Antibody therapy can enhance AngiotensinII-induced myocardial fibrosis

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Abstract

Background: Myocardial fibrosis is a pathological process that is characterized by disrupted regulation of extracellular matrix proteins resulting in permanent scarring of the heart tissue and eventual diastolic heart failure. Pro-fibrotic molecules including transforming growth factor-β and connective tissue growth factor are expressed early in the AngiotensinII (AngII)-induced and other models of myocardial fibrosis. As such, antibody-based therapies against these and other targets are currently under development.

Results: In the present study, C57Bl/6 mice were subcutaneously implanted with a mini-osmotic pump containing either AngII (2.0 μg/kg/min) or saline control for 3 days in combination with mIgG (1 mg/kg/d) injected through the tail vein. Fibrosis was assessed after picosirius red staining of myocardial cross-sections and was significantly increased after AngII exposure compared to saline control (11.37 ± 1.41%, 4.94 ± 1.15%; P <0.05). Non-specific mIgG treatment (1 mg/kg/d) significantly increased the amount of fibrosis (26.34 ± 3.03%; P <0.01). However, when AngII exposed animals were treated with a Fab fragment of the mIgG or mIgM, this exacerbation of fibrosis was no longer observed (14.49 ± 2.23%; not significantly different from AngII alone).

Conclusions: These data suggest that myocardial fibrosis was increased by the addition of exogenous non-specific antibodies in an Fc-mediated manner. These findings could have substantial impact on the future experimental design of antibody-based therapeutics.

Keywords: Myocardial fibrosis, Collagen, Antibody, Inflammation, AngiotensinII

Background

Despite advances in medical therapy, cardiovascular disease remains the cause of 33% of all deaths in the United States and 28% in Canada [1]. Furthermore, the 5-year mortality rate for patients presenting with heart failure is 50% [2]. The pathological characteristics and the final pathway leading to heart failure is the development of myocardial fibrosis [3]. Myocardial fibrosis is defined as excess deposition of extracellular matrix (ECM) proteins within the myocardium and is a significant contributor to myocardial dysfunction.

A common animal model of myocardial fibrosis is induction by exposure to AngiotensinII (AngII), a vasoregulatory hormone, which results in hypertension-mediated myocardial fibrosis [4,5]. We, and others, have previously reported that myocardial fibrosis after exposure to AngII is characterized as early as 3 days by myocardial infiltration of mononuclear cells that have been defined as bone marrow-derived, monocyte lineage, fibroblast progenitor cells (fibrocytes) [6-8]. This is followed by a significant increase in extracellular matrix deposition, the hallmark of fibrosis.

Using this model, investigators have identified the pro-fibrotic mediator transforming growth factor-β (TGFβ) as being central to the regulation of fibrosis development. In the AngII-induced model, the TGFβ pathway, including SMAD phosphorylation and CTGF production, are significantly upregulated within the first 3 days, suggesting their involvement in the initial phase of myocardial fibrosis development [4]. In support of the role for TGFβ, inhibition of TGFβ using antibodies [9] or
mice heterozygous for TGFβ knockout [10], have resulted in reduced myocardial fibrosis. However, TGFβ is a pleiotropic cytokine that has both pro-inflammatory and anti-inflammatory functions, making treatment strategies based on its reduction difficult, as serious deleterious effects have been reported, such as increased mortality after myocardial infarction [11]. Other antibody-based therapeutic strategies attempted in animal models have included targeting IL23R, platelet-derived growth factor-receptor, and the TGFβ downstream mediator connective tissue growth factor (CTGF), without any antibody-based therapy in clinical use to date [12,13].

In the present study, we show that non-specific antibody, similar to those used as controls for therapies used to treat myocardial fibrosis can in fact worsen the onset of fibrosis in the AngII-induced model. We provide evidence that the mechanism for this apparent paradoxical observation is mediated by increased pro-inflammatory cytokine production and is not dependent on the specificity of the antibody. In fact, the worsened fibrotic response to whole antibody disappeared with treatment using Fab fragments, suggesting that an Fc-mediated activation of infiltrated cells was key to the exacerbated fibrotic response.

