Role of the complement components C5 and C3a in a mouse model of myocardial ischemia and reperfusion injury

Die Rolle der Komplementfaktoren C5 und C3a in einem Mausmodell der myokardialen Ischämie und Reperfusion

Abstract

Objective: Ischemic heart disease is the leading cause of death world-wide. The complement system plays a major role in inflammation and tissue injury following myocardial ischemia and reperfusion (MI/R) injury. Systemic C5 inhibition in clinical studies has resulted in mixed results and the role of earlier complement components (e.g., C3a), upstream from C5 cleavage, has not been elucidated for MI/R injury. Therefore, we evaluated the role of C5 or C3a in a mouse model of MI/R injury.

Methods: We performed experimental MI/R with 30 min of ischemia and 4 hr of reperfusion in 8–12 wk old C57BL/6 (WT) mice. Systemic C5 or C3a inhibition was performed with an anti-C5 monoclonal antibody (BB5.1) 30 min prior to reperfusion or with a C3a receptor antagonist (C3aRA). Since the C3aRA induces neutropenia that resolves within 120 min, we administered C3aRA at two different time points in two separate groups: 30 min prior to reperfusion within the neutropenic time frame and 120 min prior to reperfusion, when the neutropenia had resolved, but C3aRA remained active. Following MI/R, cardiac function was assessed via echocardiography, serum troponin I concentrations were measured as an index of myocardial cell death and myocardial inflammation was determined via myocardial polymorpho-nuclear leukocyte (PMN) infiltration.

Results: In wild type mice, MI/R significantly decrease myocardial ejection fraction and increased serum troponin I levels and myocardial PMN infiltration compared to sham-operated animals. Systemic C5 in-hibition, 30 min prior to reperfusion, significantly protected mice from MI/R injury, confirming an important role for C5 in murine MI/R injury. Treatment with the C3aRA, 30 min prior to reperfusion (i.e., within the neutropenic time frame), protected mice significantly from MI/R related injury. In contrast, administration of the C3aRA 120 min prior to reper-fusion, when the neutropenia had resolved, but C3aRA remained active, did not prevent MI/R injury.

Conclusions: These results confirm an important role for C5 cleavage in murine MI/R injury. At the same time, they suggest a minimal role for C3a, since neutropenia rather than C3a receptor antagonism appears to be responsible for C3aRA related amelioration in MI/R injury. While C5 inhibition in the clinical setting of MI/R does not appear to be therapeutic, our results raise the possibility that inhibition of either C5a or C5b-9 may be more advantageous than inhibition of C3a or complete inhibition of C5 in humans.

Keywords: ischemia, reperfusion, I/R, cardiac, myocardial, heart, ischemic heart disease, C5, C3a, complement

Marc N. Busche[1,2]
Gregory L. Stahl[1]

1 Center for Experimental Therapeutics and Reperfusion Injury, Harvard Institutes of Medicine, Boston, MA, USA

2 Department of Plastic, Hand and Reconstructive Surgery, Hannover Medical School, Hannover, Germany

Zusammenfassung

Einleitung: Die koronare Herzerkrankung ist weltweit die führende Todesursache. Das Komplementsystem spielt eine wichtige Rolle bei der Entzündungsreaktion und dem Gewebeschaden nach myokardialer Ischämie und Reperfusion (MI/R). Die Inhibition des Komplementfaktors C5 hatte in klinischen Studien unterschiedliche Ergebnisse gezeigt, und die Rolle von Komplementfaktoren, die oberhalb der C5-Spaltung in der Komplementkaskade liegen (z.B. C3a), wurde für MI/R nicht erforscht. Daher untersuchten wir die Rolle von C5 und C3a in einem MI/R-Mausmodell.

