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RESEARCH ARTICLE | *Cardiac Regeneration and Repair*

Antibodies aggravate the development of ischemic heart failure

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[1]*University Hospital Halle, Department of Internal Medicine-III, Halle, Germany;* [2]*University Hospital Würzburg, Department of Internal Medicine-I, Würzburg, Germany;* [3]*Instituto Gulbenkian de Ciencia, Oeiras, Portugal; and* [4]*Comprehensive Heart Failure Center, Würzburg, Germany*

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Keppner L, Heinrichs M, Rieckmann M, Demengeot J, Frantz S, Hofmann U, Ramos G. Antibodies aggravate the development of ischemic heart failure. *Am J Physiol Heart Circ Physiol* 315: H1358–H1367, 2018. First published August 10, 2018; doi:10.1152/ajpheart.00144.2018.—Heart-specific antibodies have been widely associated with myocardial infarction (MI). However, it remains unclear whether autoantibodies mediate disease progression or are a byproduct of cardiac injury. To disambiguate the role of immunoglobulins in MI, we characterized the development of ischemic heart failure in agammaglobulinemic mice ($AID^{-/-}\mu S^{-/-}$). Although these animals can produce functional B cells, they cannot synthesize secretory IgM ($\mu S^{-/-}$) or perform Ig class switching ($AID^{-/-}$), leading to complete antibody deficiency. Agammaglobulinemia did not affect overall post-MI survival but resulted in a significant reduction in infarct size. Echocardiographic analyses showed that, compared with wild-type infarcted control mice, $AID^{-/-}\mu S^{-/-}$ mice exhibited improved cardiac function and reduced remodeling on *day 56* post-MI. These differences remained significant even after animals with matched infarct sizes were compared. Infarcted $AID^{-/-}\mu S^{-/-}$ mice also showed reduced myocardial expression levels of transcripts known to promote adverse remodeling, such as matrix metalloproteinase-9, collagen type I a1, collagen type III a1, and IL-6. An unbiased screening of the heart reactivity potential in the plasma of wild-type MI animals revealed the presence of antibodies that target the myocardial scar and collagenase-sensitive epitopes. Moreover, we found that IgG accumulated within the scar tissues of infarcted mice and remained in close proximity with cells expressing Fcγ receptors (CD16/32), suggesting the existence of an in situ IgG-Fcγ receptor axis. Collectively, our study results confirm that antibodies contribute to ischemic heart failure progression and provide novel insights into the mechanisms underlying this phenomenon.

NEW & NOTEWORTHY Our study sheds some light on the long-standing debate over the relevance of autoantibodies in heart failure and might stimulate future research in the field. The observation of extracellular matrix-specific antibodies and the detection of Fcγ receptor-expressing cells within the scar provide novel insights into the mechanisms by which antibodies may contribute to adverse remodeling.

antibodies; heart failure; myocardial infarction; remodeling

INTRODUCTION

Myocardial infarction (MI) is the most common cause of death worldwide, accounting for 20% of all deaths in Europe (9). It is characterized by cardiac cell death after persistent ischemia. Adult hearts have poor regenerative potential. Thus, the repair mechanisms that occur in the infarcted myocardium cannot reestablish the status quo ante, and the viable myocardium that was lost during an acute MI is subsequently replaced by collagenous scar tissue. This situation often leads to adverse cardiac remodeling and chronic heart failure (HF) in later stages. Ischemic HF is a severe condition associated with high mortality rates and characterized by ventricular wall thinning, cardiac dilation, and severe contractile impairment (10).

Immunological phenomena have a deep influence on post-MI inflammation, healing, and remodeling processes. Macrophages and neutrophils readily invade the injured heart, where they promote the clearance of necrotic debris and contribute to in situ inflammation and tissue repair (24). MI can also induce specific immune responses because B and T lymphocytes also infiltrate the injured myocardium (15, 16, 20, 26). However, how adaptive immune mechanisms contribute to the progression of HF remains largely elusive.

Heart-specific antibodies have been widely detected in the plasma of rodents and patients with MI and ischemic HF (e.g., see Refs. 4–9, 17, and 27). The cardiac antigens targeted by these autoantibodies fall within three major categories: sarcomere proteins (e.g., anti-myosin, actin, and troponin) (27, 28), cardiac receptors (e.g., anti-β_1-adrenergic receptors) (30), and damage-associated epitopes (13). However, whether these autoantibodies should be perceived as disease-modifying agents or as simple biomarkers of cardiac injury remains under dispute.

Elevated myosin-specific antibody titers have previously been correlated with a poorer prognosis in patients with MI (27). However, Dangas et al. (6) further observed higher anti-myosin antibody titers in patients with larger infarct sizes and suggested that this could simply indicate large infarct sizes. Furthermore, the finding that myosin distribution is confined to the cytoplasmic space and, therefore, is not accessible to antibody targeting has led many authors to conclude that myosin antibodies have little effect on disease progression.

Patients with MI and ischemic HF can also produce autoantibodies targeting surface receptors, including β_1-adrenergic receptors (Adrb1), on cardiomyocytes (1, 7, 30). In principle, this specificity seems to have higher clinical relevance because antibodies targeting epitopes on the second extracellular loop of the Adrb1 receptor can exert agonistic effects and thereby modify cardiac function (30). Experimental studies have demonstrated that the administration of Adrb1-specific antibodies in rats promoted dilative cardiomyopathy (1). However, de-

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spite the meaningful mechanistic potential of this finding, these antibodies were detected in only a small subgroup of patients with ischemic HF (30), and their clinical significance remains uncertain.

Recent studies have further revealed that autoantibodies already present in healthy subjects before the onset of cardiac injury (termed natural autoantibodies) can recognize damage-associated molecular patterns (DAMPs) and readily accumulate in wounded tissues, including ischemic hearts (13, 25, 32). Experiments performed in the context of ischemia-reperfusion injury showed that natural IgMs can activate the complement cascade and fuel inflammation in ischemic tissues (13, 32). These experimental observations confirm that IgMs play a role in the early steps of MI. However, the causal link between autoantibodies and late cardiac remodeling has not been established.

In the present study, we sought to assess the development of ischemic HF in agammaglobulinemic ($AID^{-/-}\mu S^{-/-}$) mice for the sake of establishing the role of autoantibodies in this model. We found that transgenic mice lacking the capacity to secrete Igs (though retaining a preserved B cell compartment) present attenuated myocardial inflammation and fibrosis in response to MI.

