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Natural IgM Blockade Limits Infarct Expansion and Left Ventricular Dysfunction in a Swine Myocardial Infarct Model

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Abstract

Background—Acute coronary syndrome is the leading cause of mortality worldwide. However, treatment of acute coronary occlusion inevitably results in ischemia-reperfusion (I/R) injury. Circulating natural IgM has been shown to play a significant role in mouse models of I/R injury. A highly conserved self-antigen, non-muscle myosin heavy chain II (NMHC-II), has been identified as a target of pathogenic IgM. We hypothesized that a monoclonal antibody (m21G6) directed against NMHC-II may inhibit IgM binding and reduce injury in a pre-clinical model of myocardial infarction (MI). Thus, our objective was to evaluate the efficacy of intravenous m21G6 treatment in limiting infarct expansion, troponin release, and left ventricular dysfunction in a swine MI model.

Methods and Results—MGH miniature swine underwent occlusion of the mid left anterior descending coronary artery for 60min, followed by 1h, 5d, or 21d reperfusion. Specificity and localization of m21G6 to injured myocardium were confirmed using fluorescently labeled m21G6. Treatment with m21G6 prior to reperfusion resulted in a 49% reduction in infarct size ($p < 0.005$) and a 61% reduction in troponin-T levels ($p < 0.05$) in comparison to saline controls at 5d reperfusion. Furthermore, m21G6 treated animals recovered 85.4% of their baseline left

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ventricular function as measured by two-dimensional transthoracic echocardiography (2D TTE) in contrast to 67.1% in controls at 21d reperfusion ($p<0.05$).

Conclusions—Treatment with m21G6 significantly reduced infarct size and troponin-T release, and led to marked preservation of cardiac function in our study. Overall, these findings suggest that pathogenic IgM blockade represents a valid therapeutic strategy in mitigating myocardial I/R injury.

Keywords

myocardial infarction; inflammation; immunology; left ventricular function

Revascularization therapy for acute coronary syndrome, *i.e.* percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG), improves overall survival¹, but does not fully prevent left ventricular dysfunction and clinical heart failure following myocardial infarction (MI).^{2–4} Despite numerous attempts to pharmacologically reduce myocyte injury following reperfusion, only cyclosporine has shown some promise in reducing infarct size in the clinic.⁵ Thus, the opportunity exists for a novel therapeutic approach that is adjunctive to revascularization therapy in order to limit infarct size, preserve cardiac function, and ultimately improve long-term outcomes.⁶ Here, we describe the first use of a monoclonal antibody (Mab) directed against a novel target exposed early in the acute phase of reperfusion. We hypothesize that the target, a conserved epitope of NMHC-II, triggers an inflammatory cascade unless its recognition by natural IgM is competitively blocked by treatment with m21G6 Mab.⁷

Previous experiments using complement or antibody deficient mice have implicated endogenous IgM antibodies as significant contributors to the pathogenesis of I/R injury.^{8, 9} These studies showed that exposure of neo-epitopes on the surface of ischemic tissues provides a binding site for a distinct fraction of circulating IgM.^{10–12} This fraction, or ‘pathogenic’ IgM clone (IgM CM-22), recognizes an epitope on NMHC-II that is exposed following reperfusion of ischemic myocardium, intestine, or skeletal muscle in mice.^{7, 13, 14} Members of the NMHC-II family are a versatile group of molecular motors involved in cell migration, cell adhesion, vesicle transport, endocytosis, exocytosis, as well as viral infection.¹⁵ The binding region of this epitope is a sequence of 12 amino acids within NMHC-II that is highly conserved across multiple species, including humans. In our murine model of MI, administration of a 12-amino acid peptide mimotope (N2 peptide, LMKNM DPLNDNV) was protective.⁷ Specifically, infarct size was reduced by 47% and serum troponin-I (cTnI) levels were reduced by 69% in comparison to saline controls. A murine anti-N2 Mab (m21G6) was additionally prepared against the conserved N2 peptide sequence and was capable of significantly reducing cTnI and infarct size. Interestingly, intravenous (IV) administration of m21G6 during reperfusion reduced IgM deposition at the site of infarct by 81%.⁷

Therefore, our prior work posits a mechanism where ischemia followed by reperfusion leads to the expression of a highly conserved self-antigen, and recognition of this antigen by the innate immune system results in a deleterious cascade of events, which may be inhibited by administration of a targeted monoclonal antibody. In this paper, we utilize a clinically

relevant large animal model of myocardial ischemia and reperfusion to demonstrate that a single pre-reperfusion dose of m21G6 Mab results not only in a significant reduction in ventricular infarct size and remodeling, but also preservation and recovery of left ventricular systolic function.