Methoden: Wir führten in 12 Wochen alten C57BL/6 (WT)-Mäusen experimentell MI/R mit 30 min Ischämie und 4 h Reperfusion durch. Systemische Inhibition der Komplementfaktoren C5 oder C3a wurde mittels eines anti-C5 monoklonalen Antikörpers (BB5.1) 30 min vor der Reperfusion oder mit einem C3a-Rezeptorantagonist (C3aRA) durchgeführt. Da der C3aRA eine Neutropenie induziert, die innerhalb von 120 min abgeklungen ist, verabreichten wir den C3aRA in zwei unterschiedlichen Versuchsgruppen zu zwei Zeitpunkten: 30 min vor der Reperfusion, innerhalb der Neutropenie, und 120 min vor der Reperfusion, wenn die Neutropenie abgeklungen war, aber der C3aRA noch aktiv war. Nach MI/R untersuchten wir die kardiale Funktion mittels Echokardiographie, bestimmten die Serumkonzentration von Troponin I als Zeichen myokardialen Zelluntergangs und die myokardiale Infiltration mit Polymorphonukleären Zellen (PMN) als Maß myokardialer Inflammation.

Ergebnisse: WT-Mäuse hatten nach MI/R im Vergleich zu sham-operierten Mäusen signifikant reduzierte Ejektionsfraktionen, während Troponin I und die myokardiale PMN-Infiltration signifikant erhöht waren. Systemische C5-Inhibierung 30 min vor der Reperfusion schützte Mäuse signifikant vor MI/R-Schädigung und bestätigt damit eine wichtige Rolle von C5 in MI/R im Mausmodell. Eine Behandlung mit dem C3aRA 30 min vor der Reperfusion, während der neutropenischen Phase, schützte die Mäuse signifikant vor MI/R-Schädigung. Eine Verabreichung des C3aRA 120 min vor der Reperfusion, wenn die Neutropenie abgeklungen war, aber der C3aRA noch aktiv war, verhinderte allerdings keine MI/R-Schädigung.

Fazit: Diese Ergebnisse bestätigen eine wichtige Rolle für C5 bei MI/R im Mausmodell. Die durch den C3aRA verursachte Neutropenie, und nicht der C3a-Rezeptorantagonismus, scheint für die Abschwächung des MI/R-Schadens verantwortlich zu sein. Damit scheint C3a bei MI/R im Mausmodell nur eine untergeordnete Rolle zu spielen. Da die Inhibition des Komplementfaktors C5 in klinischen Studien nicht erfolgreich war, sprechen unsere Ergebnisse dafür, dass die Inhibition von C5a oder C5b-9 in klinischen Studien erfolgversprechender sein könnte als die Inhibition von C3a oder eine komplette Inhibition von C5.

Schlüsselwörter: Ischämie, Reperfusion, I/R, kardial, myokardial, Herz, koronare Herzerkrankung, C5, C3a, Komplement

Introduction

Ischemic heart disease is the leading cause of death in developed countries worldwide [1]. Though reperfusion of the hypoxic myocardial tissue after ischemia is critical for reoxygenation and organ salvage, it also results in myocardial ischemia and reperfusion (MI/R) injury, causing significant myocardial damage of the reperfused tissue [2], [3], [4]. During ischemia, myocardial cells undergo intrinsic changes leading to perturbations in surface molecule expression and the formation of neoantigens, making the affected cells targets for the innate immune system [2]. This phenomenon remains a significant barrier to the otherwise restorative potential of reperfusion therapy in ischemic heart disease.

