

HEALTH AND MEDICINE

Targeted anti-IL-1 β platelet microparticles for cardiac detoxing and repairZhenhua Li^{1,2*}, Shiqi Hu^{1,2*}, Ke Huang¹, Teng Su^{1,2}, Jhon Cores^{1,2}, Ke Cheng^{1,2†}

An acute myocardial infarction (AMI) induces a sterile inflammatory response that facilitates further heart injury and promotes adverse cardiac remodeling. Interleukin-1 β (IL-1 β) plays a central role in the sterile inflammatory response that results from AMI. Thus, IL-1 β blockage is a promising strategy for treatment of AMI. However, conventional IL-1 β blockers lack targeting specificity. This increases the risk of serious side effects. To address this problem herein, we fabricated platelet microparticles (PMs) armed with anti-IL-1 β antibodies to neutralize IL-1 β after AMI and to prevent adverse cardiac remodeling. Our results indicate that the infarct-targeting PMs could bind to the injured heart, increasing the number of anti-IL-1 β antibodies therein. The anti-IL-1 β platelet PMs (IL1-PMs) protect the cardiomyocytes from apoptosis by neutralizing IL-1 β and decreasing IL-1 β -driven caspase-3 activity. Our findings indicate that IL1-PM is a promising cardiac detoxification agent that removes cytotoxic IL-1 β during AMI and induces therapeutic cardiac repair.

INTRODUCTION

Acute myocardial infarctions (AMIs), principally caused by the occlusion of a coronary artery, are a major cause of death and disability worldwide (1). MIs induce the reduction of blood flow to heart muscles and result in myocardial necrosis (2, 3). The myocardial necrosis triggers a sterile inflammatory response that contributes to adverse left ventricular (LV) remodeling and heart failure (4–6). Therefore, inhibition of the inflammatory response might serve as a potent strategy for the prevention of adverse cardiac remodeling and eventual heart failure.

During the course of an immune response, inflammasomes closely regulate the activation of caspase-1, an enzyme that is primarily responsible for processing and activating powerful proinflammatory cytokines, such as interleukin-1 β (IL-1 β) and IL-18 (7). Among these proinflammatory cytokines, IL-1 β plays a central role in the sterile inflammatory response resulting from MI by promoting the synthesis of other proinflammatory cytokines, activating profibrotic pathways, and promoting cardiomyocyte apoptosis (8). The interest in IL-1 β as a therapeutic target has led to the development of several IL-1 β blockers that interrupt IL-1 signaling. These blockers include IL-1 receptor antagonists, anti-IL-1 β -neutralizing antibodies, and decoy receptors (9). However, none of the IL-1 β blockers have been approved for clinical application in patients with MI at the present time. That includes Canakinumab (Ilaris), an IL-1 β antibody that entered the clinical stage [Anti-Inflammatory Thrombosis Outcomes Study (CANTOS)] in 2017 (10, 11). One major reason for the lack of successful clinical candidates is the risky safety profile of IL-1 β blockers. The application of IL-1 β blockers may increase the risk of fatal infections due to their lack of targeting capacity, which blunts the body's local and systemic inflammatory response to infection (12, 13). To overcome these limitations, we introduce a targeting group to IL-1 β blockers to enhance their accumulation in the site

of disease, to reduce the risk of side effects, and to improve therapeutic efficiency.

In our previous studies, we demonstrated that it is possible to take advantage of the natural infarct-homing abilities of platelet membranes by using them to decorate stem cells and particles for the targeted repair of injured hearts (14–18). Inspired by these findings, in this study, we develop a platelet-mimicking system that uses anti-IL-1 β -neutralizing antibodies. This system functions as an IL-1 β decoy that reduces the local inflammatory response in the injured heart (Fig. 1) in a targeted way. To capture the IL-1 β , a potent monoclonal antibody, Gevokizumab, was used. Gevokizumab (also called XOMA 052 and developed by XOMA Corporation) is an anti-inflammatory agent that has been used in clinical trials to treat acne vulgaris, osteoarthritis, Bechet's uveitis, pyoderma gangrenosum, and Bechet's disease (19–21). It was first modified for the binding of platelets using 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-poly(ethylene glycol) (DSPE-PEG) derivatives. Taking advantage of the infarct-homing ability of platelets, the IL-1 β decoy would be transported via the circulation to the MI area, where it would neutralize IL-1 β and, thus, prevent adverse cardiac remodeling and eventual heart failure. We anticipate that this study will create new possibilities for the successful implementation of IL-1 β blocker therapy in MI therapy.

RESULTS

Fabrication of anti-IL-1 β platelet microparticles

To create the platelet-Gevokizumab linkage, we bound *N*-hydroxysuccinimide (NHS)-terminated DSPE-PEG polymers to the platelet membranes. The Gevokizumab antibodies were then covalently bound to the NHS ends. We used SDS-polyacrylamide gel electrophoresis (PAGE) to demonstrate the binding of Gevokizumab to the DSPE-PEG polymers (fig. S1). We detected both conjugated and nonconjugated antibodies. The higher molecular weight of the conjugated antibodies made them run down the SDS-PAGE gel at a slower pace than the nonconjugated antibodies. Once the coupling of DSPE-PEG and Gevokizumab was confirmed, we built Gevokizumab-decorated platelets by mixing the coupled DSPE-PEG-Gevokizumab with platelets to form anti-IL-1 β platelet microparticles (IL1-PMs).