Methods

Animals and Experimental Design

Thirty MGH miniature swine¹⁶ underwent occlusion of the mid-left anterior descending (LAD) coronary artery for 60min using a fluoroscopically-guided percutaneous balloon catheter approach. Either normal saline or m21G6 (2mg/kg) was administered IV via an internal jugular central venous catheter just prior to balloon deflation and reperfusion. Acquisition and analysis of all infarct-related and imaging data were performed by investigators blinded to the original treatment.

All animal care and procedures were in compliance with “Principles of Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health, and protocols were directly approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee.

Surgical Procedures

All animals were loaded with oral 400mg twice daily of amiodarone over 5d prior to inducing myocardial ischemia to reduce the incidence of fatal arrhythmias post-balloon occlusion. Animals were sedated with 2mg/kg Telazol® (tiletamine/zolazepam, 1:1) IM prior to central line placement and 10mg/kg ketamine IV prior to all cardiac catheterization and imaging procedures. Additionally, animals received 1g cefazolin IV for pre-procedural antibiotic prophylaxis. General anesthesia was maintained with 1–2% inhaled isoflurane. Post-procedural pain was treated with 0.03mg/kg buprenorphine IV and/or a 25mcg/h fentanyl patch transdermally. Cardiac harvest was then performed following median sternotomy, aortic cannulation and cross clamp, with infusion of cold crystalloid cardioplegia (see Data Supplement for detailed description).

Echocardiography

For animals that were followed for 3 weeks, 2D TTE was performed at baseline, 1h, 7d, and 21d post-reperfusion. Images were obtained using a high frequency 2D probe on an iE33 xMATRIX Echocardiography System (Phillips Healthcare Inc., Andover, MA). All animals were in normal sinus rhythm during image collection. Left ventricular end-diastolic (LVED) and end-systolic (LVES) dimensions and volumes were obtained and LVEF was calculated by averaging values of both Dumesnil and modified Quinones methods.^{17–19} Left ventricular end-diastolic wall thickness measurements were recorded at two locations within the myocardial infarct zone, and the mean was compared to a remote region of non-infarcted myocardium.

Area at Risk and Infarct Size Determination

At 5d post-reperfusion, the hearts were excised and processed for area at risk (AAR) and infarct size measurements ((Supplemental Figure 1–2 and Data Supplement) as previously described.⁷

Serum Hematology and Chemistry Measurements

Serum samples from swine were collected at several time points (4h, 8h, 24h, 48h, 72h, 96h and 120h) and cardiac Troponin-T (cTnT), complete blood count (CBC) and serum chemistries were analyzed at Massachusetts General Hospital Core Chemistry Laboratory using standard methodology. C-reactive protein (CRP) levels were analyzed using ELISA.

Production and Labeling of m21G6

Production of m21G6 antibody was as previously described.⁷ For the experiments in Figure 1, the final purified m21G6 was labeled with Alexa 568 dye (Life Technologies Corp., Carlsbad, CA) (see Data Supplement for detailed description).

Immunohistochemistry

Punch biopsies of swine heart were harvested as described above, embedded in Optimal cutting temperature compound (OCT), and frozen in a dry ice-isopentane bath as previously described⁷ (see Data Supplement for details).

Exclusion Criteria

Animals were excluded from our study only if complete reperfusion of LAD was unable to be re-established or if death from an intractable ventricular arrhythmia occurred. No animals were excluded due to incomplete reperfusion, however 5 animals died due to fatal ventricular arrhythmia, and thus an overall mortality rate of 16.7% is reported.

