs (Figure 1E). Furthermore, to validate the FAP-specific activation of FCTR, co-culture experiments were conducted using MKTRs and FCTR with 3T3 fibroblasts that either lacked natural FAP expression or were genetically modified to express mouse FAP (3T3.FAP).¹⁴ After 24 hours, it was evident that FCTR effectively responded to 3T3.FAP fibroblasts, showcasing increased expression of CD69 and GARP-anchored LAP, a response not observed in MKTRs (Figure 1F). In addition, CellTrace Violet dilution assays revealed that FCTR exhibited significantly greater suppression of effector T-cell proliferation upon FAP stimulation,

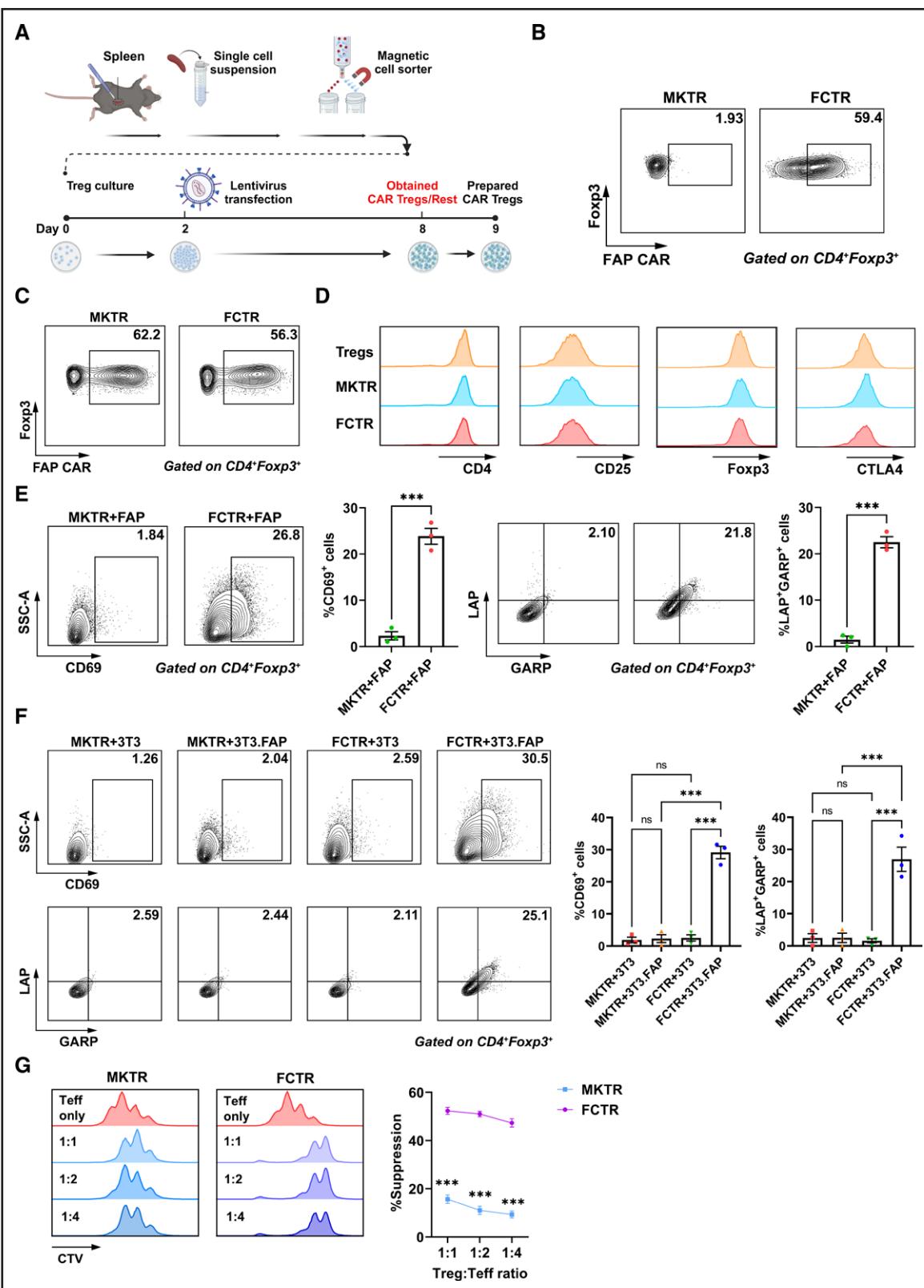


Figure 1. Generation and phenotypic assessment of the engineered regulatory T lymphocytes.

A, Schematic of CAR (chimeric antigen receptor) regulatory T lymphocyte (Treg) generation. **B** and **C**, Transduction efficiency of mock Treg (MKTR) and FAP (fibroblast activation protein) CAR Treg (FCTR) assessed by flow cytometry. **D**, Expression of CD4 (cluster of differentiation 4), CD25 (cluster of differentiation 25), Foxp3 (forkhead box P3), and CTLA4 (cytotoxic T-lymphocyte-associated protein 4) in nontransfected Tregs, MKTR, and FCTR after transfection. **E**, CD69 (cluster of differentiation 69) and GARP (glycoprotein A repetitions predominant)-anchored LAP (latency-associated peptide) expression in MKTR and FCTR after 24-hour stimulation with recombinant murine FAP. (*Continued*)

Figure 1 Continued. **F**, Flow cytometry of CD69 and GARP anchored LAP expression in MKTR and FCTR co-cultured with 3T3 cells, with or without FAP overexpression, for 24 hours. **G**, In vitro suppression assay of CellTrace Violet (CTV)-labeled effector T cells co-cultured with MKTR or FCTR at various Treg:effector T cell (Teff) ratios upon FAP stimulation. Two-tailed unpaired *t* test was used in **E**. Two-way ANOVA with Tukey multiple comparison test was performed in **F**. Two-way ANOVA followed by Bonferroni multiple comparisons within ratios (MKTR vs FCTR at each ratio) was performed in **G**. ****P*<0.001. NS indicates not significant.

compared with MKTRs (Figure 1G). These findings collectively underscore FAP-specific activation of FCTRs.

FAP-Targeting CAR Tregs Improve Cardiac Outcomes After MI

The expression of FAP by activated CFs in response to injury mirrors that of Postn (periostin), with FAP primarily originating from Postn⁺ fibroblasts.^{22,24,26} Western blot analysis showed a rapid upregulation of FAP expression beginning as early as 3 days after MI, peaking at day 7.^{21,22,26} To identify activated CFs after MI, we used a *Postn* knock-in mouse model with tamoxifen-regulated Cre activity (*Postn*^{MCM}) crossed with an Ai9 mouse line expressing tdTomato fluorescence upon Cre-mediated recombination.²⁴ By day 3 after left anterior descending ligation, a conspicuous presence of tdTomato fluorescence-labeled activated CFs was observed in the infarct and peri-infarct regions, which was absent in sham-operated controls (Figure S1A). Coexpression of FAP with tdTomato fluorescence-labeled Postn⁺ activated CFs was confirmed at day 3 and day 7 after MI, respectively (Figure S1B).

To selectively target activated CFs and their progeny, FAP-specific CAR Treg cells were adoptively transferred at day 3 after MI. The animal experiment procedure is shown in Figure 2A. As expected, CD3⁺GFP⁺ FCTRs successfully infiltrated the myocardium, colocalizing with tdTomato fluorescence-labeled activated CFs within 1 day after transfer (Figure 2B). Peak engraftment occurred at day 7 (57.96±5.36 cells/mg tissue), with near-complete clearance (>85% reduction) observed by day 14 after MI (Figure S1C). This effect was notably distinct from MI mice treated with vehicle alone or MKTRs, highlighting the precisely targeted migration of the engineered Tregs to the injury site.

To investigate the functional impact of FAP-targeting CAR Tregs during the acute phase of MI, we induced MI in C57BL/6 mice and administered vehicle (PBS), MKTRs, or FCTRs 3 days after the onset of MI. Initial assessments of infarct size, heart function, and structure ensured consistency across all groups, confirming a uniform MI induction process (Figure S2A through S2C). No significant differences in survival rate or cardiac rupture incidence were noted between MI mice treated with vehicle, MKTRs, or FCTRs, with all groups displaying minimal occurrences of heart rupture within the 14-day post-MI observation period (Figure 2C and 2D). However, a significant difference emerged in the group treated with FCTRs, which exhibited a marked reduction

in cardiac mass, as evidenced by decreases in both heart weight to body weight ratio and heart weight to tibia length ratio, distinguishing it from the control groups that received vehicle or MKTRs (Figure 2E). By day 14 after MI, vehicle control mice developed severe HF and cardiac dilation, whereas mice treated with FAP CAR Tregs exhibited significant improvements in cardiac function. These improvements were demonstrated by increased left ventricular (LV) ejection fraction (EF) and fractional shortening (FS), as well as reduced LV size, indicated by decreases in both LV end-diastolic diameter and LV end-diastolic volume. In stark contrast, MKTR-treated mice showed no functional benefits (Figure 2F and 2G). We conducted additional experiments comparing FAP CAR CD8⁺ cytotoxic T cells and FCTRs in MI at equivalent doses (2.5×10⁵ cells). Adoptive transfer of FAP CAR CD8⁺ T cells after MI failed to improve cardiac function, whereas the same dose of FCTRs significantly enhanced functional recovery (Figure S3A and S3B). These findings directly demonstrate the superiority of FCTR therapy over FAP CAR CD8⁺ T cells in post-MI repair.