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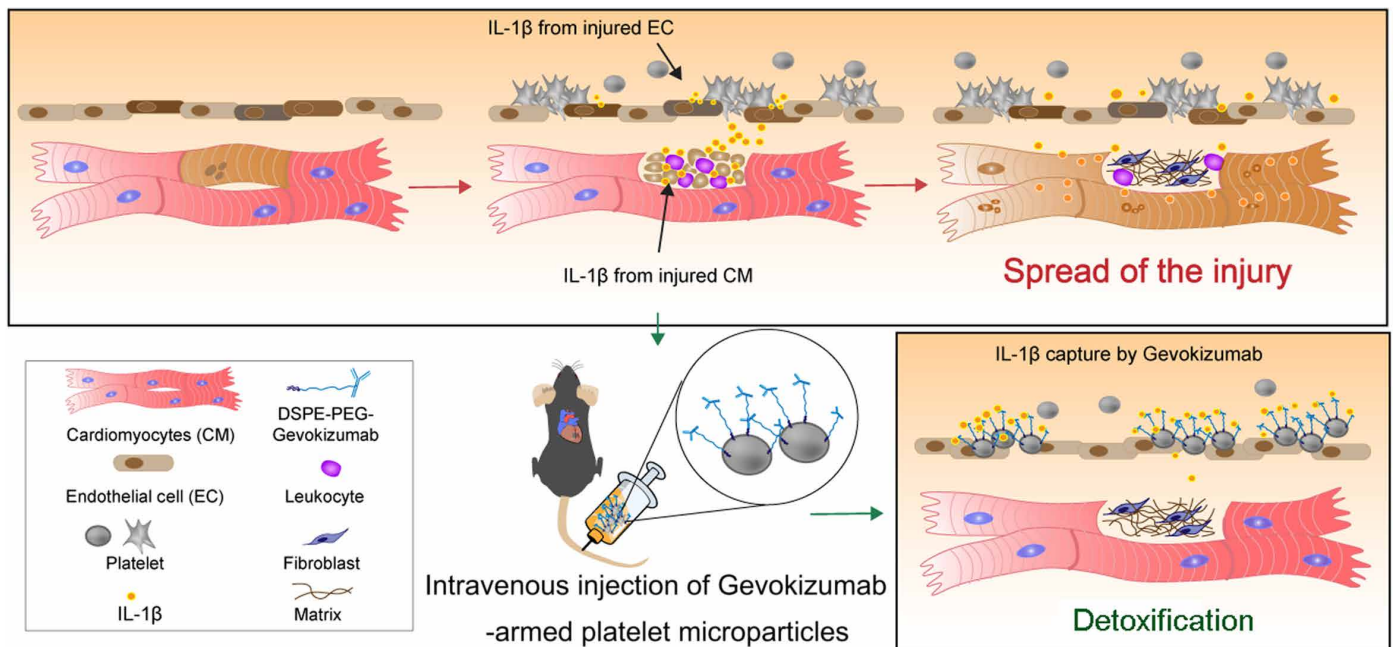


Fig. 1. Schematic diagram. Schematic illustrating the role of Gevokizumab-armed platelet microparticles as cardiac detoxification and repair agents.

Inactivated murine platelets were isolated as previously described (14–16). To prove the successful conjugation of Gevokizumab with platelets, we first used gold nanoparticle–labeled secondary antibodies to detect the Gevokizumab decorating on platelet surface. IL1-PMs were incubated with anti-CD42b antibodies (species: rabbit) overnight. Then, gold nanoparticle–labeled goat anti-mouse immunoglobulin G (IgG) antibodies (20 nm) and goat anti-rabbit IgG antibodies (20 nm) were used to bind anti-IL-1β and anti-CD42b primary antibodies, respectively. As shown in transmission electron microscope (TEM) images (fig. S2), both 20- and 10-nm gold nanoparticles were detected on the surface of platelets. The presence of 20-nm gold nanoparticles indicated that anti-IL-1β antibodies were conjugated onto platelets, while the introduction of 10-nm gold nanoparticles confirmed that the microparticles were actually derived from platelets (CD42b as a common platelet maker). Furthermore, scanning TEM and energy-dispersive x-ray mapping results confirmed that the attached nanoparticles were gold nanoparticles. In addition, we also used fluorescence-labeled secondary antibodies to detect Gevokizumab. Fluorescence microscopy and flow cytometry results showed that most of the platelets were modified by Gevokizumab with high arming efficiency (fig. S3). Then, we studied the hydrodynamic radius and zeta potential of the platelets before and after Gevokizumab decoration. The dynamic light scattering results showed a slight increase in particle size after Gevokizumab conjugation. We detected a number of much smaller particles that were mainly attributed to that part of platelets decomposed as the insertion of DSPE-PEG-Gevokizumab (fig. S4A). In contrast, there was no notable change in the zeta potential (fig. S4B). We also measured the binding ratio of Gevokizumab to platelets. Enzyme-linked immunosorbent assays (ELISAs) revealed that about 20 μg of Gevokizumab antibodies was conjugated to 10⁸ platelets (roughly 8.3 × 10⁵ antibodies per platelet) (fig. S4C). Despite the decoration of the platelet surface, expressions of platelet surface markers CD41, Glycoprotein VI (GPVI), and CD42b were not changed before and after antibody modification (fig. S5), which confirmed

the preservation of the integrity of the platelet membranes. Overall, linking anti-IL-1β antibodies to platelets had a slight effect on the platelets. To examine whether the IL1-PMs could capture IL-1β, we incubated different concentrations of fluorescence-labeled IL-1β with IL1-PMs. As shown in fig. S4D, the maximum detoxification rate that reduced the amount of IL-1β reached up to 28%.

Activity and binding ability of IL1-PM

Activated platelets tend to slowly degranulate and secrete their cytosolic and granule contents. In addition, such platelets, when introduced in vivo, are rapidly cleared by macrophagic and reticulo-endothelial activity (22). To see whether the platelets would be activated in the process of isolation and modification, we carried out platelet aggregometry, and the results confirmed the inactivation of platelets after modification (fig. S6A). In addition, we also detected the expression of P-selectin (a maker of platelet activation) using flow cytometry, and the result was consistent with the results from platelet aggregometry (fig. S6B). The first contact between circulating platelets and the vessel wall lesion (platelet tethering) was established by an interaction of the platelet receptor for von Willebrand factor (vWF) (GPIb-V-IX) with collagen-immobilized vWF (23). Direct GPVI-collagen interaction is important for initial platelet tethering and subsequent stable platelet adhesion and aggregation at sites of arterial injury. We have confirmed that IL1-PM preserved the CD42b (GPIb) and inactivated platelets should have the capacity of binding to the injured vessel as long as the binding molecules are present at the surface. Using coculture experiment, we confirmed that inactivated IL1-PM has the capacity to bind to denoted aorta (fig. S6, C to E).