Statistical Analysis

All continuous data are expressed as mean $\pm$ standard error of the mean (SEM). The data in Figure 2A–B was analyzed using an unequal-variance t test (Welch t test) and by two-sample Student t test in Figure 2C–D. Two-factor ANOVA was used to test the effects of ‘treatment’ (levels: saline or 21G6), ‘day’ (levels: 5d or 21d) and ‘treatment*day’ on AUC cTnT and %I/AAR, transformed by natural logarithm. For post hoc comparisons, Tukey’s HSD test was used ($\alpha=0.05$). Where appropriate, a t test was used to compare means between treatment groups. A two-way repeated measure ANOVA with Sidak’s multiple comparisons test was utilized to compare cardiac functional data between and within treatment groups at baseline, 7d, and 21d reperfusion. Two-tailed p values are reported throughout with a significance threshold of $p<0.05$. All statistical analyses were performed with GraphPad Prism 6.0 software (GraphPad Inc., San Diego, CA), or using the Statistics toolbox in Matlab R2012a (The Mathworks, Natick, MA).

Results

The 12-amino acid N2 sequence on the NHMC-IIB molecule is identical and conserved across multiple species, including mice, swine, and humans, and represents the binding site of m21G6.¹³ Thus, to confirm whether ischemia induces neo-epitope expression in the AAR, fluorescently labeled m21G6 was infused over a 10min period, beginning immediately prior to reperfusion. These swine were subjected to 60min mid-LAD occlusion, and followed for 1h reperfusion prior to cardiac arrest and harvest. Punch biopsies were taken from several cross-sections of both ischemic and non-ischemic left ventricle and stained with fluorescently labeled CD31 to visualize the vascular endothelium. We observed distinct co-localization of bound m21G6 and CD31, with a high degree of specificity of bound m21G6 to injured myocardium of the left ventricle. A fraction of bound m21G6 was not directly associated with CD31 or vascular endothelium. Representative images in Figure 1 obtained by confocal microscopy depict m21G6 binding preferentially to injured (*i.e.*, ischemic) myocardium of the LV as compared to non-injured (*i.e.*, non-ischemic) myocardium of the LV. Binding of m21G6 was absent in non-ischemic regions of the LV, indicating that m21G6 binding is, in fact, specific to injured, reperfused tissues where neo-epitope expression is expected to be present.

We next examined the effect of m21G6 treatment on cTnT and infarct size following 60min LAD occlusion and 5d reperfusion. Cardiac troponin-T levels were measured at baseline, every 8h for the first 24h, and then once daily for the 5d reperfusion period. Differences in both peak (most frequently at t=8h) and integrated cTnT values were then compared between saline and m21G6-treated animals. Treatment with m21G6 led to a 61% reduction in peak mean cTnT in comparison to saline controls ($3.3\text{ng/ml} \pm 0.6\text{ng/ml}$ vs. $1.3\text{ng/mL} \pm 0.13$, respectively; $p<0.05$) (Figure 2A). Integrated cTnT levels, moreover, were reduced by 47% ($70.9\text{ng}^2/\text{ml}^2 \pm 7.1\text{ng}^2/\text{ml}^2$ vs. $134.7\text{ng}^2/\text{ml}^2 \pm 14.7\text{ng}^2/\text{ml}^2$, $p<0.01$) (Figure 2B). Serum CRP levels were also analyzed via ELISA of serum samples collected at the same time points listed above, and treatment with m21G6 was similarly associated with a significant reduction in CRP release post-reperfusion. Animals treated with m21G6 experienced an approximately 2-fold increase in CRP level from baseline to peak value post-reperfusion, while those treated with saline experienced nearly a 5-fold increase in CRP level ($p=0.039$) (Supplemental Figure 3).

While cTnT and CRP levels are widespread metrics for cardiomyocyte injury and inflammation, our primary endpoint for determining the efficacy of m21G6 in mitigating reperfusion injury was infarct size as a function of the AAR (%I/AAR) measured at 5d post-reperfusion. Specifically, treatment with m21G6 reduced %I/AAR by 51%, from $48.8\% \pm 2.5\%$ in control animals to $25.0\% \pm 4.6\%$ in treated animals ($p<0.005$) (Figure 2D). As expected, there was no significant difference with respect to the AAR between treatment groups (Figure 2C), thus validating the reproducibility of ischemic territory in our model. Infarct size as a function of total area of the LV was also significantly reduced in m21G6-treated animals as compared to the saline controls ($11.0\% \pm 1.6\%$ vs. $20.2\% \pm 1.7\%$, respectively, $p<0.001$).