To explore the therapeutic potential of FAP CAR Tregs in ischemia–reperfusion injury, we established an ischemia–reperfusion model in C57BL/6 mice and promptly administered either MKTRs or FCTRs. Similar to the permanent MI model, mice treated with FCTRs showed significant reductions in both heart weight to body weight and heart weight to tibia length ratios compared with the controls at day 14 after ischemia–reperfusion injury (Figure S4A and S4B). We also demonstrated notable improvements in cardiac EF and FS, alongside reductions in LV end-diastolic diameter and LV end-diastolic volume, paralleling the cardioprotective effects observed in the permanent MI setting (Figure S4C). Taken together, these findings reveal potent beneficial effects of FCTRs in promoting cardiac repair after ischemic injury, highlighting their potential as a promising therapeutic strategy for ischemic heart disease.

Comprehensive Analysis of the Effects of FCTRs on Fibrosis, Inflammation, Cardiac Hypertrophy, and Angiogenesis After MI

To investigate the mechanisms underlying FCTR-mediated cardiac repair and functional recovery after MI, we conducted comprehensive histological and molecular analyses of myocardial tissues from all experimental groups (Figure 2A). Masson trichrome staining revealed a significant reduction in scar size and cardiac fibrosis within the peri-infarct zone of mice treated with FCTRs, surpassing

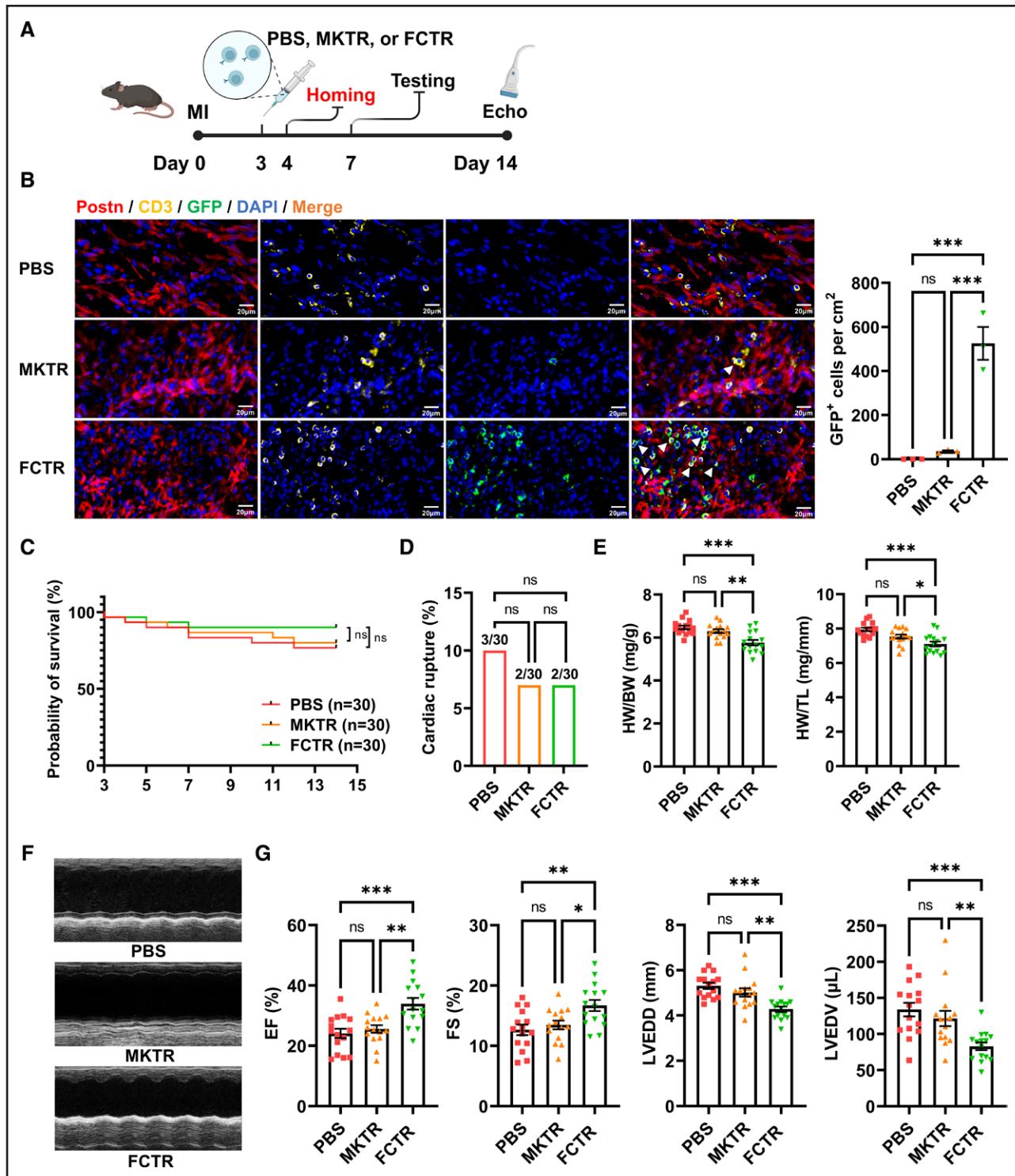


Figure 2. Homing of the engineered regulatory T lymphocytes and their impact on cardiac outcomes after myocardial infarction.

A, Experimental design for FAP (fibroblast activation protein) CAR (chimeric antigen receptor) regulatory T lymphocyte (Treg) targeting of activated cardiac fibroblasts after myocardial infarction (MI). PBS, mock Treg (MKTR), or FAP CAR Treg (FCTR) was administered on day 3 after MI. Treg homing was assessed 1 day after transfer, followed by histological and echocardiographic evaluations on days 7 and 14, respectively. **B**, Colocalization of CD3⁺GFP⁺ FCTR or CD3⁺GFP⁺ MKTR with tdTomato fluorescence-labeled Postn⁺ activated fibroblasts on day 4 after MI. Scale bars=20 μ m. Quantification depicted in the adjacent bar graphs. **C**, Kaplan-Meier survival curves of mice treated with PBS, MKTR, or FCTR up to 14 days after MI (n=30 per group). **D**, Cardiac rupture rates showed no significant differences (n=30 per group). **E**, heart weight to body weight ratio (HW/BW) and heart weight to tibia length ratio (HW/TL) measured on day 14 after MI (n=15 per group). **F**, Representative parasternal short-axis views of left ventricles in PBS-, MKTR-, or FCTR-treated mice on day 14 after MI. **G**, Echocardiographic analysis of ejection fraction (EF), fractional shortening (FS), left ventricular end-diastolic diameter (LVEDD), and left ventricular end-diastolic volume (LVEDV) on day 14 after MI (n=15 per group). Kaplan-Meier method and log-rank test were used in **C**. Fisher exact test was performed in **D**. One-way ANOVA with Tukey multiple comparison test was conducted in **B**, **E**, and **G**. *P<0.05, **P<0.01, ***P<0.001. NS indicates not significant.

both vehicle and MKTR controls (Figure 3A). This decrease in fibrosis was accompanied by downregulation of α SMA and *Col1a1* expression in the border regions of hearts receiving FCTR treatment (Figure 3B). In addition, a significant decrease in the population of myofibroblasts expressing α SMA (α -smooth muscle actin) and vimentin was observed in the peri-infarct zone of mice treated with FCTRs at day 7 after MI, compared with those treated with MKTRs or vehicle. However, the overall population of vimentin-expressing CFs remained consistent across all groups (Figure 3C and 3D). To further quantify activated CFs across experimental groups, we implemented flow cytometric validation using *Postrn*^{MCM};tdTomato reporter mice to quantify activated CFs across experimental groups. This orthogonal methodology revealed >30% reduction in activated fibroblasts (Figure S5A and S5B). These flow cytometry–validated results corroborate the fibroblast suppression phenotype observed by immunofluorescence in Figure 3C, strengthening our mechanistic interpretation. Polarization microscopy using Sirius red staining revealed reduced presence of tightly packed collagen fibers within the border regions of FCTR-treated mice, indicating a shift toward less mature and more flexible collagen formation compared with the MKTR and vehicle-treated MI groups (Figure 3E and 3F).

The reduction in myofibroblast numbers after injury is often linked to factors such as diminished proliferation, hindered cellular differentiation, enhanced apoptosis, or increased immune cell–mediated cytotoxicity. To explore these possibilities, we used immunostaining to identify the presence of the proliferation marker Ki67 and the apoptosis marker TUNEL. Our observations revealed no significant changes in the proportion of α SMA⁺vimentin⁺ CFs exhibiting Ki67 positivity (Figure S6A and S6B) or TUNEL positivity (Figure S6C and S6D). We assessed the cytotoxic activity of FAP CAR CD8⁺ T cells against MKTR and FCTR using murine FAP-transfected 3T3 fibroblasts as targets. Our data demonstrated that FAP CAR CD8⁺ T cells mediated \approx 80% specific lysis of target cells. This cytotoxic activity was significantly higher than that of either Treg group ($P<0.001$ versus MKTR; $P<0.001$ versus FCTR), both of which exhibited negligible cytotoxic activity with no significant differences between them (Figure S6E). These findings suggest that the observed decrease in cardiac fibrosis in FCTR-treated mice after MI might be primarily attributed to a reduction in myofibroblast differentiation rather than changes in proliferation, apoptosis, or cytotoxicity.