METHODS

Animals

Male mice aged 8–12 wk old were included in this study. Wild-type (WT) C57Bl6/J mice were obtained from The Jackson Laboratory, and $AID^{-/-}\mu S^{-/-}$ animals were originally produced by intercrosses of $AID^{-/-}$ and $\mu S^{-/-}$ mice at the Instituto Gulbenkian de Ciência. The double-mutant $AID^{-/-}$ $\mu S^{-/-}$ mouse strain used in this study offers a unique and original opportunity to dissect the different roles of B cells and antibodies in the context of MI. The $\mu S^{-/-}$ mutation renders B cells incapable of secreting soluble IgM molecules but has no impact on the expression of membrane IgMs (meaning that the $\mu S^{-/-}$ mice can generate functional B cells). However, because B cells can undergo Ig class switching, single-mutant $\mu S^{-/-}$ mice can still exhibit normal levels of all other Ig isotopes (IgA, IgD, IGE, and IgG). Thus, we crossed $\mu S^{-/-}$ mice with mice deficient in the enzyme activation-induced cytidine deaminase (AID), which is essential for the Ig class switching process. The resulting double-mutant animals cannot secrete Igs of any class, but they do retain the ability to produce functional B cells (19, 23). All strains shared the same genetic background. All animals were housed under specific pathogen-free conditions with a controlled light-dark cycle and a standard diet. The Landesverwaltungsamt Sachsen-Anhalt (Germany) Standing Committee on Animal Research approved all of the protocols used in this study.

Mouse Model of Permanent MI

MI was induced after left anterior descending coronary artery (LAD) permanent ligation was performed, as previously described (15, 31), in accordance with the guidelines for experimental models of MI (21). In brief, the animals were kept under isoflurane anesthesia and intubated for mechanical ventilation. They were then submitted to thoracotomy, and the LAD was then permanently ligated (MI group). In sham-operated animals, the procedure was identical except for the ligation of the LAD. Body temperature was controlled during the operation, and mice received pre- and postoperative pain management for 3 days [buprenorphine (0.1 mg/kg body wt sc)]. All surgeries were performed by an experienced veterinarian. Followup echo and end-point analyses were performed on postoperative *day 56* during the chronic remodeling phase.

Echocardiography

Cardiac echocardiographic assessments were performed on a Vevo 1100 (VisualSonics, Amsterdam, The Netherlands) using a 30-MHz probe especially developed for mice. All of the measurements were conducted by an experienced veterinarian. In brief, mice were kept under slight isoflurane anesthesia, and short-axis images were acquired at the midpapillary and apical levels of the left ventricle (B- and M-mode), as previously described (15, 22, 31).

End-Point Analyses

On *day 56* post-MI, animals were euthanized by cervical dislocation, and the blood and heart were obtained for later analyses. Blood samples were collected directly from the heart and kept at 4°C until plasma separation. The plasma was kept at −80°C until the assays were performed. Hearts were readily excised under aseptic conditions and then stored according to the requirements of downstream applications. Heart samples used for cryosectioning were stored in OCT embedding media and immediately frozen at −80°C, whereas samples intended for RNA extraction were stored in RNAlater (Thermo Scientific, Darmstadt, Germany) for 24 h and then at −80°C. The samples used for protein extraction were snap frozen (see below).

Immunofluorescence

Cryosections were cut at 12 μm thick, fixed in 4% formaldehyde, blocked with carbo-free solution, and then stained with specific antibodies according to standard protocols that were previously established in our laboratory (29). Endogenous mouse IgG already present in the infarcted area was detected using a polyclonal antibody conjugated with the fluorophore Alexa 488 (dilution 1:200, Thermo Scientific, Darmstadt, Germany). Fc-γ receptors (CD16/CD32) were stained using a monoclonal antibody (dilution 1:200, clone: 93, Biolegend). Images were acquired using an epifluorescence microscope (Axioskop 2 Plus, Zeiss, Jena, Germany) coupled with a high-resolution camera (AxioCam HRc, Zeiss) and processed using Zen Lite (Zeiss) or ImageJ (National Institutes of Health) software.

Real-Time PCR

RNA was extracted using a tissue RNA isolation kit (Qiagen RNeasy mini, Qiagen, Hilden, Germany). RNA concentration was determined using a Nanodrop system, and RNA quality was assessed based on 280/260- and 260/230-nm absorption curves. cDNA was synthesized from 300 ng RNA using an iScript kit (Bio-Rad Laboratories, Munich, Germany). Quantitative expression was monitored using a Taqman chemistry-based assay. mRNA expression levels of the target genes were calculated after normalization to the GAPDH housekeeping gene according to the $\Delta\Delta C_t$ principle (where C_t is threshold cycle). The following Taqman probes were used in this study: IL-6 (*Il6*; Mm00446190_m1), transforming growth factor (TGF)-β_3 (*Tgfb3*; Mm00436960_m1), collagen type III a1 (*Col3a1*; Mm01254476_m1), collagen type I a1 (*Col1a1*; Mm00801666_g1), matrix metalloproteinase (MMP)-2 (*Mmp2*; Mm00439498_m1), MMP-9 (*Mmp9*; Mm00442991_m1), vimentin (*Vim*; Mm013334-30m1), and smooth muscle actin (Acta2; Mm00725412_s1).

Immunoblot Assay to Screen for Heart-Specific Antibodies

Principle of the method. The heart reactivity potential of antibodies produced after MI was assessed by incubating plasma obtained from different animals with an extract of cardiac proteins (as described below; see also Refs. 10 and 11). In this experimental setting, cardiac proteins were separated according to their molecular weight via SDS-PAGE and then blotted onto nitrocellulose membranes that were then exposed to different plasma samples. A Miniblotter apparatus with 28 parallel slots (28 SL Immunetics) was assembled on the membranes containing heart antigens to allow the parallel testing of

28 plasma samples against the same membrane (Fig. 5*A*). The antibodies present in the infarcted versus sham-operated mice function as primary antibodies in a conventional Western blot assay, and heart-specific autoreactivity was revealed using a secondary anti-mouse antibody conjugated to alkaline phosphatase (Fig. 5*A*). Plasma reactivity was tested against variable heart extracts (naive hearts, myocardial scars, and collagenase-digested scars) as a strategy to dissect whether cardiac antigens were derived from cardiomyocytes or the extracellular matrix.