### Results

#### Effect of antibody on early myocardial fibrosis

The addition of non-specific antibody by tail-vein injection to the AngII model of myocardial fibrosis over 3 days resulted in a significant increase of the fibrotic area within the myocardium. The amount of fibrosis was significantly increased after mlG treatment, with 26.34 ± 3.03% of myocardium affected compared to 11.29 ± 1.20% with AngII alone, and with a more dispersed pattern of collagen deposition (Figure 1A, C, D). The mRNA expression of Col1A1, a subunit of type I collagen, the most prolific structural collagen in the myocardium, also increased in the myocardium of animals exposed to AngII, but did not increase further in the AngII + mlG group (Figure 1B).

Similarly, the amount of cellular infiltration assessed using a standardized grid-affected method after staining whole myocardial cross-sections with hematoxylin and eosin (H&E) was significantly higher in animals receiving the non-specific mlG (24.42 ± 3.67%) when compared to AngII alone (14.27 ± 1.22; *P <0.01) (Figure 2A, B, C). The phenotype of the cellular infiltrate in both groups included cells expressing both α-smooth muscle actin (SMA) and CD45 in keeping with a phenotype for fibroblasts that co-express monocyte and mesenchymal

![Figure 1 Antibody administration and fibrosis](http://www.fibrogenesis.com/content/7/1/6)
markers [14]. Fibrocytes, have been suggested to be precursors to myofibroblasts and as such important ECM producing effector cell seen in fibrotic disorders ([15]) (Figure 2D, E, F). Taken together, our findings demonstrate that antibody addition to AngII-exposed animals in fact results in worsening the fibrotic changes within the myocardium.

Increased fibrosis is not due to increased complement activation

A potential mechanism by which the addition of mIgG exacerbated fibrosis is complement-mediated cytotoxicity. In order to assess if complement is involved we stained cross-sections of myocardium using anti-C4d antibody. In the AngII alone and AngII + mIgG groups we observed positive C4d staining, primarily located in areas of cellular accumulation within the myocardium (Figure 3A, B, C). Given the similar amount of C4d in both AngII and AngII + mIgG groups, this suggests that complement activation was not specific to the addition of mIgG. Further support for the lack of deposition of IgG in the myocardium is provided by a lack of identifiable immunohistochemical staining for IgG within the myocardium of any of the groups (Additional file 1). Taken together, our findings indicate that the increased fibrotic response with the addition of mIgG is not due to complement activation.

Increased fibrosis is secondary to increased pro-inflammatory cytokines within the myocardium

The overall increase in mononuclear cells, many of which expressed markers of fibrocytes (CD45+/SMA+) within the myocardium in the AngII + mIgG group suggests that inflammatory signaling may be contributing to the enhanced cellular response to AngII. We therefore assessed the mRNA expression of two known potent pro-inflammatory mediators, tumour necrosis factor-α (TNFα) and interleukin 1β (IL1β) within the myocardium of any of the groups (Additional file 1). Taken together, our findings indicate that the increased fibrotic response with the addition of mIgG is not due to complement activation.

Figure 2 Antibody administration and cellular infiltration. Animals exposed to AngII for 3 days were concurrently administered mIgG by tail vein injection. The amount of cellular infiltrate within whole cross-sections was assessed using a grid-scoring method of whole myocardial cross-sections after H&E stain and the results reported as percentage of area affected (A). Representative H&E stained sections of AngII (B) and AngII + mIgG (C) are shown at 5x and areas of infiltration are highlighted by black borders. Immunofluorescent staining of CD45 (green) and SMA (red) indicate dual staining (yellow) within areas of infiltration in AngII (D), AngII + mIgG (E) treated myocardium, with representative images shown at 63x and an additional 4x digital magnification of an area of dual staining shown (F). *P <0.05, **P <0.01 compared to saline, †P <0.05, ‡P <0.01 compared to AngII.
mRNA expression in the myocardium of AngII animals relative to saline control (Figure 4C). However, in animals that received AngII + mIgG there was a significant reduction in TGFβ expression when compared to AngII alone that remained higher than in saline control (3.05 ± 0.35 vs. 4.48 ± 0.38 fold) (Figure 4C). Our findings suggest that the addition of mIgG may be favoring pro-inflammatory signaling (TNFα and IL1β) as opposed to anti-inflammatory signaling (TGFβ), contributing to the activation of the cells within the myocardium and ultimately resulting in increased fibrosis.