Further analysis revealed a striking reduction in the expression of inflammatory cytokines, namely IL-1 β (interleukin-1 β), IL-6 (interleukin-6), and TNF α (tumor necrosis factor- α), within damaged heart tissues at day 7 after MI, correlating with the increase in FCTRs (Figure 3G). Concomitantly, our flow cytometry analysis of cardiac-infiltrating immune cells revealed that infiltration of CD3⁺ and CD8⁺ T cells was significantly reduced in

FCTR-treated hearts at day 7 after MI (Figure 3H and 3I). In contrast, counts of CD4⁺ T cells and CD11b⁺F4/80⁺ macrophages remained unchanged across groups (Figure S7A and S7B), a finding consistent with previous reports.⁸ Taken together, these changes demonstrate potent anti-inflammatory reprogramming by FAP-targeted CAR Treg therapy.

Furthermore, immunostaining for cardiac troponin I and wheat germ agglutinin showed that mice treated with FCTRs exhibited a significant reduction in myocyte size within the peri-infarct region at day 14 after MI compared with control groups (Figure S8A and S8B). This reduction in myocyte size was accompanied by a decrease in the expression of pathological remodeling marker genes, including *BNP*, *ANP*, and β *MHC* (Figure S8C). Enhanced vascularity was observed in the peri-infarct regions of FCTR-treated mice, as assessed by CD31 staining, when compared with those treated with vehicle or MKTRs (Figure S8D and S8E). These results collectively provide compelling evidence that FCTRs can effectively mitigate cardiac injury after MI through mechanisms that include modulating fibrosis, inflammation, hypertrophy, and angiogenesis.



IL-10 Is the Main Mediator in the Cardioprotective Effect of FCTRs

To identify key mediators behind the protective effects of FCTRs, we conducted a detailed analysis of molecules and cytokine expression profiles after 24 hours of FAP stimulation compared with MKTRs. No significant differences were observed in the expression levels of LAG3 (lymphocyte-activation gene 3), PD1 (programmed cell death protein 1), P35, EBI3 (Epstein-Barr virus–induced gene), CD39 (cluster of differentiation 39), or SPARC (secreted protein acidic and rich in cysteine) between FCTRs and MKTRs (Figure S9A and S9B), but marked upregulation was noted in IL-10, TGF β (transforming growth factor- β), CTLA4, IL-13 (interleukin-13), Areg, PRF (perforin), and GZMB (granzyme B; Figure 4A and 4B; Figure S9B). IL-10 levels surged, increasing 6-fold, suggesting a key role in cardioprotection. Extended ELISA showed that FAP stimulation significantly enhanced IL-10 secretion by FCTRs, but did not markedly increase PRF, GZMB, or IFN γ (interferon- γ ; Figure 4C; Figure S9C). Consistent with this, flow cytometry confirmed that FAP activation did not alter PRF or GZMB expression (Figure S9D).

IL-10 is a well-established suppressive cytokine known for its protective functions in organ damage, including MI.^{8,11,27–29} IL-10 produced by Tregs has shown beneficial effects in diverse models of organ ischemia, underscoring its pivotal role in FCTR-mediated cardioprotection after MI.^{8,30,31} Evidence suggests that CAR Tregs improve disease control primarily by increasing IL-10 secretion in response to antigen.^{16,32–34} Our study

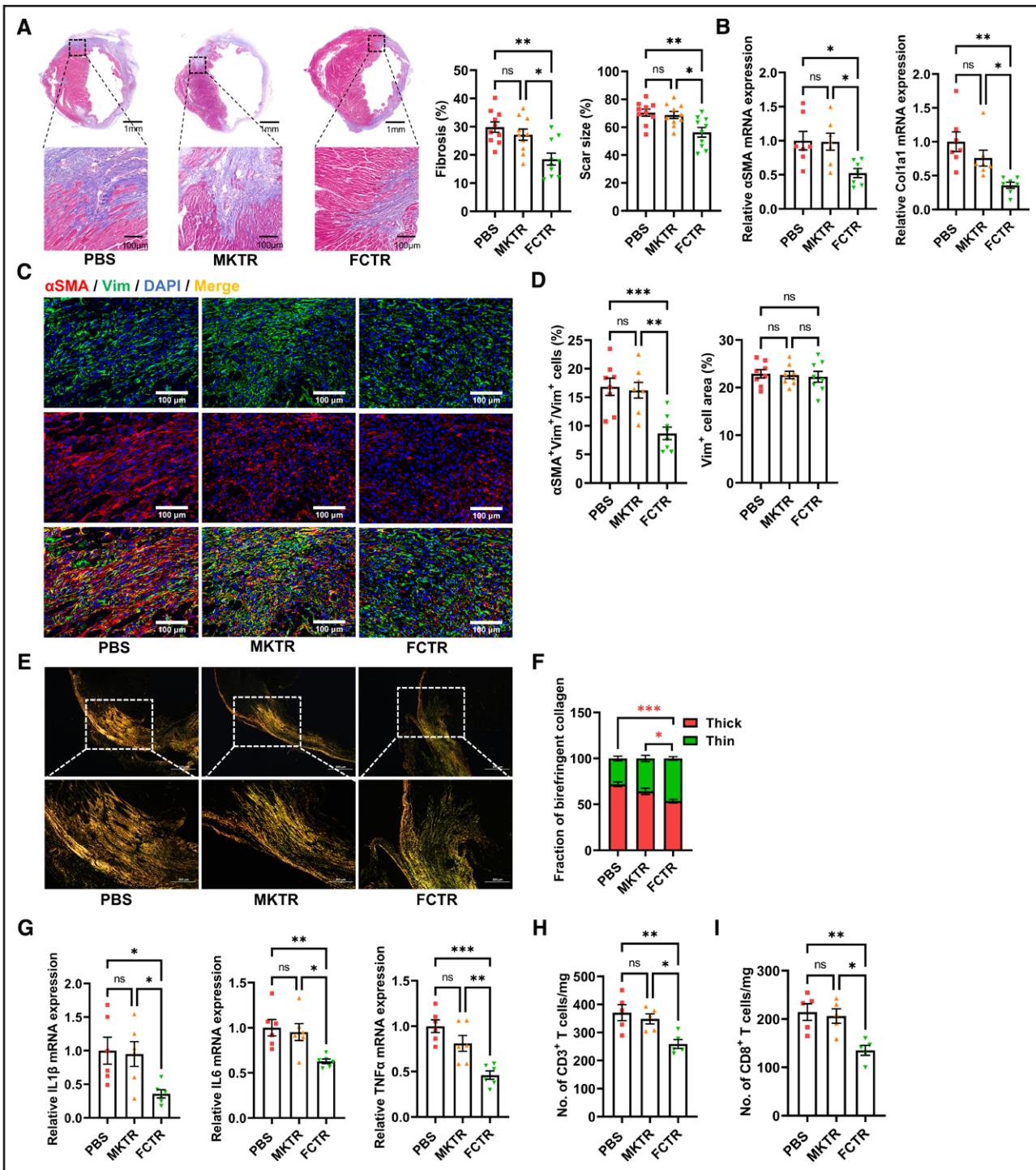


Figure 3. FAP CAR regulatory T-lymphocyte therapy attenuates cardiac fibrosis and inflammation after myocardial infarction.

A, Fibrosis and scar size assessment in peri-infarct zones by Masson trichrome staining (blue) on day 14 after myocardial infarction (MI; n=10 per group). Scale bars=1 mm (**top**) and 100 μ m (**bottom**). **B**, Reverse transcription quantitative polymerase chain reaction analysis of α SMA and $Col1a1$ mRNA expression in the peri-infarct zone on day 7 after MI (n=7 per group). **C** and **D**, Representative immunofluorescent images and quantification of the fraction of α SMA⁺/Vim⁺ fibroblasts (orange) and Vim⁺ cell area (green) in the peri-infarct zone on day 7 after MI (n=8 per group). Scale bars=100 μ m. **E** and **F**, Collagen maturity and quantity in peri-infarct areas analyzed by Sirius red polarization microscopy to differentiate thick, mature collagen fibers (red birefringent) and less mature, compliant fibers (green birefringent) on day 7 after MI (n=6 per group). Scale bars=200 μ m. **G**, Gene expression analysis of $IL-1\beta$, $IL-6$, and $TNF\alpha$ in injured hearts on day 7 after MI (n=6 per group). **H** and **I**, Flow cytometry quantified CD3⁺ (**H**) and CD8⁺ T cells (**I**) in scar tissues on day 7 after MI (n=5 per group). One-way ANOVA with Tukey multiple comparison test was performed in **A**, **B**, **D**, **F**, **G**, **H**, and **I**. *P<0.05, **P<0.01, ***P<0.001. α SMA indicates α -smooth muscle actin; CAR, chimeric antigen receptor; FAP, fibroblast activation protein; FCTR, FAP CAR regulatory T lymphocyte; IL-1 β , interleukin 1 β ; IL-6, interleukin-6; MKTR, mock regulatory T lymphocyte; NS, not significant; and $TNF\alpha$, tumor necrosis factor- α .