Preparation of heart extracts. In the present study, we tested plasma reactivity profiles against three distinct cardiac extracts representing different tissue compartments. Accordingly, hearts obtained from naïve WT mice and dissected myocardial scars obtained from infarcted mice (d56) were each homogenized with RIPA Lysis and Extraction Buffer supplemented with 10 μl Halt Protease Inhibitor Cocktail and 10 μl Halt Phosphatase Inhibitor Cocktail (ThermoFisher Scientific, Darmstadt, Germany). Samples were centrifuged (5 min, 16,110 *g*, 5°C), and the supernatant was then collected for experiments. Protein concentrations were measured by the bicinchoninic acid method (kit from Pierce, ThermoFisher) according to the manufacturer's instructions. Collagenase-treated scar extracts were also prepared using collagenase type II (1 ml, 1,000 U/ml, collagenase type II, Worthington, Troisdorf) dissolved in RPMI (VLE RPMI 1640 medium, Merck, Darmstadt, Germany). The latter extract was prepared to determine whether heart-specific antibodies would target extracellular matrix elements, which are collagenase sensitive. Extracts were then incubated in a water bath at 37°C for 30 min. Finally, heart extracts were prepared for SDS-PAGE under reducing conditions [in 60 mM Tris·HCl (pH 8.6), 40% glycerin, 8% SDS, 0.04% bromophenol blue, and 2% 2-mercaptoethanol while being boiled at 95°C for 5 min].

SDS-PAGE was performed to separate cardiac antigens (100 μg/gel total protein) according to their molecular weight in Mini-PROTEAN precast gels (4–20%, Bio-Rad Laboratories). After electrophoresis, gels containing cardiac antigens were then directly blotted onto a nitrocellulose membranes using Mini-PROTEAN Trans-Blot Cell, as previously described (10). Membranes were blocked overnight in PBS-0.2% Tween (Sigma-Aldrich, Munich, Germany) at 4°C and then mounted in a Miniblotter apparatus with 28 parallel slots (28 SL Immunetics). This Miniblotter allowed for up to 28 plasma samples to be incubated in parallel on a single membrane. Plasma samples obtained from WT infarcted mice (56 days post-MI and diluted 1:20 in PBS-0.2% Tween) were then incubated (room temperature, 2 h) with the membrane containing cardiac protein extracts. After the plasma was aspirated from the slots, membranes were removed from the Miniblotter, washed, and then incubated with goat anti-mouse IgG (γ-chain specific)-alkaline phosphatase antibodies for 90 min (1:400, Sigma-Aldrich). After successive washing steps in 0.2% PBS-Tween, the colorimetric reaction was developed using an alkaline phosphatase substrate according to the manufacturer's instructions [BCIP/NBT Color Development Substrate (5-bromo-4-chloro-3-indolyl-phosphate/nitroblue tetrazolium; Promega, Mannheim, Germany)]. The reaction was stopped by washing the membranes with distilled water. Membranes were then scanned using a gel documentation system (ImageQuant LAS400). All antibodies were used according to the guidelines on antibody use in physiology studies (3).

Statistical Analyses

All statistical analyses were performed with GraphPad Prism 6.

All results are presented as means ± SD of data obtained from 7−11 animals unless stated otherwise. The graphs display both group mean values (bars) and the distribution of each individual value. For comparisons of two groups, we used unpaired *t*-tests, whereas for multiple comparisons, we performed one- and two-way ANOVA followed by Sidak's multiple-comparison test. The survival data are shown as Kaplan-Meier curves, and the data were analyzed by log-rank tests. Differences were considered significant at $P < 0.05$. The exact *P* values are reported in all graphs.

RESULTS

Ig-Deficient Mice Show Attenuated Left Ventricular Remodeling After MI

To directly assess the contribution of antibodies to ischemic HF progression, we studied WT and AID$^{-/-}$μS$^{-/-}$ mice for 56 days in a permanent coronary ligature MI model. Ig deficiency did not influence overall post-MI survival rates (Fig. 1, *top*). However, the infarct size measured on *day 56* was significantly smaller in mice lacking antibodies than in WT control mice (WT mice: 60.0 ± 15.4% and AID$^{-/-}$μS$^{-/-}$ mice: 41.36 ± 17.9%, $P = 0.024$; Fig. 1, *bottom*).

Left ventricular function and geometry were evaluated by echocardiography on *day 56* postoperation. Echocardiographic images were taken at short-axis views (FS) at both the apical and papillary levels, which represented the infarct and border zones, respectively. No statistically significant baseline altera-

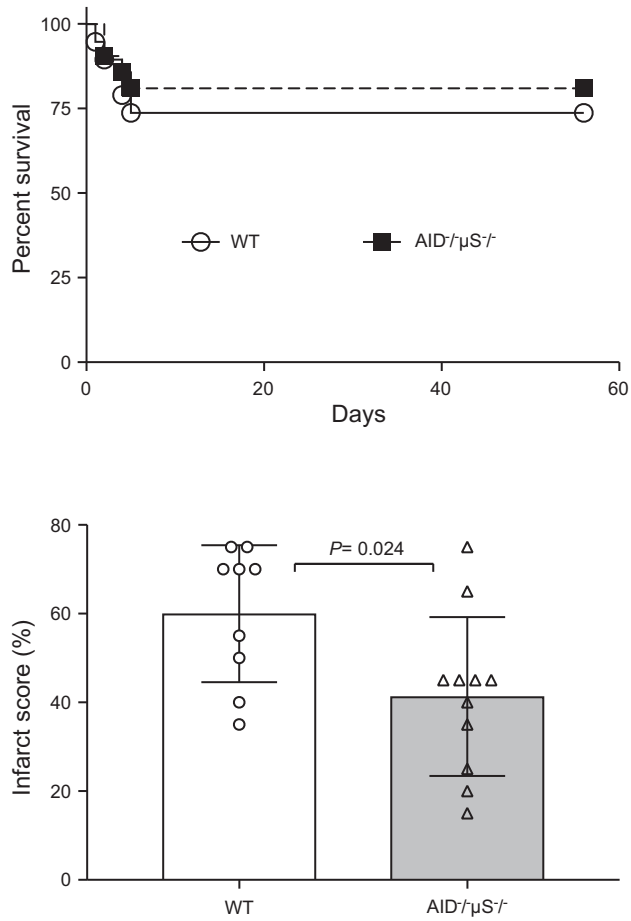


Fig. 1. Effects of agammaglobulinemia on cumulative survival (*top*) and infarct size (*bottom*). Wild-type (WT) and agammaglobulinemic (AID$^{-/-}$μS$^{-/-}$) mice were submitted to myocardial infarction (MI), and cumulative survival was followed for 56 days. Infarct size was measured by echocardiography on *day 56*. The base graphs represent means ± SD in the WT (open circles) and agammaglobulinemic mouse (AID$^{-/-}$μS$^{-/-}$; solid squares) groups. Each individual value is represented by either an open circle (WT) or an open triangle (AID$^{-/-}$μS$^{-/-}$) in the *bottom* graphs. Statistical analysis performed as shown at the *bottom*: *t*-test. The exact *P* value is indicated.