Fibrocytes enriched from PBMCs are activated by mIgG

We have previously demonstrated that a large portion of infiltrating cells after AngII exposure are monocyte-derived fibrocytes and that isolated circulating peripheral blood mononuclear cells (PBMCs) can be made to differentiate into fibrocytes under specific conditions [4,20]. This explains in part why we used human PBMCs in addition to their easy availability to evaluate the impact of exposure to IgG. One should note that human and murine FcγR subclasses are not homologous, but are able to bind IgG that originate from the opposite species as previously shown by others, which supports our experimental approach [21,22]. We therefore incubated PBMCs in vitro with mIgG for 48 h. This exposure resulted in increased production of the pro-inflammatory cytokines IL1β, TNFα, and IL6 measured by ELISA with IL6 reaching significance compared to saline control (Figure 5). This suggests that mononuclear infiltrating cells originating from the circulation can be directly affected by the presence of mIgG, suggesting a potential mechanism for our observed increase in fibrosis in vivo.

In support of our findings is previously published evidence that IgG can activate mononuclear cells, resulting in the differentiation of monocytes and the production of pro-inflammatory cytokines [23,24].

Increased fibrosis seen in animals receiving mIgG is prevented by using Fab fragments or mIgM

Fc receptor recognition and binding of the Fc fragment of IgG molecules leads to increased inflammatory signaling [25]. We therefore removed the Fc portion from the mIgG molecules, and treated AngII exposed animals with the resulting Fab fragment (Figure 6). When AngII exposed animals were treated with Fab, myocardial...
fibrosis was significantly reduced compared to AngII exposed animals treated with whole mIgG (14.49 ± 2.23 vs. 26.34 ± 3.03%; P <0.05). In order to further support our observation we tested the impact of adding whole mIgM to AngII exposure. IgM is not recognized by FcγRs, and so does not activate mononuclear cells in the circulation in an Fc-mediated manner [25]. Similar to animals that received AngII + Fab, mice exposed to AngII + mIgM did not have elevated fibrosis compared to animals exposed only to AngII. Importantly, fibrosis was significantly reduced compared to the AngII + mIgG group (12.74 ± 2.59%). Taken together, these findings suggest that the mechanism behind the exacerbation of fibrosis was Fc-mediated.

**Discussion**

There are no existing therapies available to treat established myocardial fibrosis. In the present study, animals exposed to AngII were administered a non-specific mouse IgG, such as would be used as a control in targeted antibody therapies for fibrotic diseases. Surprisingly, the introduction of antibody to this model of myocardial fibrosis increased the amount of fibrosis in the first few days of exposure. Our observations were robust as we provided direct correlation of increased fibrosis with increased cellular infiltration, as previously described [6]. We also used two different preparations of mIgG antibodies with similar results further supporting the observations (reported results were pooled). We therefore sought to investigate the potential mechanisms by which mIgG given to animals could affect myocardial fibrosis. This is particularly relevant as investigators are currently working on a multitude of targets using antibody approaches.

Evidence for the direct binding of the IgG molecules within the myocardium was assessed, as this could have contributed to antibody-dependent complement mediated cytotoxicity [26]. Complement activation occurs when complement component 1 recognizes surface bound IgG, leading to initiation of a cascade that ultimately results in cell lysis and deposition of the split product C4d [27]. C4d deposition is a well-described histological technique to identify areas of classical complement activation [26]. We found evidence of complement activation based on C4d deposition in all animals exposed to AngII with no evidence of significant differences between groups in which antibody was added. This suggests that while complement may be involved in AngII-induced myocardial fibrosis, it was not responsible for the increase in fibrosis observed after the addition of antibody. We did not explore the lectin pathway of activation in which complement activation occurs without immunoglobulin.