Infarct-homing ability of IL1-PMs

Next, we studied the infarct-homing ability of IL1-PMs in C57BL/6 mice. The mouse MI model was first constructed through left anterior descending coronary (LAD) ligation, which has been widely used by our group (24, 25). The detection of IL1-PM migration was performed

in vivo using a live imager. The mice were injected either with the Gevokizumab antibodies conjugated to the DSPE-PEG-NHS polymers or with IL1-PMs. In both cases, the antibody ends were bound to Cy5.5 amine-reactive NHS esters for signal detection. We intravenously injected IL1-PM@Cy5.5 and antibody@Cy5.5 in the mice, with or without MI. Animals were imaged at indicated time intervals to determine the infarct-homing ability of the injected agents. As shown in Fig. 2A, 8 hours after the injection of IL1-PM@Cy5.5, we observed a sustained fluorescence signal in the injured heart. The signal intensity grew from the 8- to the 72-hour time point. In contrast, no obvious fluorescence was observed in the non-MI heart, indicating the infarct signal-driven migration of platelets to the heart area. In addition, the antibodies alone did not accumulate in the injured

heart, which further confirmed the infarct-targeting ability of the platelet vehicle. We then imaged and analyzed the ex vivo tissue biodistribution (Fig. 2B). The infarcted hearts treated with IL1-PM@Cy5.5 showed stronger fluorescent signals than the IL1-PM@Cy5.5-treated non-MI hearts, the hearts treated with antibody@Cy5.5, and the other organs analyzed. In addition, most of non-platelet-conjugated antibodies (antibody@Cy5.5) accumulated in the kidneys by the study end point. The quantitative region-of-interest analysis revealed that the IL1-PM@Cy5.5-treated infarcted hearts showed eightfold higher fluorescence intensity than normal, non-infarcted hearts (Fig. 2C). In addition, the circulation lifetime of IL1-PMs and naïve platelets in normal mice was studied. As shown in fig. S7, the concentrations of both platelets and IL1-PMs decreased

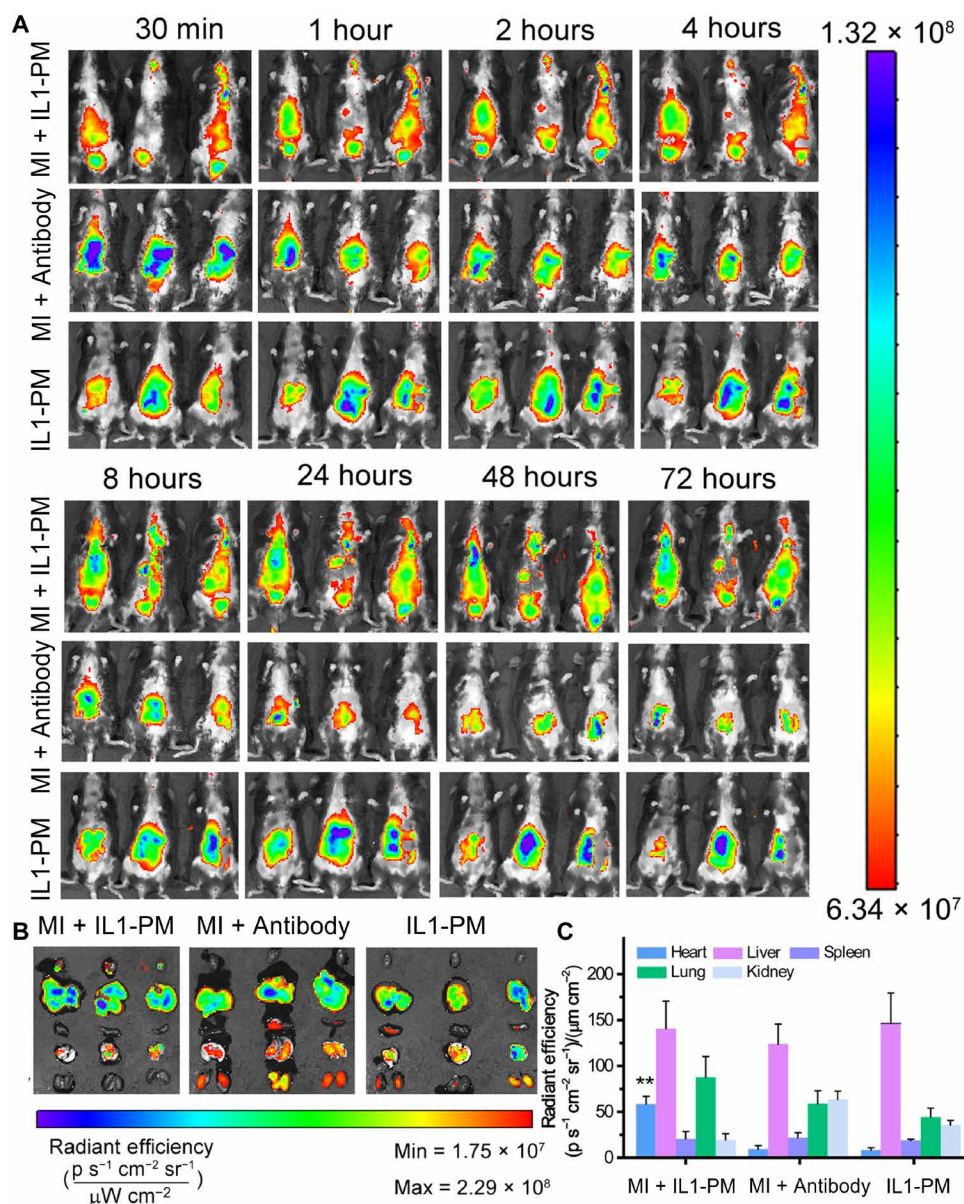


Fig. 2. Biodistribution of IL1-PMs in mice with acute MI. (A) In vivo fluorescent imaging of MI mice or sham mice at various time intervals after intravenous injection of IL1-PM@Cy5.5 or antibody@Cy5.5. **(B)** Ex vivo fluorescent imaging of the major organs excised from the treated animals. **(C)** Quantitative analysis of fluorescent intensity in the organs. Antibody, Gevokizumab; IL1-PM, Gevokizumab-armed platelet microparticles. $**P < 0.01$ indicates that the IL1-PM@Cy5.5-treated MI group is significantly different from the other groups.

with time. However, there is no significant difference between those two groups, indicating that DSPE-PEG-antibody modification has slight effects on circulation lifetime. Furthermore, all animals were subject to autopsy analysis upon euthanasia for signs of tumor growth in major organs, and those tests have returned with no abnormal findings (fig. S8A).