Over the last 30 years, the complement system has been shown to play a major role in myocardial inflammation and tissue injury following MI/R [2], [3], [4], [5], [6].
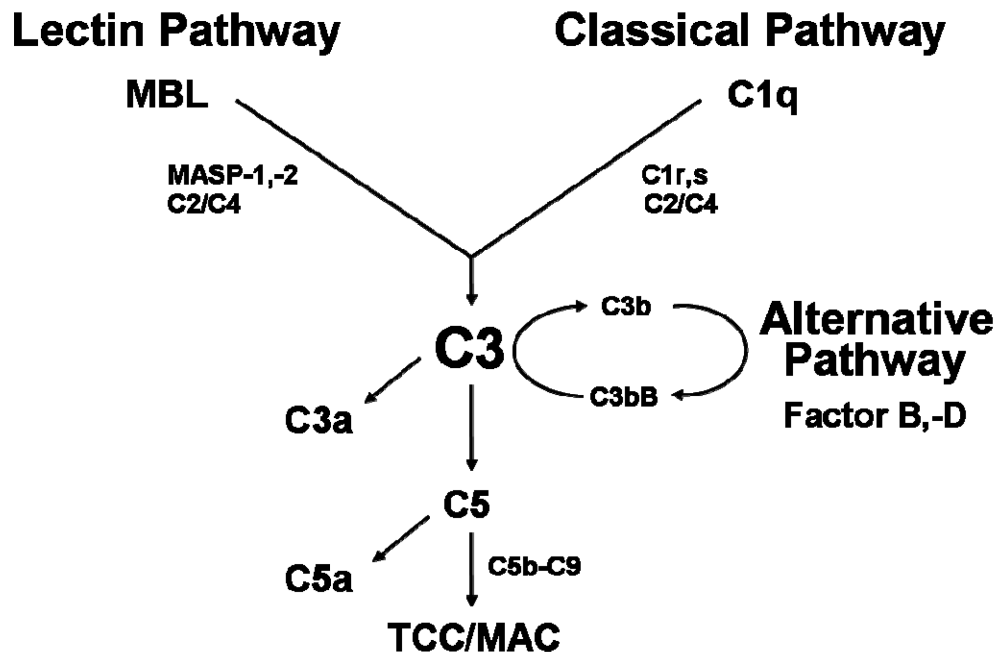
Figure 1: Complement pathways
Diagram outlining the three complement pathways. MBL = mannose-binding lectin; MASP = MBL-associated serine protease; TCC = terminal complement complex; MAC = membrane attack complex.

Complement, part of the innate immune system, can be activated by at least three separate pathways: the classical, alternative, and lectin pathways (Figure 1). The mechanism of ischemia and reperfusion (I/R) injury results from the recognition of neoantigens that have been exposed during ischemia with subsequent initiation of complement. In the classical pathway, natural antibodies interact with neoantigens and form antigen-antibody complexes. The complement subcomponent C1q can initiate classical pathway activation by recognition of these antigen-antibody complexes (Figure 1); however, C1q can also recognize apoptotic cells in the absence of antibodies [7], [8], [9]. The lectin pathway is initiated by recognition of exposed carbohydrates/ligands by mannose-binding lectin (MBL) and/or ficolins (Figure 1) [10], [11]. C3b deposition from either the classical or lectin pathway can initiate activation of the alternative pathway and serves to amplify complement activation (i.e., tick-over amplification) [3], [4], [12]. While the lectin and alternative complement pathways are historically known to activate complement in an antibody independent manner, our group recently demonstrated that MBL also interacts with IgM to activate the lectin complement pathway [13] and that lectin complement pathway activation in myocardial MI/R is dependent on both MBL and IgM [14]. Similar observations by others, also support an Ab-dependent activation of the MBL-dependent lectin pathway [15]. All three activated complement pathways converge to generate two structurally dissimilar, yet functionally similar C3 convertases (Figure 1). Each C3 convertase cleaves C3 into two fragments, the anaphylotoxin C3a and the complement component C3b, which binds to the C3 convertase to form a C5 convertase, followed by cleavage of C5 into C5a and C5b [3], [4]. The anaphylatoxin C5a is a potent activator of inflammation and a chemoattractant for inflammatory cell populations, including polymorphonuclear leukocytes (PMNs), whereas C5b can interact with C6, C7, C8, and multiple C9 units to form the terminal complement complex C5b-9. C5b-9 formation can lead to cellular activation or lysis of anucleated cells [3], [4]. Thus, complement related tissue injury may be induced directly by the terminal complement complex C5b-9, by cell-bound ligands, including C4b and C3b, and by the anaphylatoxins C3a and C5a, which can amplify injury by attracting and activating inflammatory cells [3], [4].