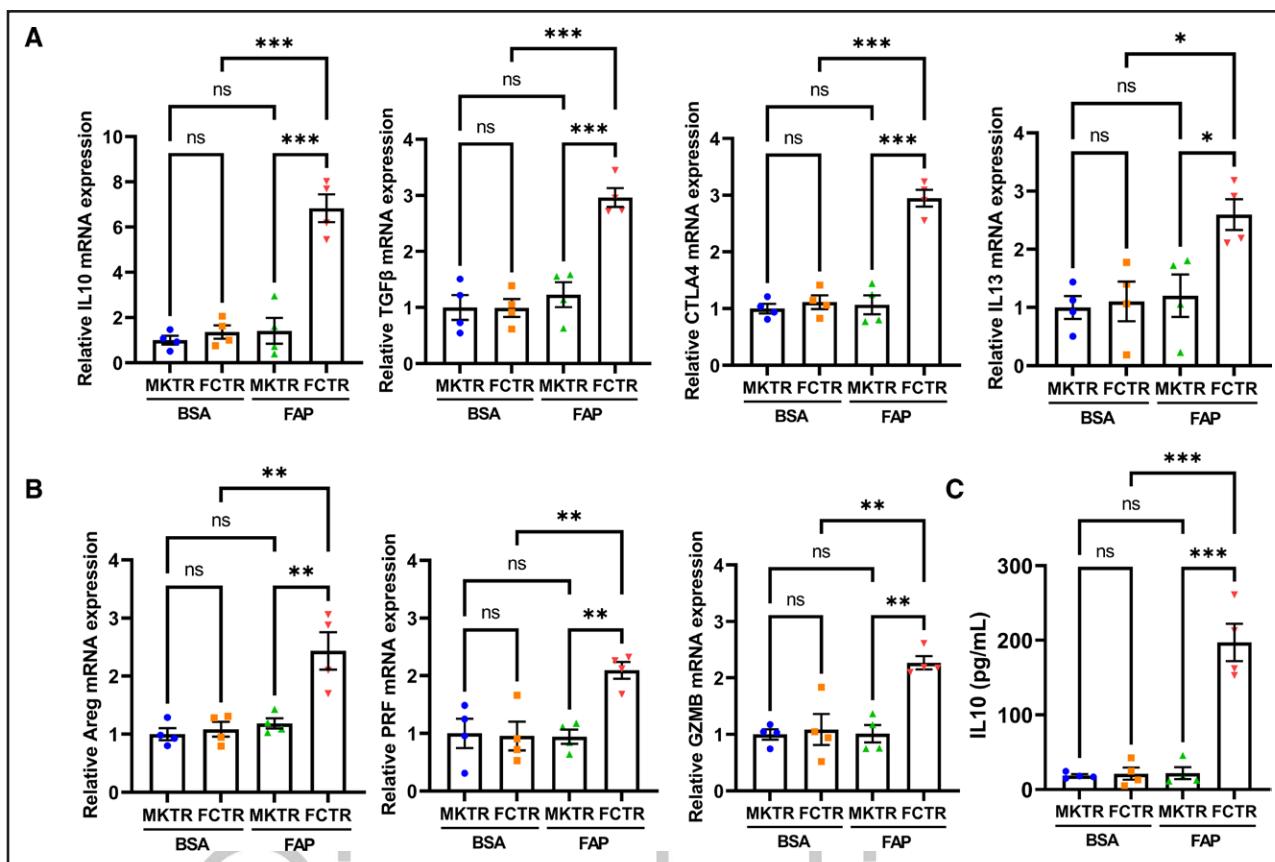


Figure 4. Differential gene expression and IL-10 secretion profiles of engineered regulatory T lymphocytes upon FAP stimulation. **A** and **B**, Expression levels of IL-10 (interleukin-10), TGF β (transforming growth factor- β), CTLA4 (cytotoxic T-lymphocyte-associated protein 4), IL-13 (interleukin-13), Areg (amphiregulin), PRF (perforin), and GZMB (granzyme B) in CAR (chimeric antigen receptor) regulatory T lymphocytes (Tregs) after 24 hours of stimulation on BSA- or FAP (fibroblast activation protein)-coated plates ($n=4$ per group). **C**, Quantification of IL-10 secretion by mock Treg (MKTR) or FAP CAR Treg (FCTR) after 24-hour stimulation with BSA or FAP using ELISA ($n=4$ per group). Two-way ANOVA with Tukey multiple comparison test was used to analyze the data in **A** through **C**. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. NS indicates not significant.

demonstrated a significant increase in IL-10 expression within FAP-activated CAR Tregs, prompting further investigation into the *in vivo* effects of IL-10 on cardiac healing and myofibroblast transformation during FAP-targeted CAR Treg therapy.

To address this question, we administered vehicle control, IL-10-expressing, or IL-10-deficient FCTRs to mice on day 3 after MI (Figure 5A). By day 14, IL-10-expressing FCTRs led to reduced heart weight to body weight and heart weight to tibia length ratios, improved heart function (elevated EF and FS values), and decreased LV end-diastolic diameter as well as LV end-diastolic volume measurements (Figure 5B through 5D). IL-10-deficient FCTRs failed to maintain these beneficial effects (Figure 5B through 5D). In addition, deletion of IL-10 reversed the reduction in cardiac fibrosis: a change attributed to the targeted migration of IL-10-expressing FCTRs to the injury site after MI (Figure 5E). Reverse transcription quantitative polymerase chain reaction analysis revealed decreased α SMA expression in the presence of IL-10-expressing

FCTRs; IL-10-deficient FCTRs showed the opposite (Figure 5F). Immunofluorescence staining for α SMA and vimentin confirmed a significant reduction in myofibroblasts in the peri-infarct zone of mice treated with IL-10-expressing FCTRs compared with vehicle-treated mice: a response that was diminished in the IL-10-deficient group (Figure 5G). These findings suggest that FCTRs promote cardiac repair by secreting IL-10, enhancing cardiac function, reducing scar formation, and inhibiting myofibroblast transformation.

Further investigation into the molecular mechanisms revealed that FCTR-derived IL-10 suppressed myofibroblast transformation by inhibiting the Smad2/3-dependent signaling pathway. Because TGF β stimulation led to a significant rise in FAP expression in CFs (Figure S10A and S10B), co-culturing TGF β -activated CFs with FCTRs resulted in the suppression of Smad2/3 phosphorylation and α SMA upregulation in activated CFs. These effects were attenuated in IL-10-deficient FCTRs, indicating that IL-10 secretion by FCTRs suppresses fibroblast differentiation into myofibroblasts through the

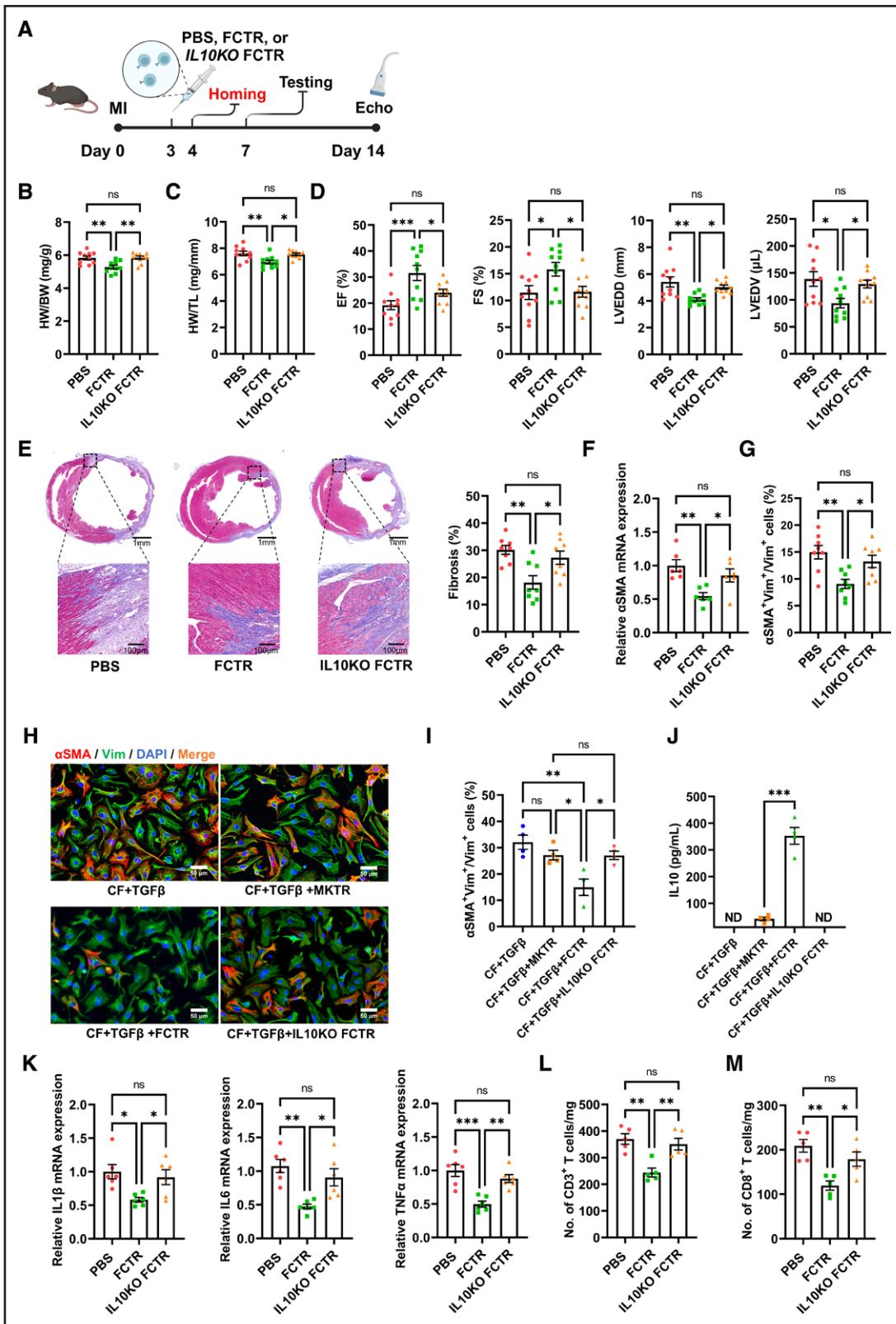


Figure 5. IL-10 mediates the cardioprotective effects of FAP CAR regulatory T lymphocytes after myocardial infarction.