Anti-inflammatory outcomes of IL1-PM treatment

We next evaluated the *in vivo* anti-inflammatory ability of intravenously administered IL1-PM. To do this, we analyzed the levels of inflammatory cytokines present in the blood and the hearts of mice 3 days after treatment using a cytokine array. We compared four different treatment groups: phosphate-buffered saline (PBS), platelets, anti-IL-1 β antibodies alone (antibody), and IL1-PM. As shown in Fig. 3A, after correcting for background intensity and normalizing to the membrane's positive control, five cytokines/proteins were found significantly changed in mouse blood after Gevokizumab and IL1-PM treatment, including IL-1 β , CXCL1, granulocyte colony-stimulating factor, IL-5, and IL-4. Compared to the antibody group, the IL1-PM group significantly reduced the level of IL-1 β , indicating the high affinity of the IL1-PMs to the IL-1 β (Fig. 3B). Furthermore, we detected the level of IL-1 β in treated heart tissues using ELISA. The results mirrored those of the blood detection results (Fig. 3C), and the neutralizing effects reached a plateau at 20 mg/kg and further increase in dose had no significant benefits (fig. S8, B and C). To that end, we used the dose of 20 mg/kg in our study. Since IL-1 β production leads to increased levels of IL-6, we also assessed whether the neutralization of IL-1 β reduced the levels of IL-6. As indicated by the cytokine array summarized in Fig. 3B, there was no significant difference in IL-6 expression levels among any of the treatment groups. To further verify this, we tested for IL-6 expression using an ELISA, which has a higher level of sensitivity than the cytokine array. The results were consistent with those of the

cytokine array. Both the antibody and the IL1-PM treatments had a negligible effect on the IL-6 levels (fig. S8D). One possible reason for the lack of dampening of IL-6 levels is the complexity of the inflammatory response, in which many cytokines are involved, including IL-1 α , IL-18, and tumor necrosis factor- α . Thus, blocking just one cytokine may not be enough to block the inflammatory cascade.

We further evaluated the anti-inflammatory effects of IL1-PM-mediated IL-1 β neutralization by quantifying the level of leukocyte infiltration in the injured heart. To do so, we looked at CD45, one of the most abundant leukocyte cell surface glycoproteins (26). We found no obvious difference in the levels of CD45 expression in heart tissue between these four treatment groups (Fig. 4A). That may be because IL-1 β may not be a key chemotaxis for leukocytes infiltrating during AMI in mice.

The next protein analyzed was caspase-1 since it is a key modulator of the inflammatory response to tissue injury, in addition to processing pro-IL-1 β to its active, mature form and inducing cardiac cell apoptosis. Caspase-1 activity was measured using two techniques: Western blot and cleavage of a fluorogenic substrate. Both techniques indicated that neutralizing IL-1 β had no effect on caspase-1 activity (Fig. 4, B and C). Furthermore, we tested for the inhibition of inflammasome in the injured hearts as a result of the IL-1 β neutralization. Heart sections were immunoassayed for apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC). However, no detectable differences were found in ASC expression between the four treatment groups, a result that was in accord with that of caspase-1 activity and CD45 expression (Fig. 4, D and E). To further test the effects of IL-1 β blockade on inflammatory response, we looked for the infiltration of macrophages and T cells in the infarct area. As shown in fig. S9, neither macrophage (F4/80⁺) nor T cell (CD3⁺) infiltrations were inhibited after neutralizing IL-1 β in mice via IL1-PMs or treating them with just antibodies, which was consistent with the results for CD45 infiltration. Together, these results

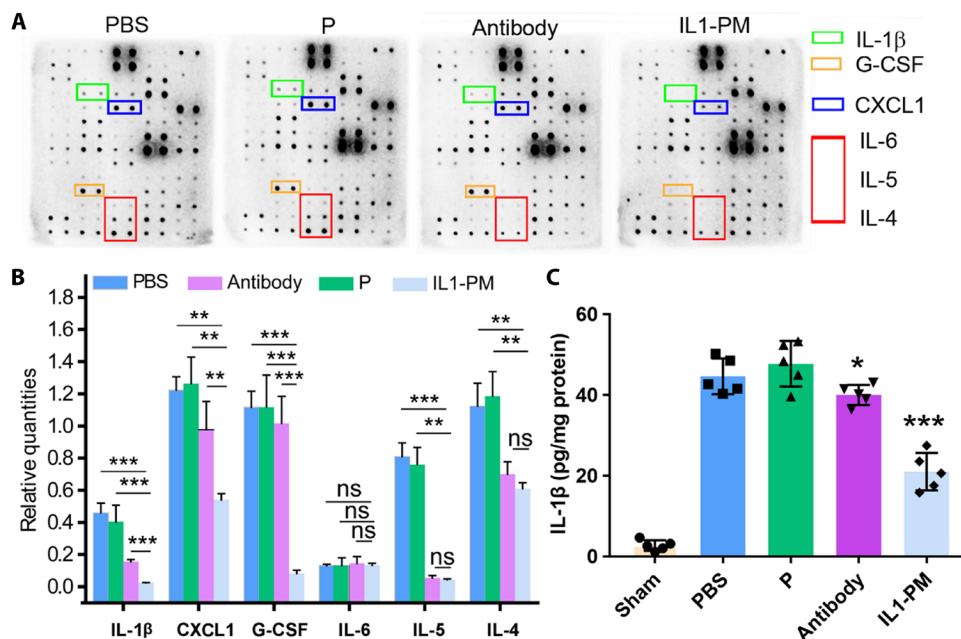


Fig. 3. Effects of IL1-PM treatment on inflammatory cytokines. (A) Cytokine array analysis of the systemic inflammatory cytokine level changes after 72 hours of treatment. (B) Quantitative summary of cytokine array analysis in (A). (C) Quantitative summary of the concentrations of IL-1 β in the heart as detected by ELISA ($n = 5$). P, platelets; G-CSF, granulocyte colony-stimulating factor; ns, not significant. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