To evaluate the effect of m21G6 on clinically relevant functional cardiac parameters, N=3 animals in each treatment group were followed for 3 weeks after reperfusion. Of note, treatment with m21G6 similarly led to a significant reduction in both peak and integrated troponin release over the initial 5d post-reperfusion (Table and Supplemental Figure 4). Additionally, treatment with m21G6 resulted in a 36% reduction in infarct size at 21d post-reperfusion compared to the saline controls ($10.5\% \pm 2.0\%$ vs. $27.7\% \pm 0.7\%$, respectively) (Table, Supplemental Figure 5) despite no significant difference in AAR/LV measurements (Table).

Hemodynamic and functional data were recorded at baseline, 1h, 7d, and 21d post-reperfusion. Resting hemodynamics were similar between the saline and m21G6 treated groups at baseline and 1h post-reperfusion. Saline treated animals had a higher resting heart rate, but a similar stroke volume to m21G6 treated animals at 21d post-reperfusion (Supplemental Table 1). Two-dimensional TTE revealed that in the saline treated group, LVEF was depressed at 7d ($45.6\% \pm 4.2\%$ vs. $57.4\% \pm 2.4\%$, $p<0.05$) and 21d post-reperfusion as compared to baseline ($38.5\% \pm 2.4\%$ vs. $57.4\% \pm 2.4\%$, $p<0.01$). In contrast, LVEF was unchanged in the m21G6 treated group at both 7d and 21d post-reperfusion (Figure 3A). Animals in both treatment groups had comparable left ventricular function within the normal range at baseline, and similarly, both saline and m21G6 treated groups subsequently experienced an acute decline in LVEF at 1h post-reperfusion ($43.3\% \pm 4.2\%$ vs. $46.8\% \pm 3.3\%$, respectively; data not shown). At 21d post-reperfusion, however, LVEF in the m21G6 treated animals was significantly improved in comparison to saline controls ($50.5\% \pm 3.1\%$ vs. $38.5\% \pm 2.4\%$, $p<0.05$) (Figure 3A).

We further investigated the effect of treatment with m21G6 on ventricular remodeling by measuring LV wall thickness in the infarct zone, as well as LV end-diastolic volumes and dimensions. At baseline and 21d post-reperfusion, end diastolic LV wall thickness measurements were obtained from the region of the infarct zone, as well as an unaffected region of the LV free-wall. In the saline treated group, LV wall thickness in the infarct zone was significantly reduced at 21d post-reperfusion compared to baseline ($5.0\text{mm} \pm 0.2\text{mm}$ vs. $7.7\text{mm} \pm 0.3\text{mm}$, $p<0.001$) (Figure 3B), while no significant decrease in LV wall thickness was observed in the m21G6 treated group ($7.3\text{mm} \pm 0.2\text{mm}$ vs. $7.7\text{mm} \pm 0.3\text{mm}$, $p=0.61$). At 21d, there was a significant difference in LV wall thickness between saline and m21G6 treated animals ($p<0.01$). Moreover, there was no difference between treatment groups in terms of LV wall thickness measurements taken from non-infarcted regions of the LV free wall (data not shown). End-diastolic volumes were significantly greater in saline treated animals at 21d post-reperfusion as compared to baseline ($56.4\text{ml} \pm 4.2\text{ml}$ vs. $31.8\text{ml} \pm 5.4\text{ml}$, $p<0.05$) (Figure 3C). In contrast, there was no significant change in end-diastolic volumes among those animals treated with m21G6 at 21d post-reperfusion as compared to baseline ($38.0\text{ml} \pm 8.7\text{ml}$ vs. $32.3\text{ml} \pm 1.6\text{ml}$, $p=0.59$). Despite a 30% increased mean LVEDV in saline vs. m21G6 treated animals at 21d post-reperfusion, this difference did not achieve statistical significance. At 21d reperfusion in the saline treated group, a significant increase was observed in LVEDd compared to baseline ($35.3\text{mm} \pm 1.3\text{mm}$ vs. $28.7\text{mm} \pm 2.0\text{mm}$, $p<0.01$). There was also a significant increase in LVEDd in the saline treated group at 21d reperfusion compared to the m21G6 treated group ($35.3\text{mm} \pm 1.3\text{mm}$ vs. $29.3\text{mm} \pm 1.8\text{mm}$, $p<0.05$) (Figure 3D).