The AngII exposure model of myocardial fibrosis is one in which myocardial inflammation potentially plays a large role based on early myocardial inflammatory cell migration and pro-inflammatory effects of AngII [6,28]. Particularly relevant are monocyte-derived fibrocytes that have been identified as an important cell type in the development of AngII-mediated myocardial fibrosis [6,8]. We observed that in animals exposed to AngII and antibody there was a significant increase in the pro-inflammatory cytokines TNFα and IL1β. This is particularly relevant as these cytokines are produced primarily by monocytes and macrophages, which can be activated by the binding of IgG to Fc gamma receptors I and IIA (FcγRs) [25,29,30]. Mouse (and human) FcγRs have higher affinity for certain IgG subclasses, which have been extensively reviewed by others [21]. The administration of
whole IgG containing all subclasses in the present study implies that we do not know which FcγR is recognizing the exogenous IgG, or if it is an activating or inhibitory receptor subtype that is involved. However, as both macrophages and fibrocytes are monocyte-derived cells, it follows that mlG can also be mediating the activation of fibrocytes that infiltrate the myocardium in our model. To test this hypothesis, we exposed PBMCs containing monocytetes to mlG. Using this in vitro assay we were able to show that the addition of mlG can activate PBMCs influencing the production of pro-inflammatory cytokines IL1β, TNFα, and IL6.

Another potential mechanism of action of mlG that could enhance the AngII-induced inflammatory response is through the ability of IgG to form aggregates [31]. The FcγRs preferentially bind monomeric (FcγI) or aggregate IgG (FcγRII, III) [32]. In general, protein aggregates interact with B cell receptors, activating B cells or targeting protein to major histocompatibility complex type II [33]. Additionally, IgG aggregates can cause clustering of FcγRs, contributing to inflammation [31]. The presence of only a small fraction of aggregated protein is capable of eliciting a response [33]. In the current study risk factors for aggregation were avoided; preparations were not heated or stored in conditions reported to contribute to protein aggregate formation and preparations were filtered prior to injection [33]. In addition, the mlG was stored at 2 mg/mL, and the dosage utilized for IV administration was 0.25 mg/mL, both of which are well below the concentration at which IgG spontaneously forms aggregates (30 mg/mL) [31]. Further support for the response elicited after mlG administration in the current study is provided by the reported inhibition of fibrocyte differentiation by aggregated IgG [32].

The treatment of AngII-exposed mice with only the Fab fragment of the mlG, which were produced by removing the Fc portion and therefore the antibodies ability to signal through FcγRs did not result in an increase in fibrosis development compared to AngII alone. Similar results were observed when animals were exposed to IgM in addition to AngII, supporting our hypothesis that the observed increase of fibrosis with IgG was based on the Fc portion interactions. Taken together, we have shown that the use of mlG in animals receiving AngII can enhance the primary inflammatory reaction within the myocardium and this appears to be mediated by Fc-mediated activation of monocyte-derived cells.

One should note that the present study was not designed to characterize the exact phenotype of infiltrated cells or whether fibrocytes or macrophages were responsible for Fc activation. However, Pilling et al. previously reported that the blockage of FcγR reduced the ability of circulating PBMCs to differentiate into fibrocytes, linking Fc activation to infiltrated cells in models of myocardial fibrosis [20]. It is also possible that stimulation of FcγRs may have a role in increasing macrophage differentiation from a pro-inflammatory to anti-inflammatory/pro-fibrotic state, ultimately resulting in an increase in fibrosis [29]. However, this has yet to be confirmed in the AngII model of myocardial fibrosis. Furthermore, our findings suggest that mlG in combination with AngII was associated with less TGFβ, suggesting a reduction in the anti-inflammatory/pro-fibrotic phenotype. This would result in an important change in the cytokine environment and balance between inflammation/cellular infiltration and resolution, potentially resulting in increased fibrosis.

While there is no established anti-fibrotic therapy in clinical use, there are a number of previous studies that have illustrated the potential applicability of neutralizing antibodies to reduce fibrosis in animal models. Such studies often report only changes between specific, targeted antibody and the non-specific control antibody treatments [34,35]. However, others have reported data suggesting that there are differences between AngII alone and AngII + IgG control antibody treatments, although these differences, such as in number and type of infiltrating cells were not investigated in depth [12].

Conclusions

The present study raises important considerations in the design of future antibody-based therapies for conditions in which inflammation can have a profound role. Our findings suggest that the Fc portion of the antibody may significantly upregulate inflammation, influencing any therapy used. This could also explain in part, why prior attempts in clinical trials using systemic administration of antibody for fibrotic disorders have largely failed [36-38]. These trials have targeted TGFβ, TGFβRII, TIMP1, and TNFα, which had promosing anti-fibrotic results with in vitro and in vivo animal models [13,39,40].