tions were observed when sham-operated WT and AID$^{-/-}$ μS$^{-/-}$ mice were compared (except for the change in fractional area at the apical level; Fig. 2*A*). However, Ig deficiency had a major impact on cardiac function and geometry in infarcted mice (Fig. 2, *A–E*). In WT mice, MI induced a severe reduction in the fractional area change (FAC) and fractional shortening (FS) at both the apical and papillary levels ($P < 0.05$; Fig. 2, *A* and *B*). In sharp contrast, contractility was better preserved in infarcted Ig-deficient mice than in MI-WT mice ($P < 0.05$ compared with MI-WT mice). Because FAC can be taken as a readout for systolic function, these results indicate that Ig deficiency exerts a cardioprotective effect in the context of MI.

Ischemic HF was also characterized by a significant increase in end-diastolic and end-systolic areas and by thinning of the anterior wall in WT animals ($P = 0.05$; Fig. 2, *C–E*). In contrast, infarcted AID$^{-/-}$$\mu$S$^{-/-}$ mice showed no significant post-MI cardiac dilative phenotype ($P = 0.05$; Fig. 2, *C* and *D*) and no significant ventricular wall thinning, both of which are hallmarks of adverse remodeling.

Because the distribution of infarct sizes was heterogeneous between the genotypes, we additionally analyzed cardiac functionality in a subgroup of animals exhibiting matched infarct sizes (>30% and <60%). AID$^{-/-}$$\mu$S$^{-/-}$ mice still exhibited better preservation of left ventricular function and geometry after infarct size normalization (Fig. 3). Collectively, these data

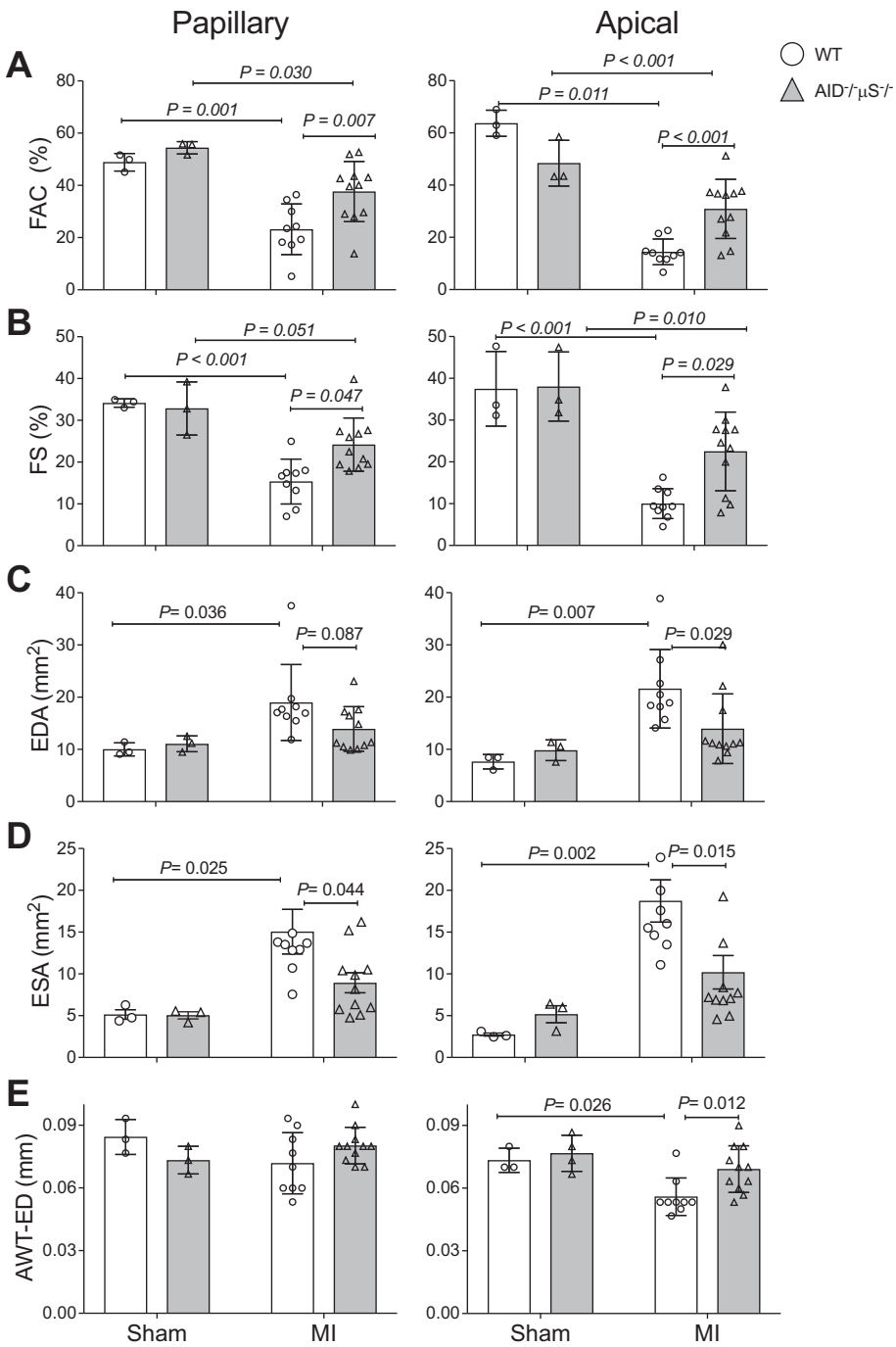


Fig. 2. Agammaglobulinemic (AID$^{-/-}$$\mu$S$^{-/-}$) mice show attenuated left ventricular remodeling after myocardial infarction (MI). Echocardiographic images were obtained on *day 56* at short-axis views at both the apical and papillary levels, which represent the infarct and border zones, respectively. *A* and *B*: fractional area change and fractional shortening (FAC and FS, respectively; both in %). *C* and *D*: end-diastolic and end-systolic area (EDA and ESA, respectively; both in mm^2). *E*: anterior wall thickness measured at end diastole (AWT-ED; in mm). The base graphs represent means ± SD in the WT (open bars) and AID$^{-/-}$$\mu$S$^{-/-}$ (gray bars) groups. Each individual value is represented by either an open circle (WT) or an open triangle (AID$^{-/-}$$\mu$S$^{-/-}$). Statistical analysis: two-way ANOVA followed by the Sidak post hoc test. The exact *P* values are indicated.