In summary, these data demonstrate that m21G6 treated animals exhibit a substantial reduction in myocardial injury and preservation of cardiac function at 21d reperfusion, along with evidence of less adverse structural ventricular remodeling in comparison to saline treated controls.

Discussion

While it is well established that revascularization therapy results in myocardial salvage, the process of reperfusion itself also leads to cardiomyocyte death which can limit the benefits of revascularization in terms of long term improvement in LV systolic function. This phenomenon, referred to as “lethal reperfusion injury”, accounts for a considerable component of myocardial infarct size and represents a target for therapeutic intervention.^{6, 20} In our study, we developed a consistent and reproducible swine model of myocardial I/R injury via percutaneous balloon catheter occlusion of the mid-LAD for 60min. This model closely parallels clinically relevant techniques of coronary revascularization, including PCI and CABG, during which administration of therapies for reperfusion injury are feasible. Using this model, we showed that a single treatment with 2mg/kg of m21G6 at the time of reperfusion led to a comparative reduction in infarct size and adverse ventricular remodeling with improved recovery of systolic function.

The literature supports that myocardial infarction followed by revascularization results in activation and migration of numerous acute inflammatory cells in the infarct zone, including neutrophils, monocytes, and macrophages, which release cytokines and mediate tissue damage. In this early phase, up to 72h reperfusion, infarct expansion occurs, followed by breakdown of irreversibly injured myocardium. During the late phase, myocardial scar formation takes place primarily from 1–4 weeks reperfusion. This process leads to adverse left ventricular remodeling, including LV dilation to increase filling volumes and hypertrophy to reduce wall stress. The final result is impaired systolic function.²¹ By binding exposed N2 neo-epitopes in revascularized myocardium and altering the acute inflammatory response, we found that m21G6 is able to limit tissue necrosis. Fold-change increase in CRP levels was significantly lower in animals treated with m21G6 prior to reperfusion, which indicates that m21G6 ameliorates tissue damage triggered by acute inflammatory mediators and complement activation. C-reactive protein is known to localize to infarcted myocardium and may work in conjunction with activated complement in promoting inflammation, thrombosis, and injury.²² In fact, high CRP levels post MI correlate to unfavorable outcomes according to prior studies.²³ Although the rise in serum CRP supports increased systemic inflammation, the myocardial tissue sections were not analyzed directly for markers of local inflammation. In addition, LV wall thickness in the infarct zone remained essentially unchanged in m21G6-treated animals, but was reduced in the saline treated animals by 35%. Moreover, saline controls experienced a 30% increase in LVEDV and a 17% increase in LVEDd at 21d reperfusion, suggesting that m21G6 treatment mitigates adverse ventricular remodeling.

To date, several studies have confirmed a critical role for natural antibodies in the initiation of acute inflammation in myocardial I/R injury as well as other I/R models.^{7, 24–29} Our previous work has demonstrated the importance of a specific pathogenic clone of IgM in the

induction of I/R injury and proposes a mechanism whereby exposure of a neo-epitope on NMHC-II expressed on reperfused tissues provides a binding site for natural IgM (Figure 4).⁷ Blockade of this pathway by specifically inhibiting the binding of pathogenic IgM to the N2 epitope represents a novel and unexploited pathway for therapeutic intervention in the treatment of I/R injury. As we have demonstrated, m21G6 binding to exposed N2 epitopes on NMHC-II following reperfusion prevents binding of circulating pathogenic IgM binding and subsequent complement activation, thereby moderating innate immune system activation (Figure 4). Remarkably, a single dose administration of m21G6 at 2mg/kg, with a serum half-life in swine of approximately 51h, was sufficient to dampen this acute inflammatory signaling pathway and subsequently decrease infarct size at 5d post-reperfusion and improve cardiac function at 21d post-reperfusion. Further investigation (data not shown) suggests similar efficacy with the humanized m21G6 in this model, thereby validating this approach for clinical evaluation. Given the prolonged half-life of m21G6, an extended treatment window may reveal similar efficacy and provide greater flexibility in administration following acute MI.