In summary, we were able to demonstrate that non-specific antibody administered to animals exposed to AngII can result in worsening of myocardial fibrosis by increasing pro-inflammatory cytokine production in the myocardium via Fc portion mediated activation.

Methods

Animals

All work was performed in accordance with the Canadian Council on Animal Care and approved by the local Dalhousie’s University Committee on Laboratory Animals. Male C57Bl/6 mice between 7 and 8 weeks of age were purchased from the Jackson Laboratory (Bar Harbour, ME, USA) and were housed within the Carleton Animal Care Facility at Dalhousie University. Mice were provided food and water ad libitum for 1 week prior to experimentation.
Antibody preparation

Two preparations of mlgG were used and the results were pooled as there was no significant difference between the preparations for any of the parameters tested. Non-specific mlgG antibody was purchased from Sigma or isolated from mouse serum. Whole mlgG was fragmented and Fc portion removed using the Pierce Fab Preparation kit (Fisher), following the manufacturer’s instructions. In brief, mlgG in PBS was prepared in dilution buffer and desalted. The mlgG was then mixed with agarose-immobilized papain in a spin column and incubated for 3 h followed by centrifugation to elute digested antibody. The Fab portion was purified from the digest using a Protein A column, after which Fc fragments and remaining whole IgG were eluted. Protein concentrations were measured by absorbance at 280 nm using a Nanodrop 2000 spectrophotometer (Thermo Fisher), and all elution fractions were run on a non-reducing gel to assess purity (Additional file 2).

AngII infusion and immunoglobulin intravenous injection

Animals were randomly assigned to the following groups; 1- AngII (2.0 μg/kg/min; Sigma Aldrich, Oakville, ON, Canada; n = 7), 2- AngII + mlgG (1 mg/kg/d; n = 6), 3- AngII + Fab (1 mg/kg/d; n = 4), 4- AngII + mlgM (1 mg/kg/d; Biolegend, San Diego, CA, USA; n = 4) and additional saline controls (saline +/- mlgG or Fab; n = 10). Animals had mini osmotic pumps containing AngII or saline implanted under general anesthesia as previously reported [6]. In short, animals were anesthetized with isoflurane (Baxter Healthcare Corp., New Providence, NJ, USA) in oxygen at which point a 1 to 2 cm mid-scapular skin incision was made and a mini osmotic pump (Alzet, Palo Alto, CA, USA) containing AngII or saline was inserted subcutaneously. The pumps remained in for 3 days during which the animals were provided food and water ad libitum and observed for signs of morbidity. Mouse IgG, Fab, and IgM were diluted in sterile saline and equalized to 100 μL, which was injected via the tail vein daily starting on the day of pump insertion surgery, for a total of three doses.

Tissue harvest

Hearts from experimental animals were harvested and weighed, then divided into three sections. The base section of the heart was processed for histological examination with the apical section split vertically and these pieces were snap frozen in liquid nitrogen immediately for molecular analysis.

Histological analysis

Hearts were processed for histological assay by fixation with 10% formalin for 24 h or protecting with sucrose/OCT followed by snap freezing. Formalin fixed tissue was paraffin-embedded and serially sectioned on a microtome (5 μm). Basic myocardial histology and cellular infiltration were examined using heart cross-sections stained with hematoxylin and eosin. A blinded observer quantified the infiltrating cells by counting the number of grids affected within an image of an entire heart cross-section at 50x magnification (1 section per animal), based on a previously published grid-scoring method to quantify the degree of cellular infiltration between groups [6]. Collagen was detected using Sirius red and fast green stains and was semi-quantified as previously described [4]. Briefly, the entire heart cross-section at 5x was analyzed using image analysis software to calculate the percentage of red pixels over the area of the cross-section.

Immunohistochemistry for C4d (Hycult Biotech, Plymouth Meeting, PA, USA) was performed on paraffin embedded tissues, which were deparaffinized and treated for antigen retrieval prior to staining. Briefly, endogenous peroxidases were quenched with 3% hydrogen peroxide; endogenous biotin was blocked (DAKO Biotin Blocking System, DakoCytomation); and non-specific staining was blocked with normal goat serum. Sections were incubated with primary antibody, followed by a specific biotin-conjugated secondary antibody. The antibody complexes were then conjugated to an Avidin-biotin complex (Vectastain ABC kit; Vector, Burlington, CA, USA) and developed using 3,3′ diaminobenzidine as the chromogen (DAB; DakoCytomation). Light microscopy was performed and pictures were captured using a Zeiss AxiosplanII with an AxioCAM HRC colour camera. Images were analyzed in Adobe Photoshop 5.0.