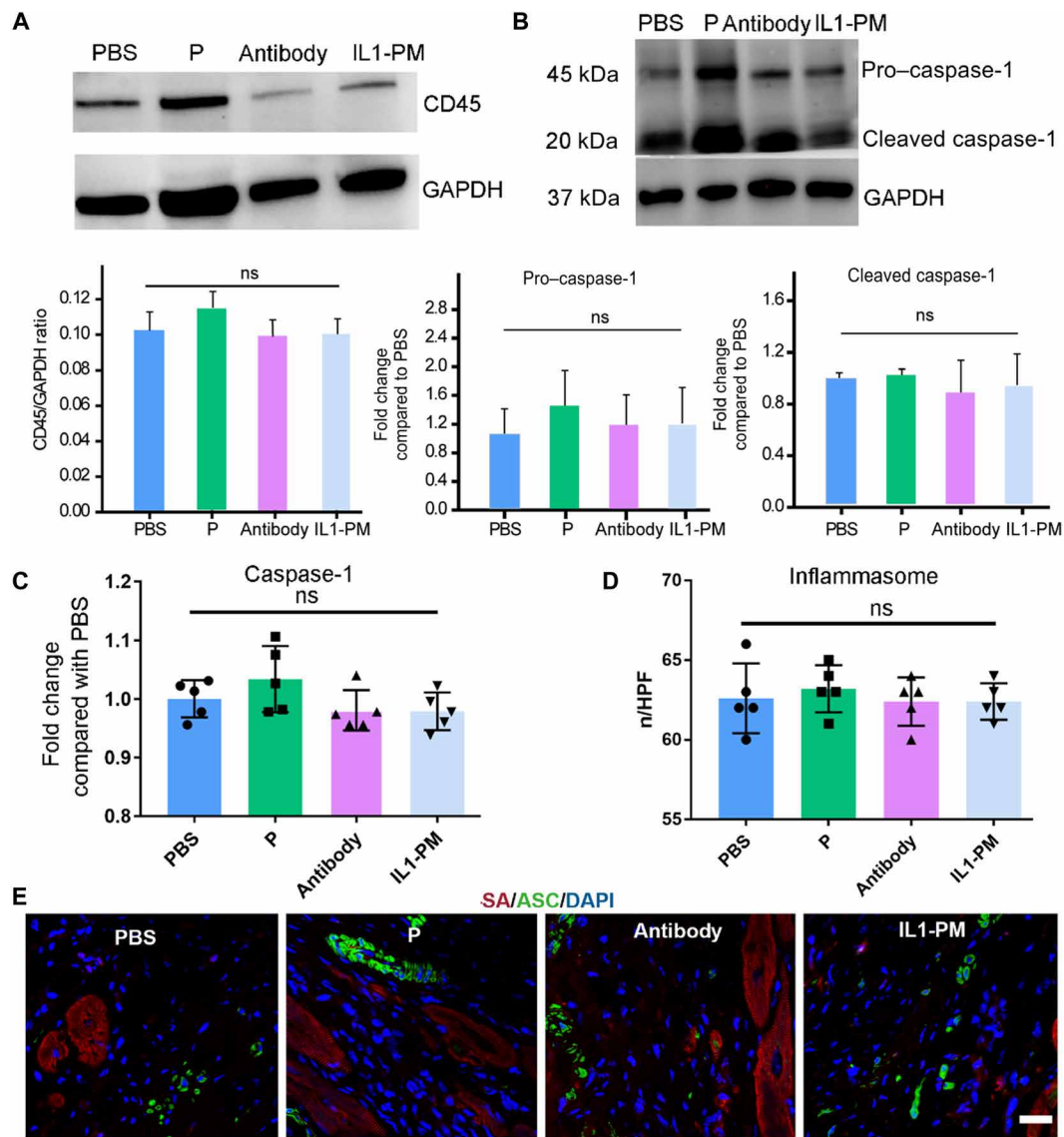


Fig. 4. Anti-inflammatory ability of IL1-PMs in heart tissue. Western blot results for CD45 (A) and cleaved caspase-1 (B) presence in the plasma 72 hours after surgery ($n = 3$). (C) Histogram summarizing caspase-1 (YVAD-AMC cleavage) activity normalized to the PBS group ($n = 5$). (D) Quantification of the number of ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain)-positive inflammasomes. (E) Representative image of the formation of ASC-containing inflammasomes 72 hours after MI. HPF, high-power field; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SA, sarcomeric actin; DAPI, 4',6-diamidino-2-phenylindole. Scale bar, 20 μ m.

confirmed that IL-1 β blockade had no significant effect on the formation of active inflammasomes or on the systemic inflammatory response. We found that neutralizing IL-1 β did not change the level of caspase-1 activity as well, indicating that the attenuation of cardiac remodeling was independent of caspase-1 activity. The lack of correlation can be attributed to the fact that caspase-1 acts upstream of the IL-1 β in the inflammatory pathway and it appears that neutralizing downstream IL-1 β had no feedback effect on the caspase-1. These results prompted us to investigate the effects of IL-1 β blockade on downstream enzymes instead.