A, Schematic of the in vivo experiment designed to explore the crucial role of IL-10 (interleukin-10) in FAP (fibroblast activation protein) CAR (chimeric antigen receptor) regulatory T lymphocytes (Tregs; FCTR). **B** and **C**, Heart weight to body weight ratio (HW/BW) and heart weight to tibia length ratio (HW/TL) in PBS, FCTR, or IL-10 knockout (KO) FCTR-treated mice on day 14 after myocardial infarction (MI; n=10 per group). **D**, Echocardiographic analysis of ejection fraction (EF), fractional shortening (FS), left ventricular end-diastolic diameter (LVEDD), and left ventricular end-diastolic volume (LVEDV) on day 14 after MI (n=10 per group). (Continued)

Figure 5 Continued. **E**, Fibrosis assessment in peri-infarct zones by Masson trichrome staining (blue) on day 14 after MI (n=8 per group).

Scale bars=1 mm (**top**) and 100 μ m (**bottom**). **F**, Reverse transcription quantitative polymerase chain reaction analysis of α SMA mRNA expression in the peri-infarct zone on day 7 after MI (n=6 per group). **G**, Quantifications of α -smooth muscle actin (α SMA) $^+$ myofibroblasts (α SMA $^+$ Vim $^+$ /Vim $^+$ cardiac fibroblasts [CFs]) in the peri-infarct zone 7 days after MI were performed using immunofluorescence (n=8 per group). **H** and **I**, Neonatal mouse primary CFs were treated with transforming growth factor- β (TGF β) for 24 hours to stimulate FAP expression. Representative immunofluorescent images and quantification of myofibroblasts (α SMA $^+$ Vim $^+$ /Vim $^+$ CFs) co-cultured with PBS control, mock Treg (MKTR), FCTR, or IL-10 KO FCTR for an additional 24 hours (n=4 per group). Scale bars=50 μ m. **J**, IL-10 secretion in co-culture supernatants measured by ELISA (n=4 per group). **K**, Gene expression of *IL-1 β* , *TNF α* , and *IL-6* in the peri-infarct zone was analyzed on day 7 after MI (n=6 per group). **L** and **M**, Flow cytometry quantified CD3 $^+$ (**L**) and CD8 $^+$ T cells (**M**) in scar tissues on day 7 after MI (n=5 per group). One-way ANOVA with Tukey multiple comparison test was performed in **B** through **I** and **K** through **M**. A 2-tailed unpaired *t* test was performed in **J**. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. ND indicates not detected; and NS, not significant.

Smad2/3-dependent signaling pathway (Figure S10C and S10D; Figure 5H through 5J).

In addition, IL-10 plays a crucial role in improving cardiac remodeling and promoting tissue repair after MI by stimulating M2 macrophage polarization.^{35,36} Our analysis of M2 marker gene expression in infarcted hearts treated with vehicle, IL-10-expressing, or IL-10-deficient FCTRs revealed that IL-10-expressing FCTRs upregulated M2 markers and promoted a prohealing F4/80 $^+$ CD206 high M2 macrophage phenotype. In contrast, IL-10 deletion abolished these effects, underscoring the essential role of IL-10 in FCTR-mediated M2 macrophage polarization and cardiac repair (Figure S11A and S11B).

Moreover, IL-10-expressing FCTRs were associated with a significant reduction in inflammatory cytokines (*IL-1 β* , *IL-6*, and *TNF α*) and a decrease in CD3 $^+$ and CD8 $^+$ T-cell infiltration within the injured heart after MI. These beneficial effects were markedly attenuated in the absence of IL-10 (Figure 5K through 5M). These findings collectively suggest that FAP CAR Treg treatment elicits a robust anti-inflammatory response mediated by IL-10 secretion in the infarcted heart.

Areg, a member of the epidermal growth factor family, promotes tissue regeneration, angiogenesis, and protection against injuries, such as ischemic stroke and ischemic peripheral artery diseases, when produced by Tregs.³⁷⁻³⁹ The exact role of Areg in FCTR-mediated cardioprotection is not fully understood, but our study revealed that Areg deletion does not significantly affect the protective effects of FCTRs after MI (Figure S12A and S12B). This suggests that Areg may not be a major contributor to the cardioprotective effects of FCTRs in the context of MI.

Building upon previous evidence, our study demonstrates that engineered FCTRs exhibit precise tropism toward activated FAP $^+$ CFs, enabling targeted delivery to injury sites. The IL-10 secreted by these FCTRs serves as the primary mediator of cardioprotection (Figure 6). Mechanistically, FCTRs attenuate Smad2/3 signaling through paracrine IL-10 secretion, thereby modulating myofibroblast differentiation and extracellular matrix remodeling. Furthermore, they promote macrophage polarization toward a proreparative M2 phenotype and facilitate the resolution of postinfarction inflammatory responses. These mechanisms culminate in attenuated

adverse ventricular remodeling and improved cardiac function after MI.

Evaluating the Safety Profile of FCTR Therapy in Cardiac Repair

We conducted a thorough evaluation of potential toxicities associated with FCTR cell therapy. Despite reports of FAP CAR T cell-mediated side effects, such as cachexia and anemia,^{18,26} we observed no changes in body weight or blood cell counts after FCTR therapy (Figure S13A and S13B). In addition, no histological effects were found in various noncardiac organs or tissues, including skin, skeletal muscle, femur, kidney, lung, liver, testis, and pancreas (Figure S13C), suggesting a favorable safety profile in our experimental model.

DISCUSSION

In cardiac disease research, the pursuit of innovative strategies to repair the heart after MI has consistently been at the forefront of scientific endeavors. Despite advancements in immunotherapies, including antifibrotic vaccines, cytokine-targeting biologics, and fibroblast-specific CAR T cells, post-MI cardiac repair remains hindered by the inability to simultaneously resolve fibrosis and inflammation.^{17,19,21,22,40} Our study introduces FAP-specific CAR Tregs as a mechanistically distinct therapeutic paradigm. These engineered Tregs exhibit a dual capacity to mitigate fibrotic burden by suppressing myofibroblast differentiation and to reprogram the cardiac immune microenvironment through enhanced M2 macrophage polarization and suppression of excessive inflammatory responses. By integrating spatiotemporal precision with immunomodulatory function, FCTRs significantly improve functional recovery in both permanent MI and ischemia-reperfusion injury models, offering a transformative framework for CAR Treg-based precision immunotherapies in cardiology.

Spatiotemporal immunofluorescence mapping revealed synchronized FAP $^+$ /tdTomato $^+$ coexpression (Postn $^+$ fibroblasts) initiating at 72 hours after MI (Figure S1A and S1B), consistent with fibrotic activation dynamics in cardiovascular pathologies.^{21,22,26} Using tdTomato fluorescence as a surrogate marker for FAP, we

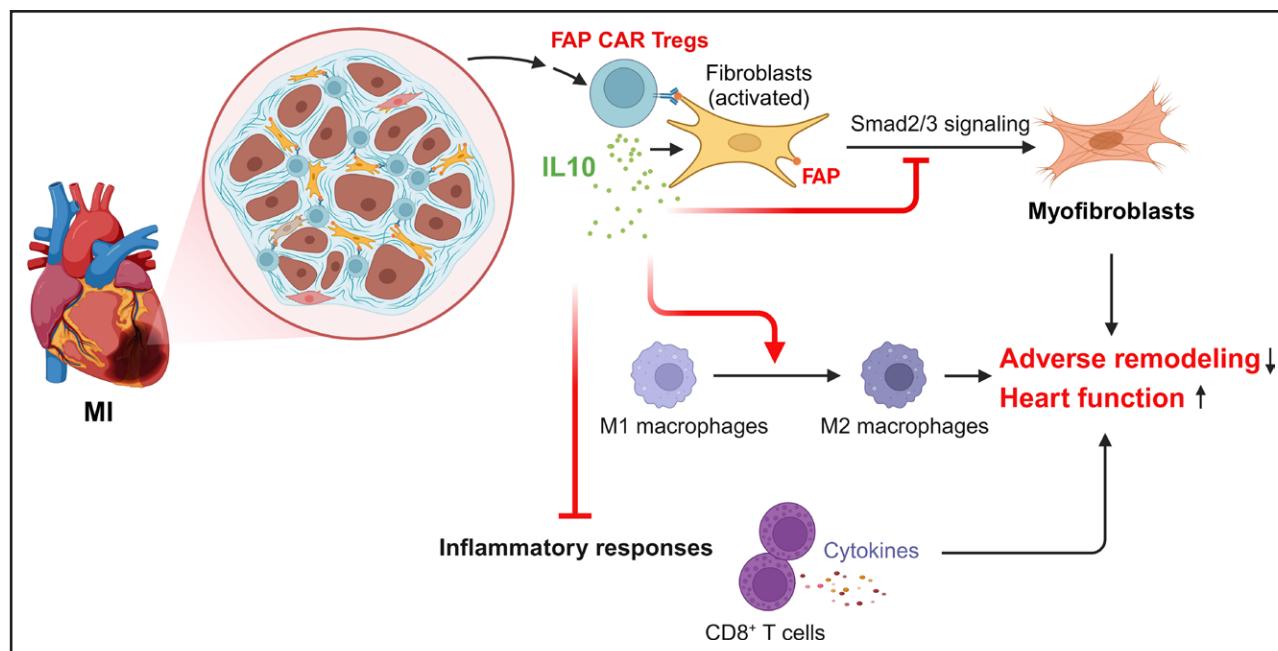


Figure 6. Schematic representation of the mechanisms underlying FAP CAR regulatory T lymphocyte-mediated cardioprotection.