Immunofluorescence staining

Sucrose protected, frozen sections were fixed in 4% paraformaldehyde, permeabilized with 0.03% Triton X-100, blocked against non-specific antibody binding with 10% normal goat serum, and stained for CD45 (BD Biosciences, Mississauga, ON, Canada) and alpha-smooth muscle actin (SMA; Sigma). Antibodies were detected using anti-host specific Alexa fluorescently labeled secondary antibodies (Invitrogen). Images were captured with a Zeiss Axiosvert 200 inverted microscope with a Hamamatsu ORCA-R2 digital camera with an AttoArc 2 HBO 100 W lamp.

Relative quantitative polymerase chain reaction (qPCR)

Total RNA was isolated from snap frozen heart sections using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol. First strand cDNA was synthesized from RNA using iScript cDNA Synthesis Kit (Biorad, Hercules, CA, USA). The qPCR was completed using iQ SYBR Green Supermix (Biorad) and the iQ Multi-colour Real-Time PCR Detection System thermocycler
(Biorad) was used for detection. Efficiency curves and no-template control samples were run with each thermodenaturing. Melt curves were run after cycling to ensure target specificity. Primers were designed against mRNA sequences using PrimerBlast [41] and are listed. TGFβ, forward 5′-GCTTCCAAGGAAAGGTAGG-3′, reverse 5′-CTCTTGTAGTCCTCAGATCC-3′; CTGF, forward 5′-TCACCTCAGACACTGTGTTTCG-3′, reverse 5′-TAGGGAGTTGTCTGTCAGACG-3′; CollA1, forward 5′-CAACAGTCTTTCACTCAAGC-3′, reverse 5′-GTGGAGGGAGTTTACACGAC-3′; TNFa, forward 5′-TCTCATGCACCACCATCAAGACT-3′, reverse 5′-ACCACCTCTCCCTTGCAAGAATCA-3′; IL1β, forward 5′-TCTCGGGAAGAAGCTGCGT-3′, reverse 5′-CCCACACGTTGACATCTAGT-3′; 18S (control), forward 5′-TCAACATTTGATGATGTCCG CCGT-3′, reverse 5′-TCTTTGAGTTGTGATGCCGTTTCT-3′. Expression was normalization to the 18S ribosomal gene using the Pfaffl method.

Cell isolation and culture
PBMCs were isolated from healthy volunteers. The average donor age was 27.3 ± 2.5 years and the group included four men and eight women, none of who had any history of hypertension, cardiovascular disease, or treatment for hypertension. Briefly, whole blood was collected in EDTA containing Vacutainer tubes, diluted in Dulbecco’s phosphate buffered saline (pH8; Invitrogen) containing 2% fetal bovine serum (FBS; Invitrogen) and processed over Ficoll-Paque Plus gradient (GE Healthcare, Waukesha, WI, USA). The PBMC containing layer was isolated, washed, and cells plated in RPMI media with 10% FBS, 2 mM L-glutamine, 100 μg/mL streptomycin, and 100 U/mL Penicillin.

Isolated PBMCs were treated with mlgG (25 μg/mL) for 48 h. Supernatant was collected, centrifuged to remove cell debris and then stored at -80°C. Relative production of TNFα, ILβ, and IL6 were assessed using commercial ELISA kits, following the manufacturer’s instructions (IL1β and TNFa, Abcam; IL6, BD Biosciences, Mississauga, ON, Canada).

Statistical analysis
Data are represented as mean ± SEM. One-way ANOVA tests were completed using the Bonferroni post-test to compare the experimental groups to the relative controls. All qPCR results were evaluated based on the one-tailed T-test to compare changes in relative mean expression. All statistical calculations were computed using GraphPad Prism4 software and significance was determined if P ≤ 0.05.