IL1-PMs inhibit cardiomyocyte apoptosis

Caspase-3 is an effector of apoptosis that is located downstream of IL-1 β and is activated by it (27, 28). To evaluate the detoxification

efficiency of IL1-PMs, we measured the caspase-3 activity in the heart tissue, as a result of each of the four treatments, using Western blot and caspase-3 fluorometric assays. Figure 5A indicates that the level of cleaved caspase-3 (activated caspase-3) is reduced in the heart after antibody and IL1-PM treatment compared to the PBS and nonconjugated platelet controls. Furthermore, the IL1-PM treatment is a more effective inhibitor than the antibody treatment (Fig. 5B). In addition to caspase-3, we also detected the IL1-PM-driven inhibition of apoptosis using a terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) staining assay (Fig. 5, C and D). As expected, the IL1-PM treatment had the highest impact on the apoptosis of cardiomyocytes when compared with the other treatment groups, including antibody treatment alone, a result that was consistent with the results of

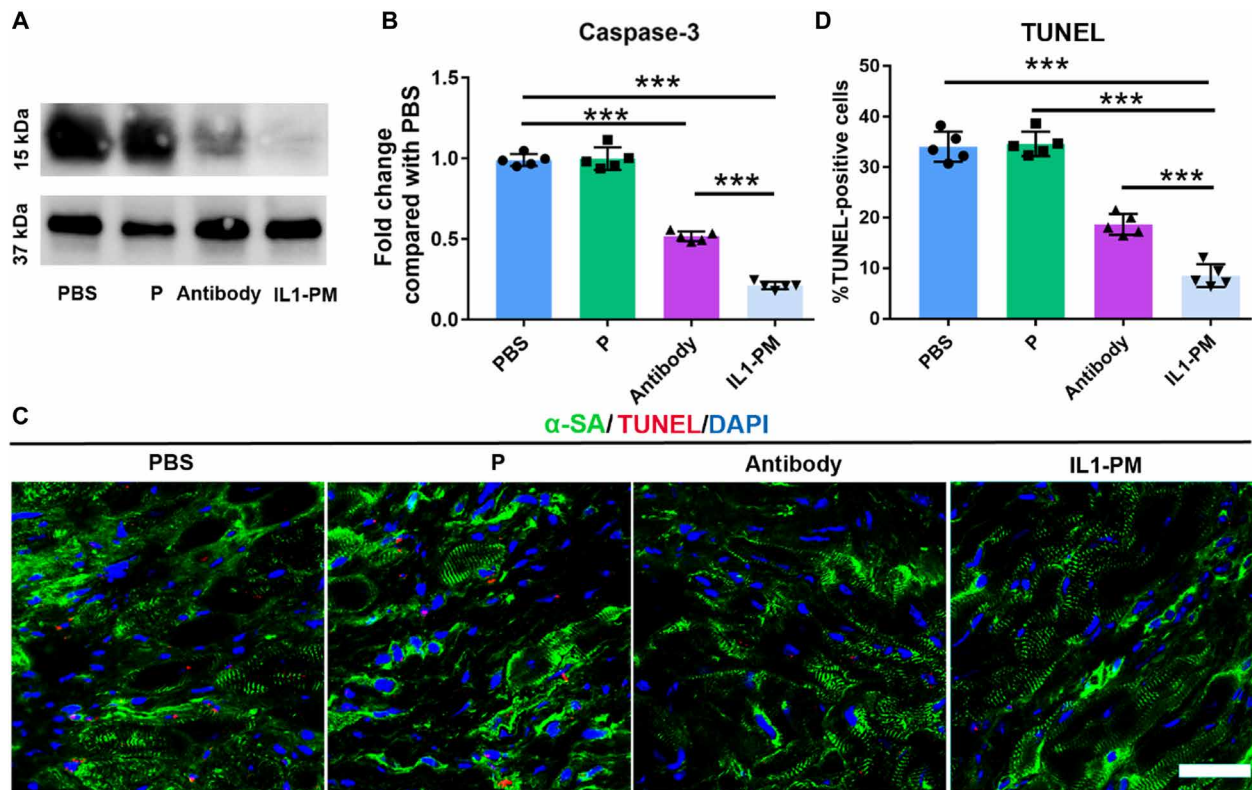


Fig. 5. IL1-PM treatment reduces cardiac apoptosis. (A) Expression of apoptosis-associated protein (caspase-3) analyzed by Western blot 72 hours after treatment. (B) Caspase-3 activity was evaluated using a fluorometric assay kit ($n = 5$). (C) TUNEL staining for cardiomyocyte apoptosis in the infarcted heart 3 days after MI. Scale bar, 20 μm . (D) Quantification of cardiomyocyte apoptosis. $***P < 0.001$.

caspase-3 inhibition. From these data, it is reasonable to conclude that the IL-1 β -neutralizing ability of IL1-PM reduces caspase-3 activity, which then more efficiently inhibits cardiomyocyte apoptosis.

IL1-PMs attenuate cardiac remodeling

After having demonstrated the capacity of IL1-PM to neutralize IL-1 β and protect cardiomyocytes, we then measured its effects on cardiac remodeling. We first investigated changes in heart morphometry using Masson's trichrome staining and found reduced collagen accumulation in the scarred segment of the myocardium (Fig. 6A). After quantification, we found that IL1-PM treatment was the most successful at protecting the heart and yielded more viable myocardium, with the smallest scar size, when compared to the antibody-treated group or the controls (Fig. 6, B and C). The attenuation of cardiac remodeling was reflected by a decrease in LV end-diastolic volume (LVEDV) and LV end-systolic volume (LVESV), accompanied by an increase in LV ejection fraction (LVEF) and LV fractional shortening (LVFS) as determined by transthoracic echocardiography (Fig. 6, D to G). These results confirmed that the neutralization of IL-1 β led to caspase-3 inhibition, reducing adverse cardiac remodeling after an AMI. Moreover, although some benefits were noted from antibody therapy alone, the introduction of infarct-homing platelets improved the anti-inflammatory efficacy of the therapy.