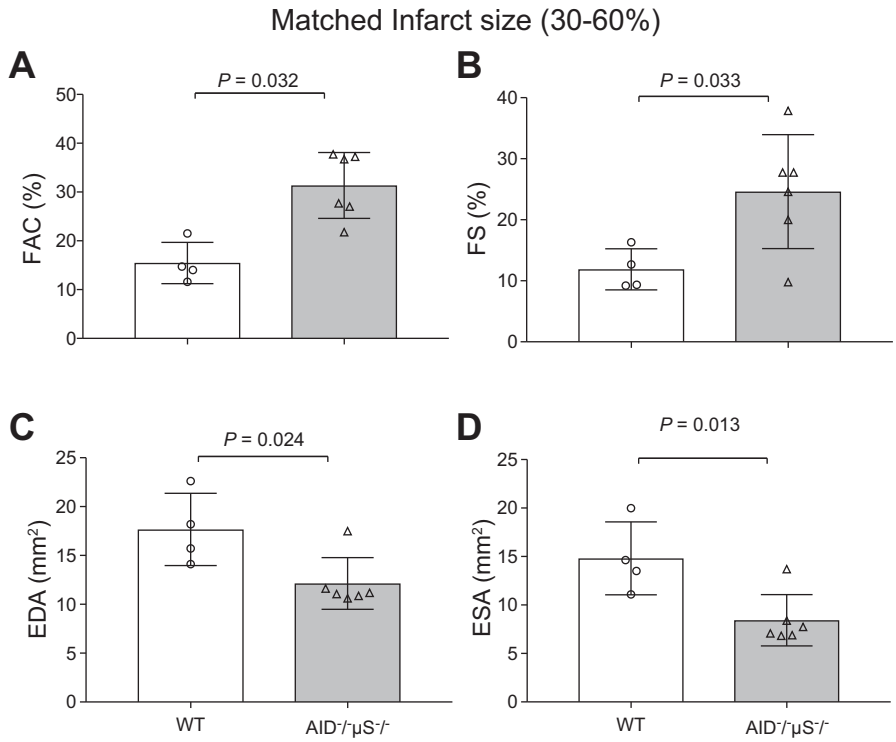


Fig. 3. Echocardiographic parameters in infarct size-matched animals. Postmyocardial infarction (post-MI) cardiac function and morphology were compared in subgroups of animals exhibiting similar infarct sizes (30–60%) according to the data shown in Fig. 1, *bottom*. *A* and *B*: fractional area change and fractional shortening (FAC and FS, respectively; both in %). *C* and *D*: end-diastolic (EDA; *C*) and end-systolic areas (ESA; *D*) (both in mm^2). The bar graphs represent means ± SD of the WT (open bars) and agammaglobulinemic mouse ($AID^{-/-}\mu S^{-/-}$; gray bars) groups. Each individual value is represented by either an open circle (WT) or an open triangle ($AID^{-/-}\mu S^{-/-}$). Statistical analysis: two-way ANOVA followed by the Sidak post hoc test. The exact *P* values are indicated.

show that Ig deficiency is correlated with attenuated adverse remodeling in response to MI.

Attenuated Inflammation and Extracellular Matrix Formation in Ig-Deficient Mice

Next, we used quantitative PCR to analyze the expression levels of genes related to inflammation, matrix proteinase activity, and collagen production, as these are critical components of chronic myocardial remodeling. *Il6*, *Tgfb3*, *Col1a1*, *Col3a1*, *Vim*, and *Acta2* transcripts were significantly upregulated in response to MI in WT animals (Fig. 4). In contrast, Ig-deficient mice that were submitted to MI showed significantly reduced expression of *Il6* and procollagen transcripts on *day 56* post-MI.

Overall, and in accordance with the echocardiographic findings, these gene expression analyses indicate that Ig deficiency results in attenuated inflammatory and fibrotic responses at chronic stages after MI.

Myocardial Scars Are Targeted by Autoantibodies and Harbor Cells Expressing Fc Receptors

To explore which Ig reactivities could be involved in the progression of HF, we used an unbiased screening approach to detect heart-specific antibodies. First, we compared the reactivity profile of plasma obtained from MI versus sham-operated mice (*day 56*) against naive heart extracts (Fig. 5*B*). As shown in Fig. 5, heart-reactive antibodies were detected in 10 of 21 tested MI plasma samples (47%). In sharp contrast, plasma obtained from sham-operated mice showed nearly no reactivity against cardiac antigens (19% of animals). Overall, post-MI antibody responses were rather mild and restricted to a limited number of cardiac antigens (Fig. 5*B*).

To further dissect these findings, we tested the reactivity of MI plasma samples against protein extracts prepared from naive hearts, dissected scar tissues (*day 56* post-MI), or collagenase-digested scars (shown in Fig. 5*C*). The rationale underlying this experimental design was to further dissect the source of the antigens detected in the injured myocardium. Healthy heart extracts are composed of proteins derived from cardiomyocytes, the vasculature, resident cells, and extracellular matrix components. In contrast, protein extracts prepared from dissected scars were composed mostly of extracellular matrix components, which are sensitive to collagenase digestion. All three types of protein extracts were run in parallel on the same gel and then transferred onto the same nitrocellulose membrane so that the plasma reactivity profiles could be directly compared.

As shown in Fig. 5*C*, some infarcted animals produced antibodies targeting epitopes that were present in viable myocardium but not in dissected scars (e.g., marked by red squares in 2; Fig. 5*C*). Our interpretation is that this reactivity might be related to antigens expressed mainly by cardiomyocytes (e.g., contractile elements or cardiac receptors). Surprisingly, we also detected antibodies that reacted with antigens present in the dissected scars, which were composed mainly of extracellular components (e.g., plasma 2, 6, and 9, marked by blue squares in Fig. 5*B*). Moreover, these scar epitopes were also sensitive to collagenase digestion, providing strong evidence that they might indeed be derived from extracellular matrix proteins. Taken together, this approach revealed that the heart-specific antibodies produced by infarcted mice could be divided into two major categories: antibodies targeting the viable myocardium and antibodies targeting extracellular matrix components, with the latter being a highly overlooked topic.