Non-muscle myosin¹⁵ has been implicated in smooth muscle proliferation associated with atherosclerosis and hypertension³⁰ but has not been previously identified as a potential target in myocardial reperfusion injury. The precise mechanism whereby the cytoplasmic N2 epitope becomes accessible to circulating IgM has yet to be elucidated. However, in non-myocardial tissues, it is of interest that during apoptosis in a human Jurkat T cell line, NMHC-A is cleaved in a caspase-specific manner.³¹ It has also been reported in Jurkat cells that NMHC-IIA is cleaved and exposed during apoptosis where it is recognized by a subset of chronic lymphocytic leukemia IgM antibodies.³² Thus apoptotic cells of any lineage within the infarct zone could provide a nexus for a damaging inflammatory cascade and may contribute to myocardial cell death in the setting of MI.³³

IgM with injury-inducing specificity for the N2 epitope on NMHC-II, is present in all species that have been investigated, including humans, rats, mice and swine (data not shown), and the N2 binding region is 100% conserved across all of these species. In this study, fluorescently labeled m21G6 exhibits a high degree of binding specificity to ischemic LV tissue, while undetectable in non-ischemic LV tissues (Figure 1). It is also important to note that there were no m21G6 treatment-related adverse effects on other organs as determined by histological evaluation of the liver, lung, kidney, spleen, or mediastinal lymph nodes in any of the treatment groups (*i.e.*, 1h, 5d, and 21d post-reperfusion). Serum hematology, chemistries, and liver enzymes were also within normal ranges at all time points and across all treatment groups (data not shown).

While revascularization therapy (*i.e.*, PCI or CABG) dramatically improves overall survival, the full benefits of revascularization are tempered by I/R injury, as some estimates indicate that I/R injury may account for as much as 50% of the final myocardial infarct size according to several animal studies.⁶ This is supported by our murine model in which RAG-1^{-/-} mice, which are antibody deficient, were reconstituted with pathogenic IgM and subjected to I/R. Infarct sizes (%I/AAR) were significantly increased in RAG-1^{-/-} mice reconstituted with pathogenic mouse IgM as well as human IgM purified from 10 separate individuals when compared with the RAG-1^{-/-} saline controls (data not shown).⁷ We have

also established in our murine model that m21G6 is capable of blocking human pathogenic IgM and reducing infarct size and cTn release which not only suggests that the specificity of m21G6 is conserved across species¹² but also that a humanized version of m21G6 may have therapeutic potential. These data demonstrate that circulating pathogenic IgM is specific for the N2 epitope and is capable of restoring myocardial I/R injury to a level similarly seen in C57BL/6 mice. However, because neither the RAG-1^{-/-} mice nor the swine used in the present study were not completely protected from injury, other mechanisms of injury are likely also involved and may include, for example, mitochondrial permeability pore opening³⁴, the Reperfusion Injury Salvage Kinase (RISK) pathway³⁵, as well as other intrinsic and extrinsic pathways. In dose-response curves in C57BL/6 mice with normal antibody levels, as well as in this swine study, we observe a maximal reduction in infarct size due to reperfusion injury to be approximately 50%.

Overall, we have shown that m21G6 has a strong therapeutic effect compared to saline controls in a large-animal model by mitigating myocardial necrosis and infarct size, while also enhancing recovery of cardiac function and inhibiting adverse remodeling. Although numerous pre-clinical studies have identified therapeutic strategies for targeting myocardial I/R injury, many of these therapies have had disappointing results in human clinical trials.³⁶ We recognize that pre-clinical large animal studies, such as ours, do not capture the complex pathophysiological context of human disease and thus have limited potential for translation to the bedside. However, our experimental model does adhere to current guidelines for the pre-clinical assessment of novel cardioprotective agents including timing of drug administration, duration of both ischemia and reperfusion, and evaluation of the appropriate acute and long-term study endpoints.^{37, 38} Furthermore, the data presented here as well as in our rodent model suggest that this therapeutic approach may have potential for successful clinical translation for multiple reasons: 1) the novel pathway of injury, the presence of pathogenic IgM, and the N2 epitope binding sequence are all conserved across multiple species, including humans; 2) experimental models performed previously with rodents, and here with large animals, have demonstrated a robust effect of m21G6 in reducing I/R injury; and 3) the therapeutic window may extend for up to 90 min post-reperfusion.⁷ Therefore, we believe that further development and clinical investigation of the humanized m21G6 is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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WHAT IS KNOWN

- Revascularization therapy for STEMI improves overall survival, but still results in left ventricular dysfunction and clinical heart failure in a substantial proportion of patients.
- “Lethal reperfusion injury” accounts for a considerable component of myocardial infarct size and represents a target for therapeutic intervention.
- Despite numerous attempts to reduce myocyte injury following reperfusion, however, only cyclosporine in one study has shown some promise in reducing infarct size in the clinic, thus the need for novel approaches.