DISCUSSION

Cardiovascular disease remains as the primary killer in Western societies. Stem cell transplantation provides a promising method for

cardiac regeneration, but current therapies are limited by inefficient interaction between potentially beneficial cells and the injured tissue that is highly inflammatory. Ideal approaches are required to not only bring more "seeds" (therapeutic cells) to their targets but also improve the "soil," the inflammatory post-MI heart. IL-1 β plays a key role in triggering the inflammatory cascade in the infarcted myocardium. Thus, in this work, we developed an anti-IL-1 β antibody-platelet conjugate that serves as a cardiac detoxification and anti-inflammatory therapeutic for the treatment of AMI. Platelet microparticles were chosen as the antibody carriers because of their innate ability to find cardiac injury. Circulating platelets can bind to vessel lesion through the interaction of GPIb (CD42b) with vWF, which subsequently induces platelet activation in the infarct area. Later on, GPIIb/IIIa activation and P-selectin expression further trigger platelet aggregation. Ziegler *et al.* (29) developed scFvanti-GPIIb/IIIa functionalized positron emission tomography (PET) tracer for the detection of minimal cardiac ischemia through imaging of activated platelets in the infarct area. Furthermore, they also synthesized bispecific antibodies to deliver both anti-inflammatory molecular (30) and peripheral blood mononuclear cells for the treatment of MI (31). These studies provided the foundation that platelets were both promising targets and carriers in the treatment of heart injury (32). Our findings indicate that neutralizing IL-1 β protects cardiomyocytes from apoptosis by attenuating caspase-3 activity downstream of IL-1 β production and inhibiting the development of ventricular dilation after an AMI. Supported by our results, we believe that IL-1 β is a potential therapeutic target for the patients with AMI and that the conjugation of platelets with inflammation-neutralizing

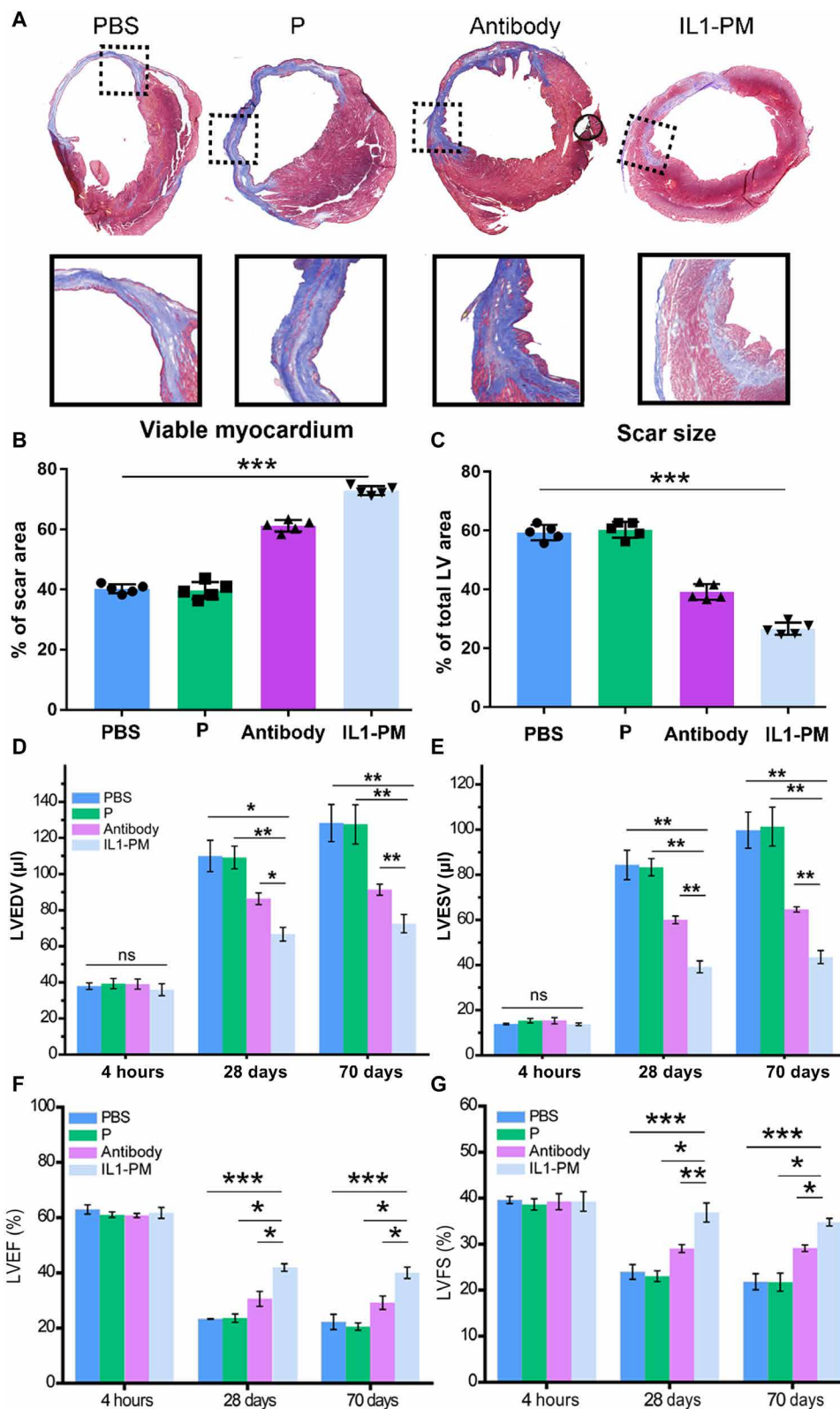


Fig. 6. IL1-PM treatment attenuates cardiac remodeling. (A) Representative Masson's trichrome staining of myocardial sections 70 days after treatment. Quantitative analyses of (B) viable myocardium and (C) scar size from the Masson's trichrome images. IL1-PM groups versus other three groups. (D) LVEDV and (E) LVESV measured by echocardiography 4 hours, 28 days, and 70 days after treatment ($n = 5$). (F) LVEFs and (G) LVFSs measured by echocardiogram at baseline (4 hours after MI), 28 days, and 70 days after treatment ($n = 5$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

antibodies provides a promising strategy for the treatment of cardiac injury.