Schematic representation illustrates the intricate mechanisms by which FAP (fibroblast activation protein) CAR (chimeric antigen receptor)-expressing regulatory T lymphocytes (Tregs) alleviate adverse cardiac remodeling and promote heart healing through IL-10 (interleukin-10) release. The detailed depiction highlights the therapeutic efficacy of FAP CAR Treg therapy in the context of myocardial infarction (MI), emphasizing the crucial role of IL-10 in mediating the protective effects of engineered Tregs. Schematics created using BioRender (<https://BioRender.com>).

identified this phase as the optimal therapeutic window. Intravenous administration of FCTRs during this period triggered rapid homing to infarcted myocardium, in which FCTRs formed immunoregulatory clusters around activated CFs (Figure 2B). This targeted localization mirrors the homing patterns of FAP CAR T cells observed in hypertensive models,^{17,19} suggesting a universal mechanism for FAP-specific CAR immune cell trafficking to fibrotic niches. We propose that this self-guided homing establishes focal immunoregulatory units, directly linking cellular localization to functional reprogramming of injury-responsive fibroblasts and immune cells, thereby creating a spatially coordinated repair microenvironment.

The FCTR-treated group exhibited significant reductions in LV size and improvements in cardiac function at 2 weeks after MI compared with MKTR controls, demonstrating the therapeutic potential of these engineered cells in attenuating pathological remodeling and enhancing myocardial recovery. FCTRs increased EF from 24.06% to 33.95% (41.13% relative improvement) and FS from 12.65% to 16.69% (31.91% enhancement; Figure 2G). These gains exceed established efficacy benchmarks for Treg-based MI therapies, including the ≈40% EF increase and ≈25% FS increase reported for nontargeted Tregs.^{5,6,8,21}

Inflammation and fibrosis are indispensable for necrotic tissue clearance and scar maturation after MI, but their dysregulation drives maladaptive remodeling

and impedes recovery.^{41,42} Myofibroblasts, characterized by high expression of FAP and Postn, emerge as central mediators of fibrosis and represent a rational therapeutic target.^{27,43} Although FAP-targeted CAR T cells have demonstrated efficacy in eliminating activated fibroblasts in preclinical cancer and hypertensive injury models,^{14,17,18,23} their clinical translation for MI is constrained by the essential role of CFs in acute-phase scar formation and ischemic repair. This limitation underscores the advantage of engineered Tregs, which modulate fibrotic and inflammatory responses without ablating CFs, highlighting the superior potential of using FCTRs, rather than FAP CAR T cells, in the context of MI because of their ability to support beneficial repair processes.^{5,6,10} As expected, our data revealed that FCTR deployment significantly reduced cardiac fibrosis and promoted the formation of a functional, collagen-stabilized scar, effects not observed with FAP CAR CD8⁺ T cells (Figure 3A, 3E, and 3F; **Figure S3A and S3B**). Mechanistically, they suppressed excessive myofibroblast differentiation and activity, as evidenced by reduced αSMA⁺ cells and collagen I/III deposition (Figure 3C and 3D; Figure 5G).

Meanwhile, we found that, beyond fibrosis mitigation, FCTRs orchestrated a proreparative immune milieu through 2 synergistic mechanisms: (1) they polarized macrophages toward an F4/80⁺CD206^{high} M2 phenotype (**Figure S11A and S11B**); and (2) they reduced CD3⁺ and CD8⁺ T-cell infiltration and attenuated levels

of proinflammatory mediators (*IL-1 β* , *IL-6*, and *TNF α*) in injured myocardium (Figure 3G through 3I). This dual capacity to simultaneously resolve fibrosis and temper inflammation establishes FCTRs as an attractive strategy for ischemic heart disease. These beneficial functions do not require the target cells to express FAP; the CAR primarily serves as a trigger for Treg activation and a homing mechanism to the injury site.

Current evidence confirms detrimental effects of conventional polyclonal CD3+ and CD8+ T-cell therapies on postischemic cardiac repair.^{44,45} Because no studies have reported on the role of FAP CAR CD8+ cytotoxic T cells in the context of MI, we compared FAP CAR CD8+ cytotoxic T cells and FCTRs at equivalent doses (2.5×10^5 cells). Whereas FAP CAR CD8+ T cells failed to improve cardiac function after MI, FCTRs significantly enhanced functional recovery (Figure S3A and S3B), clearly establishing the superiority of FCTR therapy for post-MI repair.

After MI, latent TGF β is rapidly activated by reactive oxygen species and proteases, with additional synthesis by infiltrating platelets, leukocytes, and fibroblasts.⁴⁶ Although TGF β bioactivity increases early, proinflammatory mediators initially delay myofibroblast transformation until tissue debris is cleared.⁴⁶ This sequence of events explains the notable absence of detectable myofibroblasts in the early stages (the first 2 days) after MI. As inflammation resolves, TGF β signaling drives myofibroblast differentiation and extracellular matrix preservation.^{43,46} Our study mechanistically demonstrates that FCTR-derived IL-10 plays a central role in suppressing pathological fibrosis. Adoptive transfer of FCTRs resulted in marked elevation of IL-10 within infarcted myocardium, which inhibited TGF β -induced Smad2/3 phosphorylation in CFs (Figure S10C and S10D). This pathway blockade effectively prevented CF-to-myofibroblast transition, as evidenced by reduced α SMA+ cell populations and collagen deposition (Figure 5F through 5I). Our data establish Smad2/3 as the principal profibrotic mediator downstream of TGF β in post-MI hearts, aligning with findings in other cardiomyopathy models.^{43,47}

CAR directs Treg localization and activation near FAP+ fibroblasts; activated FCTRs exert potent antigen-nonspecific bystander effects, including immunosuppression and proregenerative activities that are not antigen-specific. The antifibrotic potency of IL-10 observed in our study extends beyond direct fibroblast modulation. As demonstrated in Figure 5K through 5M; Figure S11A and S11B, IL-10 secretion by engineered Tregs orchestrated another dual therapeutic mechanism: (1) polarizing macrophages toward a prohealing F4/80+CD206 high M2 phenotype; and (2) suppressing proinflammatory cytokine production (*IL-1 β* , *IL-6*, *TNF α*) and CD3+ and CD8+ T cell infiltration. This aligns with previous findings showing that exogenous IL-10 improves LV function by reducing collagen deposition,²⁷ whereas IL-10 deficiency exacerbates inflammatory

responses and infarct expansion.²⁷ Our work establishes that endogenous IL-10 production from precisely localized Tregs, rather than systemic delivery, creates a preoperative niche, mirroring the cardiac IL-10 surge observed in CD28 superagonist-treated mice.⁶ This engineered Treg-centric IL-10 delivery strategy offers 3 key advantages over conventional approaches: first, targeted enrichment of IL-10 within damaged heart tissues maximizes therapeutic efficacy while minimizing off-target effects; second, sustained IL-10 exposure through engineered Tregs overcomes the short half-life limitations of recombinant protein therapies; third, the self-amplifying nature of M2 macrophage-derived IL-10 establishes a positive feedback loop that prolongs antifibrotic and anti-inflammatory activity. These beneficial functions do not require target cells to express FAP; the CAR primarily serves as a site-directed homing mechanism and activation trigger for subsequent Treg functions.

Furthermore, our study demonstrated an excellent safety profile, with no treatment-related adverse effects observed throughout the investigation. This finding is consistent with previous studies examining various CAR Treg therapies across multiple disease models, which have consistently reported an absence of adverse effects.^{13,32} We propose that this favorable safety profile stems from 4 key factors: (1) the optimized CAR construct design, which enhances specificity, increases activation precision, and reduces cellular heterogeneity, thereby mitigating risks of cytokine release syndrome, neurotoxicity, and off-target tissue damage¹³; (2) the use of a low cell dose, which minimizes the potential for adverse effects; (3) the targeted localization of FCTRs to the infarcted heart, in which FAP expression is significantly upregulated, effectively limiting off-target activity and preventing systemic immunosuppression; and (4) the self-limiting persistence, which provides sufficient therapeutic duration during remodeling while avoiding chronic toxicity.