Additional files

**Additional file 1: Anti-mlgG immunohistochemistry.** Immunohistochemical staining against mlgG was used to assess the amount of bound IgG in myocardium of animals exposed to saline (A), AngII (B), and AngII + mlgG (C) for 3 days. Representative images are shown at 20x.

**Additional file 2: Non-reducing gel of digested Fab.** Non-reducing PAGE (10%) was run to assess the quality of digestion of whole mlgG and isolation of the Fab fragment.

**Abbreviations**
AngII: Angiotensin II; CTGF: Connective tissue growth factor; ECM: Extracellular matrix; H&E: Hematoxylin and eosin; IL1β: Interleukin 1β; IL6: Interleukin 6; mlgG: Mouse immunoglobulin G; mlgM: Mouse immunoglobulin M; PBMC: Peripheral blood mononuclear cell; qPCR: Real-time quantitative polymerase chain reaction; SMA: α-smooth muscle actin; TGFβ: Transforming growth factor-β; TNFa: Tumor necrosis factor-a.

**Competing interests**
The authors declare that they have no competing interests.

**Authors’ contributions**
NR designed the study, acquired and interpreted data, and drafted the manuscript. AG assisted in study design, assisted with data acquisition and interpretation, and assisted in drafting the manuscript. AF and MS participated in data acquisition and analysis. DB assisted in design of in vitro experiments and acquired related data. Additionally, critical revision of the manuscript was carried out by MS, AF, and TB. JL assisted in study design and critical review of the manuscript. JFL participated in study design and drafting of the manuscript. All authors have approved the final version of the manuscript.

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**References**
1. Statistics Canada: Mortality, Summary List of Causes. Ottawa, ON: Statistics Canada; 2008:6.
2. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, Lichtman JH, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Solomon EZ, et al: Executive summary: heart disease and stroke statistics–2012 update: a report from the American Heart Association. Circulation 2012, 125:185–197.
3. Berk BC, Fujiwara K, Lehoux S: ECM remodeling in hypertensive heart disease. J Clin Invest 2007, 117:568–575.
4. Rosin NL, Falkenham A, Sopel MJ, Lee TD, Legare JF: Regulation and role of connective tissue growth factor in AngII-induced myocardial fibrosis. Am J Pathol 2013, 182:714–726.
5. Rosin NL, Sopel M, Falkenham A, Myers TL, Legare JF: Myocardial migration by fibroblast progenitor cells is blood pressure dependent in a model of angII myocardial fibrosis. Hypertens Res 2012, 35:449–456.
6. Sopel MJ, Rosin NL, Lee TD, Legare JF: Myocardial fibrosis in response to Angiotensin II is preceded by the recruitment of mesenchymal progenitor cells. Lab Invest 2010, 91:565–578.
7. Sopel M, Falkenhain A, Oner A, Ma J, Lee TD: Fibroblast progenitor cells are recruited into the myocardium prior to the development of myocardial fibrosis. Int J Exp Pathol 2012, 93:115–124.

8. Haudek SB, Cheng J, Du J, Wang Y, Hermosillo-Rodriguez J, Trial J, Taffet GE, Entman ML: Monocytic fibroblast precursors mediate fibrosis in angiotensin-II-induced cardiac hypertrophy. J Mol Cell Cardiol 2010, 49:499–507.

9. Koitabashi N, Danner T, Zaiman AL, Pinto YM, Rowell J, Mankowski J, Zhang H, Kleinewietfeld M, Fokuhl V, Dechend R, Muller DN: Hypertension signaling inhibition ameliorates angiotensin II-induced cardiac damage. Hypertension 2012, 60:1430–1436.

10. Brooks WW, Conard CH: Myocardial fibrosis in transforming growth factor beta[1] heterozygous mice. J Mol Cell Cardiol 2000, 32:187–195.

11. Frantz S, Hu K, Adamek A, Wolf J, Sallam A, Maier SK, Lonning S, Ling H, Enl G, Bauersachs J: Transforming growth factor beta inhibition increases mortality and left ventricular dilatation after myocardial infarction. Basic Res Cardiol 2008, 103:485–492.

12. Marko L, Kukan H, Park J, Qadri F, Spallek B, Binger KJ, Bowman EP, Kleinewietfeld M, Fokuhl V, Dechend R, Muller DN: Interferon-gamma signaling inhibition ameliorates angiotensin II-induced cardiac damage. Hypertension 2012, 60:1430–1436.