Additional studies need to be done before this work can be translated. First, since activated platelets would induce aggregation, we need to keep the isolated platelets inactivated before application. Second, in the past decades, researchers have developed therapeutics that target various inflammatory pathways, such as pexelizumab for targeted cleavage of the C5 component, anti-P-selectin monoclonal antibody to inhibit P-selection (33), and Canakinumab to block IL-1 β . However, most of these anti-inflammatory strategies failed in clinical trials. Possible reasons for the failure are off-target effects and the inappropriate timing of administration. Third, in this work, antibodies were inserted into platelets using DSPE-PEG linkers with high grafting efficiency as indicated by our data. Nonetheless, future effort needs to be taken on improvement of the purity of platelets and lipid-modified antibodies to maintain an acceptable loading efficiency batch to batch. In addition, it is worth mentioning that our targeting strategy is based on the platelet platform, which is a biologic with high instability and contamination risk. Nonetheless, blood or platelet transfusion is common in current medical practice; therefore, the risk of using platelet derivatives can be managed. It will be beneficial to further fine-tune our system by developing a targeting strategy that does not rely on the use of live platelets, such as the use of defined platelet binding molecules for targeting. Fourth, owing to the semiquantitative nature of *in vivo* imaging system (IVIS), future pharmacokinetic studies should use more quantitative methods such as PET to determine the actual numbers of IL1-PMs in various organs. In addition, in the clinic, most of the patients with acute MI will receive percutaneous coronary intervention and then receive some other treatments. Thus, a LAD ligation model is not perfect to mimic the clinical status of the patients, while an ischemia/reperfusion (I/R) model may be more appropriate for the study of MI. Thus, in the future, an I/R model in both small and larger animal should be used for the study of MI.

MATERIALS AND METHODS

Gevokizumab-conjugated platelet microparticles (IL1-PMs)

DSPE can bind with the membranes of cells, liposomes, and platelets. The surface of the platelets was functionalized with anti-IL-1 β antibodies. The inactivated platelets were isolated according to our previous studies (14–16). Then, 10⁸ platelets were dispersed in 0.5 ml of PBS buffer with 1 \times 10⁻³ M EDTA and 2 \times 10⁻⁶ M prostaglandin E₁ (PGE₁), and then, DSPE-PEG–Gevokizumab was added at different concentrations. The mixture was stirred for 3 hours. Unconjugated DSPE-PEG–Gevokizumab was removed by centrifugation at 800g for 10 min. The IL1-PMs were then washed twice with PBS using centrifugation at 800g for 10 min.

Quantification of antibodies on platelets

The IL1-PM were resuspended in 100 μ l of deionized water and ultrasonicated to lyse the platelets and release the DSPE-PEG–Gevokizumab. The quantity of antibodies conjugated to the platelets was measured using ELISA. The numbers of antibodies per platelet were calculated using the equation

$$N(\text{antibody}) = \frac{m_{\text{antibody}} * NA}{M_w} / \text{Number of platelets}$$

Determining antibody arming efficiency on platelets

IL1-PMs were first incubated with anti-CD42b antibodies (species: rabbit) overnight. Then, gold nanoparticle–labeled goat anti-mouse IgG antibodies (20 nm) and goat anti-rabbit IgG antibodies (20 nm) were used to bind anti-IL-1 β and anti-CD42b primary antibodies, respectively. In addition, fluorescein isothiocyanate–labeled goat anti-mouse IgG antibodies were also used to confirm the presence of anti-IL-1 β on the surface of platelets. The free IgG antibodies were removed through centrifugation (10 min at 800g). The prepared samples were examined using TEM and fluorescence microscopy.

Studies on platelet activation

The expression of P-selectin was determined using flow cytometry. Freshly prepared platelets were activated with collagen as a positive control. Then, positive control and IL1-PM samples were incubated with phycoerythrin-labeled anti-P-selectin (12–0626–80, Thermo Fisher Scientific) overnight. Samples were washed three times with PBS.

Mouse model of MI

All animal work was compliant with the Institutional Animal Care and Use Committee of North Carolina State University. A MI model was constructed according to our previous work. (14–17)

Infarct-homing ability and biodistribution of IL1-PM

IL1-PM@Cy5.5 or antibody@Cy5.5 was intravenously injected into MI mice or normal (non-MI) mice ($n = 3$) at a dose of 2 mg of antibodies per kilogram of body weight. Animals were imaged after various time intervals for biodistribution analysis. Circulation lifetime of IL1-PM and platelets in normal mice was studied. A total of 10⁸ 1,1'-dioctadecyl-3,3',3'-tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt (DiD)–labeled IL1-PMs or platelets were intravenously injected into normal mice. At various time points, blood was drawn and measured for fluorescence intensity at 670 nm for evaluation of circulation lifetime of the agents.

Cytokine array analysis of systemic inflammatory cytokines

Plasma levels of cytokines were measured 3 days after treatment using a RayBiotech Inc. Supplier Diversity Partner MOUSE CYTOKINE ARRAY C3 (4), according to the manufacturer's instructions. In addition, the plasma levels of IL-6 were tested using an ELISA kit.

Statistical analysis

All experiments were performed independently at least three times. Results are shown as means \pm SD. Comparisons between any two groups were performed using the two-tailed, unpaired Student's *t* test. Comparisons among more than two groups were performed using one-way analysis of variance (ANOVA), followed by the post hoc Bonferroni test. Single, double, and triple asterisks represent $P < 0.05$, 0.01, and 0.001, respectively; $P < 0.05$ was considered statistically significant.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at <http://advances.sciencemag.org/cgi/content/full/6/6/eaay0589/DC1>

Supplementary Materials and Methods

Fig. S1. Gevokizumab modification.

Fig. S2. TEM characterization of IL1-PM.

Fig. S3. Arming of Gevokizumab onto platelets.

Fig. S4. Confirming antibody conjugation and detoxification rate.

Fig. S5. Platelet markers on IL1-PM.

Fig. S6. Inactivated platelet microparticles can bind to damaged vasculatures.

Fig. S7. Circulation lifetime of IL-1-PM in normal mice.

Fig. S8. Toxicity of IL-1-PM treatment and quantification of IL-1 β and IL-6 concentrations in the heart.

Fig. S9. Inflammatory response after treatments.

[View/request a protocol for this paper from Bio-protocol.](#)

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