Our study establishes FAP-targeting CAR Tregs with enhanced IL-10 expression as an innovative strategy to improve cardiac repair and functional recovery after MI. Figure 6 integrates established concepts from previous literature; all depicted key mechanisms are directly supported by our experimental findings. This study delivers the first proof-of-concept that these engineered cells address a key unmet need in cardiology, simultaneous resolution of fibrosis and inflammation, and establish a translational platform for developing precision immunotherapies targeting fibrotic diseases in diverse organ systems.

Future studies should use single-cell RNA sequencing of infarct-zone Tregs, comparing FCTRs versus mock/polyclonal controls from matched cardiac microenvironments, to define CAR-specific transcriptional programs governing cardiac tissue repair. This methodology will resolve CAR-specific transcriptional signatures by identifying differentially expressed genes mediating

tissue repair after MI, advancing next-generation precision therapies.

ARTICLE INFORMATION

Received August 13, 2025; accepted December 8, 2025.

Affiliations

Department of Cardiology (M.Z., Y.Q., T.Z., M.L., T.T., N.X., S.N., B.L., Z.Z., J.J., M.G., J.L., X.C.), Hubei Key Laboratory of Biological Targeted Therapy (M.Z., Y.Q., T.Z., M.L., T.T., N.X., S.N., B.L., Z.Z., J.J., M.G., J.L., X.C.), Hubei Provincial Engineering Research Center of Immunological Diagnosis and Therapy for Cardiovascular Diseases (M.Z., Y.Q., T.Z., M.L., T.T., N.X., S.N., B.L., Z.Z., J.J., M.G., J.L., X.C.), Department of Integrated Traditional Chinese and Western Medicine (D.H.), and Department of Ultrasound (L.Z.), Union Hospital, and Division of Cardiology, Department of Internal Medicine, Tongji Hospital (C.C.), Department of Immunology, School of Basic Medicine (W.W.), and Departments of Epidemiology and Biostatistics (C.W.) and Nutrition and Food Hygiene (Z.S.), School of Public Health, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. Clinical Research Center for Medical Imaging in Hubei Province, Wuhan, China (L.Z.). Hubei Province Key Laboratory of Molecular Imaging, Wuhan, China (L.Z.).

Acknowledgments

The authors thank EditSprings (<https://www.editsprings.cn>) for linguistic assistance.

Author Contributions

Drs M. Zhang and Qin: study design, experimentation, analysis, and drafting; Drs Zhou and Liu: methodology; Drs Tang, Xia, Nie, Lv, Zhu, Jiao, Gu, and Li: data and revision support; Drs Chen, Hu, W. Wang, L. Zhang, and C. Wang: resources and supervision; Dr Cheng: project supervision and manuscript review.

Sources of Funding

This work was supported by grants from the National Natural Science Foundation of China (grants 82030016, 82230011, and 82450004 to Dr Cheng, and 81600187 to Dr Zhang).

Disclosures

None.

Supplemental Material

ARRIVE checklist

Methods

Tables S1–S2

Figures S1–S13

Uncropped gel blots

References 48–50

REFERENCES

1. Tsao CW, Aday AW, Almarzooq ZI, Anderson CAM, Arora P, Avery CL, Baker-Smith CM, Beaton AZ, Boehme AK, Buxton AE, et al; American Heart Association Council on Epidemiology and Prevention Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics: 2023 update: a report from the American Heart Association. *Circulation*. 2023;147:e93–e621. doi: 10.1161/CIR.0000000000001123
2. Frangogiannis NG. The extracellular matrix in myocardial injury, repair, and remodeling. *J Clin Invest*. 2017;127:1600–1612. doi: 10.1172/JCI87491
3. Sun K, Li YY, Jin J. A double-edged sword of immuno-microenvironment in cardiac homeostasis and injury repair. *Signal Transduct Target Ther*. 2021;6:79. doi: 10.1038/s41392-020-00455-6
4. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, Caforio ALP, Crea F, Goudevenos JA, Halvorsen S, et al; ESC Scientific Document Group. 2017 ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the task force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J*. 2018;39:119–177. doi: 10.1093/eurheartj/ehx393
5. Saxena A, Dobaczewski M, Rai V, Haque Z, Chen W, Li N, Frangogiannis NG. Regulatory T cells are recruited in the infarcted mouse myocardium and may modulate fibroblast phenotype and function. *Am J Physiol Heart Circ Physiol*. 2014;307:H1233–H1242. doi: 10.1152/ajpheart.00328.2014
6. Weirather J, Hofmann UD, Beyersdorf N, Ramos GC, Vogel B, Frey A, Ertl G, Kerkau T, Frantz S. Foxp3+ CD4+ T cells improve healing after myocardial infarction by modulating monocyte/macrophage differentiation. *Circ Res*. 2014;115:55–67. doi: 10.1161/CIRCRESAHA.115.303895
7. Nahrendorf M, Swirski FK. Regulating repair: regulatory T cells in myocardial infarction. *Circ Res*. 2014;115:7–9. doi: 10.1161/CIRCRESAHA.114.304295
8. Alshabaki YK, Nayer B, Lu YZ, Salimova E, Lau SN, Tan JL, Amann-Zalcenstein D, Hickey PF, Del Monte-Nieto G, Vasanthakumar A, et al. Tregs delivered post-myocardial infarction adopt an injury-specific phenotype promoting cardiac repair via macrophages in mice. *Nat Commun*. 2024;15:6480. doi: 10.1038/s41467-024-50806-y
9. Delgobo M, Weiß E, Ashour D, Richter L, Popokowski L, Arampatzis P, Stangl V, Arias-Loza P, Mariotti-Ferrandiz E, Rainer PP, et al. Myocardial milieu favors local differentiation of regulatory T cells. *Circ Res*. 2023;132:565–582. doi: 10.1161/CIRCRESAHA.122.322183
10. Xia N, Lu Y, Gu M, Li N, Liu M, Jiao J, Zhu Z, Li J, Li D, Tang T, et al. A unique population of regulatory T cells in heart potentiates cardiac protection from myocardial infarction. *Circulation*. 2020;142:1956–1973. doi: 10.1161/CIRCULATIONAHA.120.046789
11. Hofmann U, Frantz S. Role of T-cells in myocardial infarction. *Eur Heart J*. 2016;37:873–879. doi: 10.1093/euroheartj/ehv639
12. Raffin C, Vo LT, Bluestone JA. T(reg) cell-based therapies: challenges and perspectives. *Nat Rev Immunol*. 2020;20:158–172. doi: 10.1038/s41577-019-0232-6
13. Eskandari SK, Daccache A, Azzi JR. Chimeric antigen receptor Treg therapy in transplantation. *Trends Immunol*. 2024;45:48–61. doi: 10.1016/j.it.2023.11.005
14. Wang LC, Lo A, Scholler J, Sun J, Majumdar RS, Kapoor V, Antzis M, Cotner CE, Johnson LA, Durham AC, et al. Targeting fibroblast activation protein in tumor stroma with chimeric antigen receptor T cells can inhibit tumor growth and augment host immunity without severe toxicity. *Cancer Immunol Res*. 2014;2:154–166. doi: 10.1158/2326-6066.CIR-13-0027
15. MacDonald KG, Hoeppli RE, Huang Q, Gillies J, Luciani DS, Orban PC, Brody R, Levings MK. Alloantigen-specific regulatory T cells generated with a chimeric antigen receptor. *J Clin Invest*. 2016;126:1413–1424. doi: 10.1172/JCI82771
16. Doglio M, Ugolini A, Bercher-Brayer C, Camisa B, Toma C, Norata R, Del Rosso S, Greco R, Ciceri F, Sanvitto F, et al. Regulatory T cells expressing CD19-targeted chimeric antigen receptor restore homeostasis in systemic lupus erythematosus. *Nat Commun*. 2024;15:2542. doi: 10.1038/s41467-024-46448-9
17. Aghajanian H, Kimura T, Rurik JG, Hancock AS, Leibowitz MS, Li L, Scholler J, Monslow J, Lo A, Han W, et al. Targeting cardiac fibrosis with engineered T cells. *Nature*. 2019;573:430–438. doi: 10.1038/s41586-019-1546-z
18. Tran E, Chinnasamy D, Yu Z, Morgan RA, Lee CC, Restifo NP, Rosenberg SA. Immune targeting of fibroblast activation protein triggers recognition of multipotent bone marrow stromal cells and cachexia. *J Exp Med*. 2013;210:1125–1135. doi: 10.1084/jem.20130110
19. Rurik JG, Tombacz I, Yadegari A, Mendez Fernandez PO, Shewale SV, Li L, Kimura T, Soliman OY, Papp TE, Tam YK, et al. CAR T cells produced in vivo to treat cardiac injury. *Science*. 2022;375:91–96. doi: 10.1126/science.abm0594
20. Fu X, Khalil H, Kanisicak O, Boyer JG, Vagnozzi RJ, Maliken BD, Sargent MA, Prasad V, Valiente-Alandi I, Blaxall BC, et al. Specialized fibroblast differentiated states underlie scar formation in the infarcted mouse heart. *J Clin Invest*. 2018;128:2127–2143. doi: 10.1172/JCI98215
21. Wang J, Du H, Xie W, Bi J, Zhang H, Liu X, Wang Y, Zhang S, Lei A, He C, et al. CAR-macrophage therapy alleviates myocardial ischemia–reperfusion injury. *Circ Res*. 2024;135:1161–1174. doi: 10.1161/CIRCRESAHA.124.325212
22. Yoshida S, Hayashi H, Kawahara T, Katsuki S, Kimura M, Hino R, Sun J, Nakamaru R, Tenma A, Toyoura M, et al. A vaccine against fibroblast activation protein improves murine cardiac fibrosis by preventing the accumulation of myofibroblasts. *Circ Res*. 2025;136:26–40. doi: 10.1161/CIRCRESAHA.124.325017
23. Benmbebarek MR, Karches CH, Cadilhac BL, Lesch S, Endres S, Kobold S. Killing mechanisms of chimeric antigen receptor (CAR) T cells. *Int J Mol Sci*. 2019;20:1283. doi: 10.3390/ijms20061283
24. Kanisicak O, Khalil H, Ivey MJ, Karch J, Maliken BD, Correll RN, Brody MJ, Lin SCJ, Aronow BJ, Tallquist MD, et al. Genetic lineage tracing defines myofibroblast origin and function in the injured heart. *Nat Commun*. 2016;7:12260. doi: 10.1038/ncomms12260
25. Boroughs AC, Larson RC, Choi BD, Bouffard AA, Riley LS, Schiferle E, Kulkarni AS, Cetrulo CL, Ting D, Blazar BR, et al. Chimeric antigen receptor