13. Leask A: Potential therapeutic targets for cardiac fibrosis: TGFbeta, angiotensin, endothelin, CCN2, and PDGF, partners in fibroblast activation. Circ Res 1990, 106:1675–1680.

14. Bellini A, Mattoli S: Ambivalent effect of immunoglobulins on the complement system: activation versus inhibition. Mol Immunol 2008, 45:407–407.

15. Mestas J, Hughes CC: On mice and not men: differences between mouse and human immunology. J Immunol 2004, 172:2731–2738.

16. Murray PJ, Wynn TA: Protective and pathogenic functions of macrophage subsets. Nat Rev Immunol 2011, 11:723–737.

17. Haudek SB, Trial J, Xia Y, Gupta D, Pilling D, Entman ML: Fc receptor engagement mediates differentiation of cardiac fibroblast progenitor cells. Proc Natl Acad Sci U S A 2008, 105:10179–10184.

18. Debiets JM, Van de Winkel JG, Caepuers JL, Dieteren IE, Buurman WA: Cross-linking of both Fc gamma RI and Fc gamma RII induces secretion of proinflammatory factor by human monocytes, requiring high affinity Fc-Fc gamma R interactions. Functional activation of Fc gamma RII by treatment with proteases or neutranidase. J Immunol 1990, 144:1304–1310.

19. Sutterwala FS, Noel GJ, Salgame P, Mosser DM: Reversal of proinflammatory responses by ligation the macrophage Fc gamma receptor type I. J Exp Med 1998, 188:217–222.

20. Nelin R: Interactions between immunoglobulin G molecules. Immunol Lett 2010, 132:1–5.

21. Pilling D, Tucker NM, Gomer RH: Aggregated IgG inhibits the differentiation of human fibrocytes. J Leukoc Biol 2006, 79:1242–1251.

22. Alapati D, Rong M, Chen S, Hrehre D, Rodriguez MM, Lipson KE, Wu S: Connective tissue growth factor antibody therapy attenuates hypertrophia-induced lung injury in neonatal rats. Am J Respir Cell Mol Biol 2011, 45:1169–1177.

23. Kuwahara F, Kai H, Tokuda K, Kai M, Takeshita A, Egashira K, Imazumi T: Transforming growth factor-beta function blocking prevents myocardial fibrosis and diastolic dysfunction in pressure-overloaded rats. Circulation 2002, 106:130–135.

24. Haverhilj MJ, Tien Dijke P: Exploring anti-TGF-beta therapies in cancer and fibrosis. Growth Factors 2011, 29:140–152.

25. Mann DL, McMurray JJ, Packer M, Swedberg K, Borer JS, Colucci WS, Dijan J, Drexler H, Feldman A, Kober L, Krum H, Liu P, Nieminen M, Tavazzi L, van Veldhuisen DJ, Waldenstrom A, Warren M, Westheim A, Zannad F, Fleming I: Targeted anticytokine therapy in patients with chronic heart failure: results of the Randomized Enercept Worldwide Evaluation (RENEWAL). Circulation 2004, 109:1594–1602.

26. Chung ES, Packer M, Lo KH, Fascinamade AA, Willerston JT, Anti TNFACHFL: Randomized, double-blind, placebo-controlled, pilot trial of infliximab, a chimeric monoclonal antibody to tumor necrosis factor-alpha, in patients with moderate-to-severe heart failure: results of the anti-TNF Therapy Against Congestive Heart Failure (ATTACH) trial. Circulation 2003, 107:3313–3314.

27. Westermann D, Van Limhout S, Dhayat S, Dhayat N, Schmidt A, Noutsias M, Song X, Spillmann F, Rad A, Schultheiss HP, Tschoppe C: Tumor necrosis factor-alpha antagonism protects from myocardial inflammation and fibrosis in experimental diabetic cardiomyopathy. Basic Res Cardiol 2007, 102:500–507.

28. Brocks B, Kraft S, Zahn S, Noll S, Pan C, Schauer M, Kebs B: Generation and optimization of human antagonistic antibodies against TIMP-1 as potential therapeutic agents in fibrotic diseases. Hum Antibodies 2006, 15:115–124.

29. Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL: Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. Nucleic Acids Research 2012, 40:134.