WHAT THE STUDY ADDS

- The study describes the first use of a monoclonal antibody (m21G6) directed against a novel cytoplasmic target exposed early in the acute phase of reperfusion in a swine model.
- The target, a conserved epitope of non-muscle myosin heavy chain-II (NMHC-II), triggers an inflammatory cascade unless its recognition by natural IgM is competitively blocked with m21G6.
- Treatment with m21G6 significantly reduces infarct size, troponin-T release and at day 21 post-reperfusion, preserves cardiac function.

Ischemic left ventricle

Non-ischemic left ventricle

Figure 1. Specificity of m21G6 in the swine model of MI

Animals were subjected to 1h LAD occlusion and 1h reperfusion. Prior to reperfusion, fluorescently labeled m21G6 (2mg/kg) (arrow heads, red) was injected IV. Tissue sections were also stained with CD31 (green) to visualize vasculature and analyzed by confocal microscopy. Scale bar, 10 μ m.

Figure 2. Effect of m21G6 on serum cTnT and infarct size at 5d reperfusion in the swine MI model

Swine were subjected to 1h LAD occlusion and 5d reperfusion. Prior to reperfusion either saline or m21G6 (2mg/kg) was injected IV. (A) Peak cTnT levels. (B) Integrated cTnT levels over 5d reperfusion (area under the curve). (C) AAR expressed as a percentage of the LV. (D) Myocardial infarct size size expressed as a percentage of the AAR. #, $p<0.05$; *, $p<0.01$; **, $p<0.001$. For all panels, $N=11$ and $N=5-6$ for saline and m21G6, respectively.

Figure 3. Effect of m21G6 on cardiac function over 21d reperfusion

Swine were subjected to 1h LAD occlusion and 21d reperfusion. Prior to reperfusion either saline or m21G6 (2mg/kg) was administered IV. At the indicated reperfusion times, (A) left ventricular ejection fraction (LVEF), (B) LV wall thickness, (C) left ventricular end diastolic volume (LVEDV), and (D) left ventricular end diastolic dimension (LVEDd) were measured. #, $p < 0.05$; *, $p < 0.01$; **, $p < 0.001$. For all panels, $N = 3/\text{group}$.

Figure 4. The N2 Pathway

During myocardial infarction, hypoxia and reperfusion-induced neo-antigen expression of NMHC-II at the cell surface triggers an IgM-complement cascade leading to cell death. Murine 21G6 masks this neo-antigen thereby blocking IgM binding and salvaging myocardium and preventing loss of cardiac function.

Table 1
Comparison of the effect of m21G6 on serum cTnT and infarct size at 5d and 21d reperfusion in swine MI model

Swine were subjected to 1h LAD occlusion and either 5d or 21d reperfusion. Prior to reperfusion either saline or m21G6 (2mg/kg) was injected IV.

	Saline				m21G6			
	Peak cTnT	AUC TnT	%I/AAR	%AAR/LV	Peak cTnT	AUC TnT	%I/AAR	%AAR/LV
Day 5	3.3 ± 0.6	134.7 ± 14.7	48.8 ± 2.5	41.7 ± 2.7	1.3 ± 0.1 ^a	70.9 ± 7.1 ^b	25.0 ± 4.6 ^c	44.7 ± 3.0
Day 21	3.1 ± 0.5	163.2 ± 21.9	27.7 ± 0.7	52.0 ± 5.2	0.9 ± 0.1 ^d	37.6 ± 2.6 ^e	10.5 ± 2.0	44.0 ± 1.3

Data is presented as mean ± SEM and analyzed by two-factor ANOVA testing effects of treatment and day. For post-hoc comparisons, Tukey's HSD was used.

^{a,b,c} , p<0.05 vs 5d saline;

^{d,e} , p<0.05 vs 21d saline.