The profile of Igs collected from MI animals indicated that they bear heart reactivity potential. Thus, we next sought to

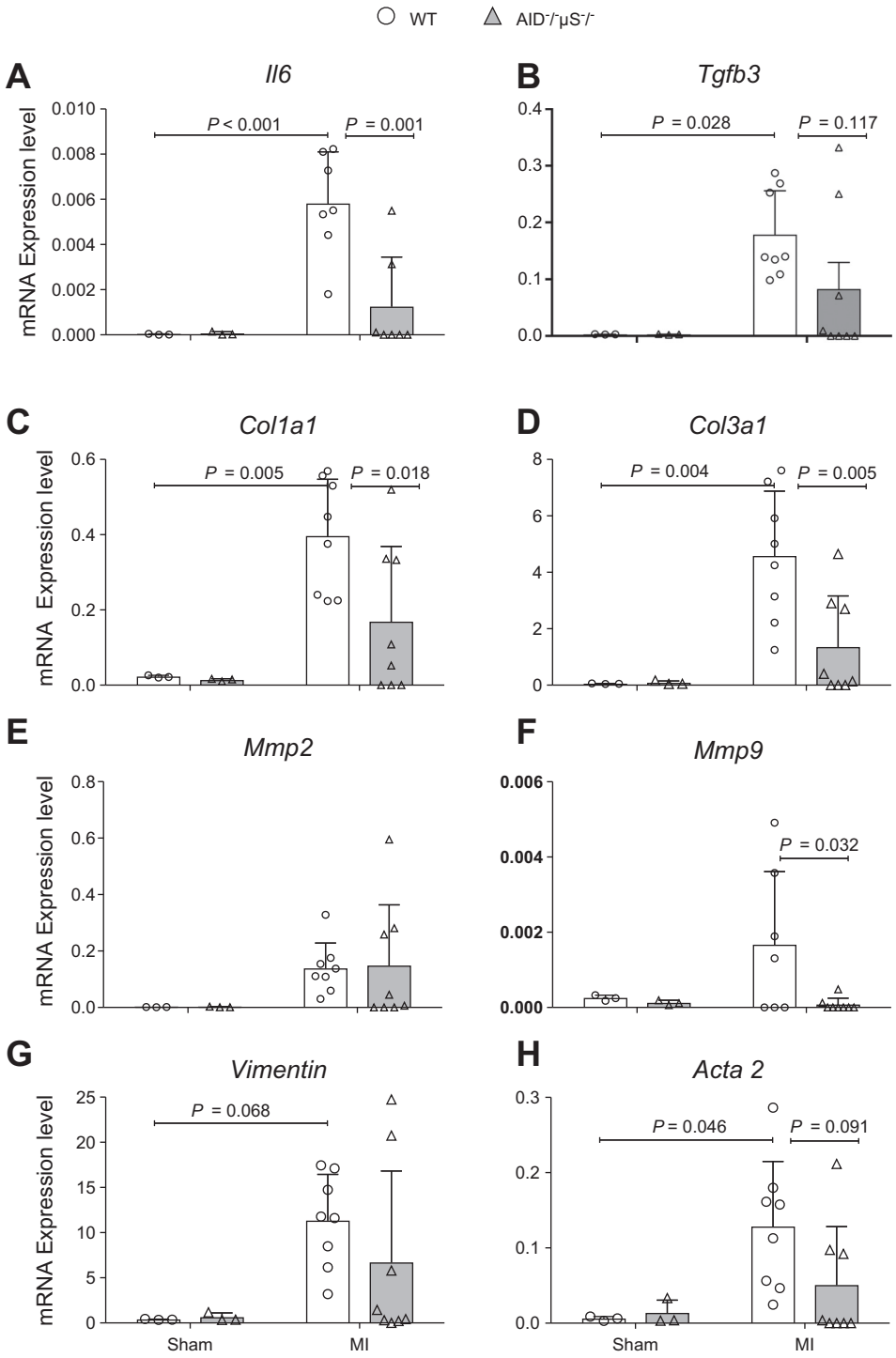
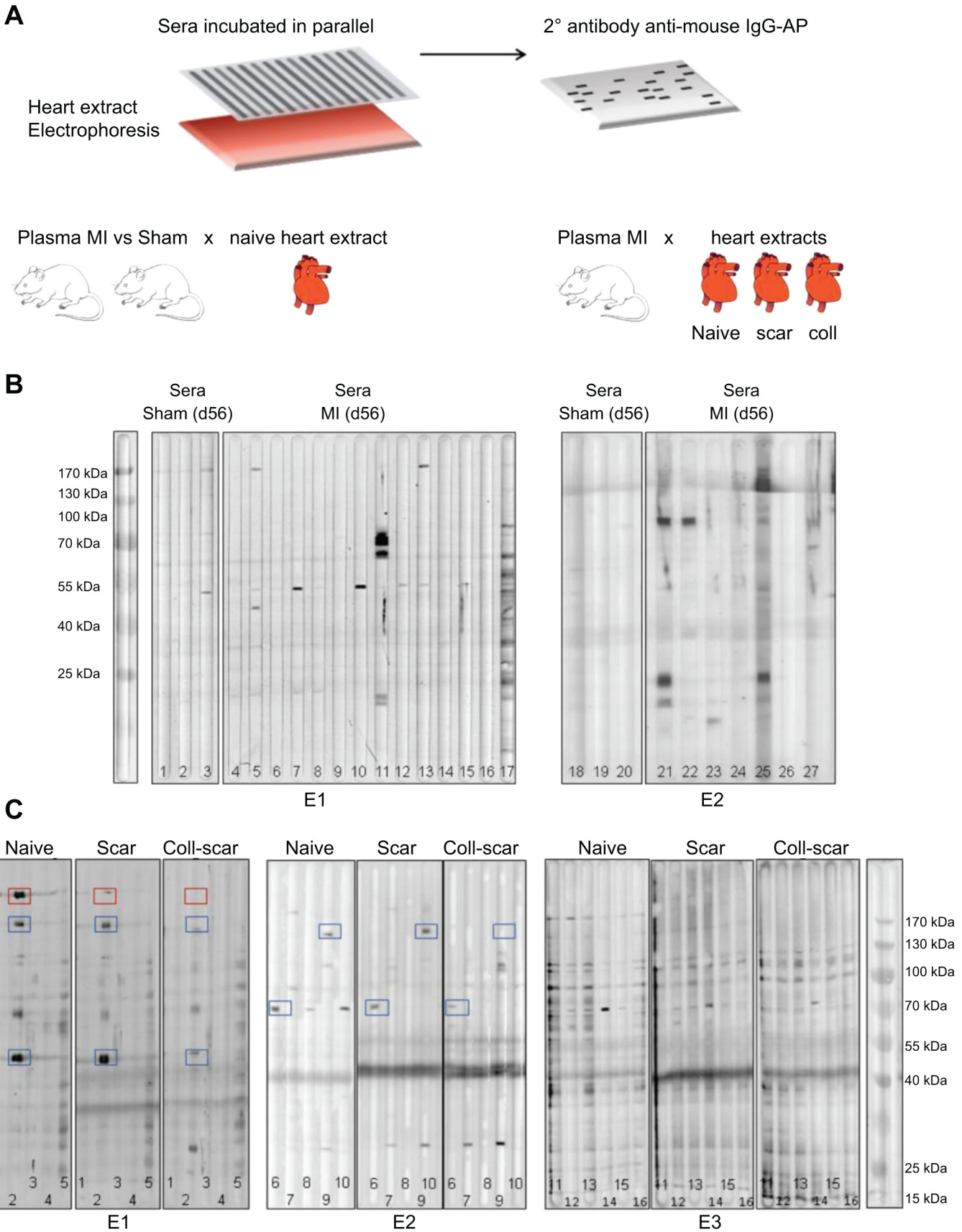