- costimulation domains modulate human regulatory T cell function. *JCI Insight*. 2019;5:e126194. doi: 10.1172/jci.insight.126194
26. Sun Y, Ma M, Cao D, Zheng A, Zhang Y, Su Y, Wang J, Xu Y, Zhou M, Tang Y, et al. Inhibition of Fap promotes cardiac repair by stabilizing BNP. *Circ Res*. 2023;132:586–600. doi: 10.1161/CIRCRESAHA.122.320781
 27. Jung M, Ma Y, Iyer RP, DeLeon-Pennell KY, Yabluchanskiy A, Garrett MR, Lindsey ML. IL-10 improves cardiac remodeling after myocardial infarction by stimulating M2 macrophage polarization and fibroblast activation. *Basic Res Cardiol*. 2017;112:33. doi: 10.1007/s00395-017-0622-5
 28. Verma SK, Garikipati VNS, Krishnamurthy P, Schumacher SM, Grisanti LA, Cimini M, Cheng Z, Khan M, Yue Y, Benedict C, et al. Interleukin-10 inhibits bone marrow fibroblast progenitor cell-mediated cardiac fibrosis in pressure-overloaded myocardium. *Circulation*. 2017;136:940–953. doi: 10.1161/CIRCULATIONAHA.117.027889
 29. Ouyang W, Rutz S, Crellin NK, Valdez PA, Hymowitz SG. Regulation and functions of the IL-10 family of cytokines in inflammation and disease. *Annu Rev Immunol*. 2011;29:71–109. doi: 10.1146/annurev-immunol-031210-101312
 30. Liesz A, Suri-Payer E, Veltkamp C, Doerr H, Sommer C, Rivest S, Giese T, Veltkamp R. Regulatory T cells are key cerebroprotective immunomodulators in acute experimental stroke. *Nat Med*. 2009;15:192–199. doi: 10.1038/nm.1927
 31. Sharir R, Semo J, Shaish A, Landa-Rouben N, Entin-Meer M, Keren G, George J. Regulatory T cells influence blood flow recovery in experimental hindlimb ischaemia in an IL-10-dependent manner. *Cardiovasc Res*. 2014;103:585–596. doi: 10.1093/cvr/cvu159
 32. Mukhatayev Z, Dellacecca ER, Cosgrove C, Shivde R, Jaishankar D, Pontarolo-Maag K, Eby JM, Henning SW, Ostapchuk YO, Cedercreutz K, et al. Antigen specificity enhances disease control by Tregs in vitiligo. *Front Immunol*. 2020;11:581433. doi: 10.3389/fimmu.2020.581433
 33. Blat D, Zigmund E, Alteber Z, Waks T, Eshhar Z. Suppression of murine colitis and its associated cancer by carcinoembryonic antigen-specific regulatory T cells. *Mol Ther*. 2014;22:1018–1028. doi: 10.1038/mt.2014.41
 34. Skuljec J, Chmielewski M, Happel C, Habener A, Busse M, Abken H, Hansen G. Chimeric antigen receptor redirected regulatory T cells suppress experimental allergic airway inflammation, a model of asthma. *Front Immunol*. 2017;8:1125. doi: 10.3389/fimmu.2017.01125
 35. Shirakawa K, Endo J, Kataoka M, Katsumata Y, Yoshida N, Yamamoto T, Isobe S, Moriyama H, Goto S, Kitakata H, et al. IL (interleukin)-10-STAT3-galectin-3 axis is essential for osteopontin-producing reparative macrophage polarization after myocardial infarction. *Circulation*. 2018;138:2021–2035. doi: 10.1161/CIRCULATIONAHA.118.035047
 36. Yap J, Irei J, Lozano-Gerona J, Vanaprucks S, Bishop T, Boisvert WA. Macrophages in cardiac remodelling after myocardial infarction. *Nat Rev Cardiol*. 2023;20:373–385. doi: 10.1038/s41569-022-00823-5
 37. Zaiss DMW, Gause WC, Osborne LC, Artis D. Emerging functions of amphiregulin in orchestrating immunity, inflammation, and tissue repair. *Immunity*. 2015;42:216–226. doi: 10.1016/j.immuni.2015.01.020
 38. Ito M, Komai K, Mise-Omata S, Iizuka-Koga M, Noguchi Y, Kondo T, Sakai R, Matsuo K, Nakayama T, Yoshie O, et al. Brain regulatory T cells suppress astrogliosis and potentiate neurological recovery. *Nature*. 2019;565:246–250. doi: 10.1038/s41586-018-0824-5
 39. Liu J, Pan L, Hong W, Chen S, Bai P, Luo W, Sun X, He F, Jia X, Cai J, et al. GPR174 knockdown enhances blood flow recovery in hindlimb ischemia mice model by upregulating AREG expression. *Nat Commun*. 2022;13:7519. doi: 10.1038/s41467-022-35159-8
 40. Fritzsche E, Volk HD, Reinke P, Abou-El-Enein M. Toward an optimized process for clinical manufacturing of CAR-Treg cell therapy. *Trends Biotechnol*. 2020;38:1099–1112. doi: 10.1016/j.tibtech.2019.12.009
 41. Snider JC, Riley LA, Mallory NT, Bersi MR, Umbarkar P, Gautam R, Zhang Q, Mahadevan-Jansen A, Hatzopoulos AK, Maroteaux L, et al. Targeting 5-HT(2B) receptor signaling prevents border zone expansion and improves microstructural remodeling after myocardial infarction. *Circulation*. 2021;143:1317–1330. doi: 10.1161/CIRCULATIONAHA.120.051517
 42. Fan Z, Guan J. Antifibrotic therapies to control cardiac fibrosis. *Biomater Res*. 2016;20:13. doi: 10.1186/s40824-016-0060-8
 43. Khalil H, Kanisicak O, Prasad V, Correll RN, Fu X, Schips T, Vagozzi RJ, Liu R, Huynh T, Lee SJ, et al. Fibroblast-specific TGF- β -Smad2/3 signaling underlies cardiac fibrosis. *J Clin Invest*. 2017;127:3770–3783. doi: 10.1172/JCI94753
 44. Santos-Zas I, Lemarié J, Zlatanova I, Cachanado M, Seghezzi JC, Benamer H, Goube P, Vandestienne M, Cohen R, Ezzo M, et al. Cytotoxic CD8+ T cells promote granzyme B-dependent adverse post-ischemic cardiac remodeling. *Nat Commun*. 2021;12:1483. doi: 10.1038/s41467-021-21737-9
 45. Wernly B, Paar V, Aigner A, Pilz PM, Podesser BK, Förster M, Jung C, Pinon Hofbauer J, Tockner B, Wimmer M, et al. Anti-CD3 antibody treatment reduces scar formation in a rat model of myocardial infarction. *Cells*. 2020;9:295. doi: 10.3390/cells9020295
 46. Frangogiannis NG. The inflammatory response in myocardial injury, repair, and remodelling. *Nat Rev Cardiol*. 2014;11:255–265. doi: 10.1038/nrcardio.2014.28
 47. Henderson NC, Rieder F, Wynn TA. Fibrosis: from mechanisms to medicines. *Nature*. 2020;587:555–566. doi: 10.1038/s41586-020-2938-9
 48. Vimond N, Lasselin J, Anegon I, Guillonneau C, Bezie S. Genetic engineering of human and mouse CD4(+) and CD8(+) Tregs using lentiviral vectors encoding chimeric antigen receptors. *Mol Ther Methods Clin Dev*. 2021;20:69–85. doi: 10.1016/j.omtm.2020.11.008
 49. Cavasin MA, Sankey SS, Yu AL, Menon S, Yang XP. Estrogen and testosterone have opposing effects on chronic cardiac remodeling and function in mice with myocardial infarction. *Am J Physiol Heart Circ Physiol*. 2003;284:H1560–H1569. doi: 10.1152/ajpheart.01087.2002
 50. Lindsey ML, Brunt KR, Kirk JA, Kleinbongard P, Calvert JW, de Castro Bras LE, DeLeon-Pennell KY, Del Re DP, Frangogiannis NG, Frantz S, et al. Guidelines for in vivo mouse models of myocardial infarction. *Am J Physiol Heart Circ Physiol*. 2021;321:H1056–H1073. doi: 10.1152/ajpheart.00459.2021