Fig. 4. Attenuated inflammation and extracellular matrix formation in agammaglobulinemic ($AID^{-/-}\mu S^{-/-}$) mice. Expression levels of genes related to inflammation and cardiac remodeling were assessed by quantitative PCR. *A*: interleukin-6 (*Il6*). *B*: transforming growth factor-β_3 (*Tgfb3*). *C* and *D*: pro-collagen-1α (*Col1a1*; *C*) and pro-collagen-3α (*Col3a1*; *D*). *E* and *F*: matrix metalloproteinase-2 (*Mmp2*; *E*) and matrix metalloproteinase-9 (*Mmp9*; *F*). The base graphs show means ± SD of the WT (open bars) and $AID^{-/-}\mu S^{-/-}$ (gray bars) groups. *G* and *H*: vimentin (*Vim*) and smooth muscle actin (*Acta2*). Each individual value is represented by either an open circle (WT) or an open triangle ($AID^{-/-}\mu S^{-/-}$). Statistical analysis: two-way ANOVA, followed by the Sidak post hoc test. The exact *P* values are indicated.

measure the presence of IgM and IgG in perfused heart extracts. Strikingly, the amount of IgG (but not IgM) recovered from myocardial samples was significantly higher after MI than in healthy extract tissues (Fig. 6, *top left*). These findings indicate the existence of in situ humoral immune responses in remodeling hearts. An immunofluorescence analysis confirmed that cardiac IgGs also accumulate in the scar zone (Fig. 6, *bottom left*). We interpret these data as further supporting the notion that in the chronic phase post-MI, autoantibodies against myocardial/scar epitopes are induced. Finally, we also found that these scar tissues harbored cells expressing FcγR CD16/32, which are factors that mediate the effector functions of IgGs (Fig. 6, *bottom right*). The colocalization of IgG and FcγR within the scar further supports the notion that antibodies aggravate the progression of ischemic heart failure.

DISCUSSION

The presence of heart-reactive autoantibodies in the plasma of HF patients and rodents is a widely described phenomenon



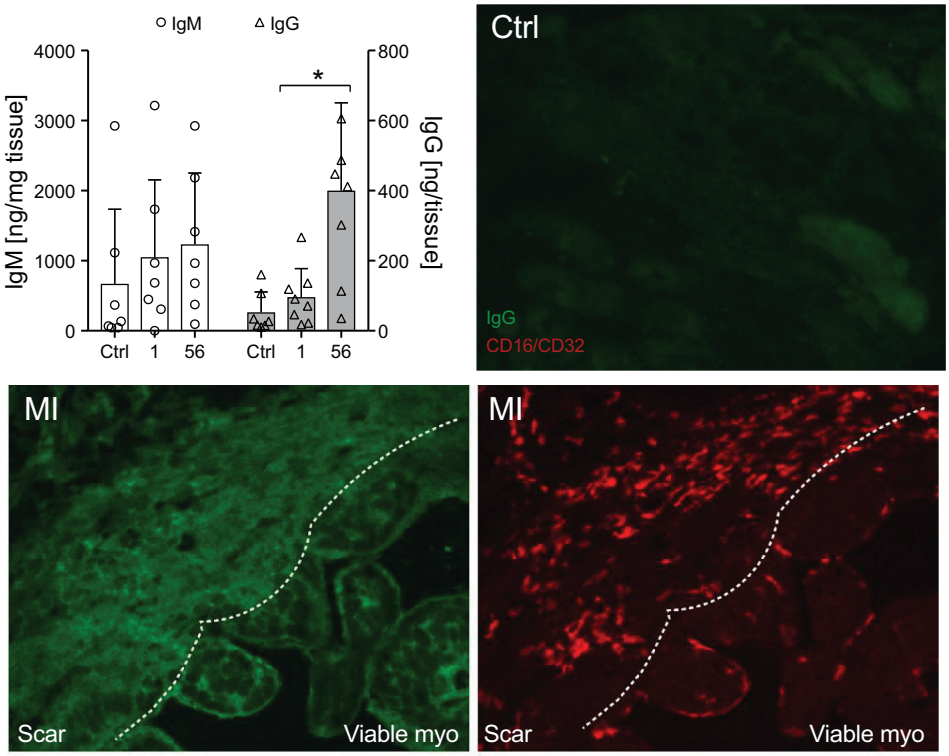


Fig. 6. IgG and Fc receptor-γ (FcRγ)-expressing cells accumulate in the scar during chronic remodeling. Total IgM and total IgG were detected in perfused heart extracts obtained from healthy, myocardial infarction (MI) *day 1*, and MI *day 56* wild-type (WT) mice (*top left*). *Top right* and *bottom*: immunofluorescence in heart slices prepared from WT infarcted mice (*day 56*). *Top right*: control slices showing tissue baseline levels of autofluorescence. *Bottom left*: IgG accumulation within the scar. *Bottom right*: the remodeling scar harbors a significant number of cells expressing FcRγ. Scar antibodies and FcRγ-expressing cells remain in close proximity within the scar. The dashed line in the *bottom* images indicates the infarct border zone.

that has been documented for many decades (e.g., see Refs. 4–9, 17, and 27). However, the functional relevance of these autoantibodies and their putative contribution to disease progression has remained largely controversial. Moreover, in the last decade, a series of studies have revealed that B cells are involved in the development of post-MI inflammation (20). Yet the question of whether B cells influence post-MI outcomes via antibody-mediated versus independent mechanisms has remained largely unresolved. This is a particularly difficult issue to dissect because mice lacking the gene segments necessary to build an Ig molecule also show a complete B cell deficiency phenotype (e.g., the μMT and JhT mouse strains). This is because the expression of membrane-bound Igs is a necessary prerequisite for B cell survival (18). In the present study, we circumvented this limitation by performing MI experiments on animals able to express membrane-bound Igs. Hence, these mice could generate functional B cells but were completely lacking the capacity to secrete soluble antibodies. Our results indicate that antibodies critically mediate the development of ischemic HF in mice, as Ig deficiency resulted in smaller infarct sizes, the long-term preservation of systolic function, and the attenuation of cardiac remodeling (i.e., ventricular dilation and wall thinning). To the best of our knowledge, this is the first study to use this double-mutant mouse strain in the field of cardiology.

In the present study, we decided to focus on the role of antibodies on chronic remodeling because previous studies have already established the role of IgMs in mediating cardiac injury during the early post-MI phase (4, 13). Although we could not identify the specific contribution of IgMs and IgGs to the phenotype described, our findings suggest that both isotopes might be relevant. On the one hand, the reduction in infarct size observed in $AID^{-/-}\mu S^{-/-}$ mice suggests that antibodies act in the early steps after coronary ligation when infarct expansion normally occurs as a result of secondary damage (12). The antibody-mediated effects in these early stages of MI require the actions of preformed Igs. Research performed in the field of ischemia-reperfusion injury has revealed that the natural IgMs that are formed before the onset of cardiac disease can indeed recognize dying cells and fuel local inflammation in a complement-mediated manner (4, 13). Furthermore, our observation that agammaglobulinemia attenuated cardiac remodeling even when animals with matched infarct sizes were compared indicates that antibodies also participate in the chronic stages of this disease. These long-term effects indicate that IgGs play a role in these processes.

Fig. 5. Unbiased screening of myocardial infarction (MI)-induced heart-specific antibodies. *A*: experimental design. Plasma samples obtained from infarcted and sham-operated wild-type (WT) mice (*day 56*) were incubated with cardiac proteins and separated according to their molecular weight by SDS-PAGE. They were then blotted onto nitrocellulose membranes and incubated with plasma obtained from MI or sham-operated animals. Two different immunoblot settings were tested in this study. *B*: plasma obtained from WT sham-operated (p1–p3, p18–p20) or infarcted (p4–17, p21–27) mice were incubated with a protein extract prepared from a healthy heart. The images represent the results of two independent experiments (*E1* and *E2*), and each column indicates the heart reactivity profile of plasma obtained from different mice (p1–p27). *C*: MI plasma (*day 56*) was tested against protein extracts prepared from healthy hearts (healthy), post-MI scars (scar), and collagenase-treated scars (collagenase). Red boxes indicate reactivity bands that targeted epitopes present in viable myocardium but not in dissected scars. Blue boxes indicate epitopes present in dissected scars that are sensitive to collagenase degradation. The images represent the results of three independent experiments (E1–E3), and each column indicates the heart-reactivity profile of a different sample obtained from WT infarcted animals (p1–p16).

Small clinical trials performed in the early 2000s reported that plasmapheresis followed by immunoadsorption of specific IgGs improved cardiac function in patients with HF (9). Our findings strongly support the notion that pathogenical antibodies contribute to ischemic HF progression. However, we could not determine which antibody specificities are responsible for promoting cardiac damage and fueling adverse remodeling after MI. Future studies will be necessary to answer these questions, and we expect the results presented in this study may help to revive interest in the biology of cardiac antibodies.

Most of the literature currently available on heart-specific antibodies focuses on cardiomyocyte-derived epitopes (e.g., see Ref. 8). Antigens expressed on endothelial cells, fibroblasts, or extracellular matrix components have been highly overlooked. Our unbiased screening approach revealed that WT infarcted mice produce antibodies targeting collagenase-sensitive epitopes in the scar. These findings might help to expand the concept of "cardiac antigens" by stressing that cardiomyocytes are not the only source of antigens in the heart. Importantly, we observed that IgGs can accumulate within the scar in WT infarcted mice. In addition, we found that myocardial scars harbor cells expressing FcγR (CD16/CD32). These receptors interact with IgGs, and their cross-linking can lead to the activation of various cell types (2, 3). Haudek et al. (14) reported that cardiac fibroblasts express FcγR and that their activation mediates the differentiation of fibroblasts.

We acknowledge that the specificities and mechanisms underlying the pathogenic roles of antibodies in HF progression were not fully defined in this study. However, the associative evidence presented here suggests a previously undescribed antibody-FcγR axis within the myocardial scar. In future work, we might investigate whether antibody deposition in the myocardial scar could lead to fibroblast activation and thereby promote the maintenance of active remodeling processes.

Collectively, our results confirm that antibodies can mediate the progression of ischemic HF and provide novel insights into the mechanisms underlying this phenomenon. Our findings may hold clinical significance, and we hope that this work will restimulate further research in the field of heart-specific antibodies.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.K., J.D., S.F., U.H., and G.R. conceived and designed research; L.K., M.R., and G.R. performed experiments; L.K., M.H., M.R., and G.R. analyzed data; L.K., M.H., M.R., J.D., S.F., U.H., and G.R. interpreted results of experiments; L.K., M.H., and G.R. prepared figures; L.K., M.H., J.D., S.F., U.H., and G.R. drafted manuscript; L.K., M.H., J.D., S.F., U.H., and G.R. edited and revised manuscript; L.K., M.H., M.R., J.D., S.F., U.H., and G.R. approved final version of manuscript.

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