To attenuate MI/R injury experimentally, the complement system has been inhibited on several levels. Previous studies have shown that C5 inhibition protects against I/R injury of the brain [16], lung [17] and myocardium [18]. However, in clinical studies, systemic C5 inhibition with pexelizumab has resulted in mixed results. Early studies suggested a pexelizumab-related protection with impressive improvement of the survival rate in MI and coronary artery bypass graft surgery [19], [20], [21]. As a result, two large Phase 3 clinical trials were initiated, which showed a non-significant trend toward a positive effect of the study drug, but failed to meet the primary endpoints [22], [23], [24].

While the anaphylatoxin C5a has been shown to be a potent activator of inflammation [3], [4], the role of the anaphylatoxin C3a, an early complement component upstream from C5 cleavage, has shown mixed results in animal models of I/R injury. In mouse models of renal and cerebral I/R injury, C3a appears to play a key role in mediating I/R injury [25], [26], while Proctor et al. suggested only a minor role for C3a in GI/R injury [27]. Despite this, the role of the anaphylatoxin C3a in MI/R injury

has not been elucidated. We therefore evaluated the role of C5 and C3a in a mouse model of MI/R injury.

Methods

Administration of anti-C5 monoclonal antibody and C3a receptor antagonist

All animals used were male mice aged 8–12 wk old. C57BL/6 [wild type (WT)] mice were obtained from Charles River Laboratories (Wilmington, MA, USA). All procedures were reviewed and conducted in accordance with the Institute's Animal Care and Use Committee (IACUC). Experiments were performed according to the standards and principles set forth in the National Institutes of Health [28]. One group of mice was anesthetized with isoflurane and injected via the penile vein with 1 mg/kg of a C3a receptor antagonist (C3aRA; Calbiochem, San Diego, CA, USA) 120 min prior to reperfusion to achieve C3a receptor antagonism and avoid the associated neutropenia as described [27]. Two additional groups were treated during the MI/R procedure 30 min prior to reperfusion with 1 mg/kg of the C3aRA or 50 mg/kg of a C5 monoclonal antibody (BB5.1), as we have described [16]. Sham operated mice and mice undergoing MI/R without C3aRA or C5 monoclonal antibody treatment, received vehicle (e.g. PBS).

Experimental MI/R

Experimental MI/R was performed as we have previously described [5], [14], [29]. In brief, mice were initially anesthetized with sodium pentobarbital (60 mg/kg, i.p.), intubated and ventilated with positive pressure on a small animal ventilator (Model 683, Harvard Apparatus, Holliston, MA, USA). Anesthesia was then maintained with isoflurane (1–3 MAC). After creating a longitudinal incision through the skin of the lateral left chest, the overlying chest muscles were retracted using 5-0 black-braided silk suture (Ethicon, Somerville, NJ, USA). The chest was opened within the third intercostal space, and the chest wall retracted using 5-0 black-braided silk suture (Figure 2 B). An 8-0 black-braided silk suture (U.S. Surgical, Norwalk, CT, USA) was passed underneath the left anterior descending coronary artery (LAD), 2 mm from the tip of the left atrium. To induce ischemia, a 1- to 2-mm piece of 0-0 suture (Deknatel, Fall River, MA, USA) was placed on the LAD, and the 8-0 suture tightened to occlude the artery (Figure 2 A and B). After 30 min of ischemia, the ligation was loosened and the 0-0 suture removed and the myocardium reperfused for 4 hr. The chest wall was closed using 5-0 black-braided silk sutures. The overlying chest muscles were allowed to retract, and the skin was sutured using 5-0 black-braided silk sutures. An electrocardiogram (modified lead III) was evaluated before, during and after ischemia and used to establish comparable ischemia and reperfusion in every mouse. Only mice demonstrating increased ST elevation (>4 mm) during ischemia were included in the study.
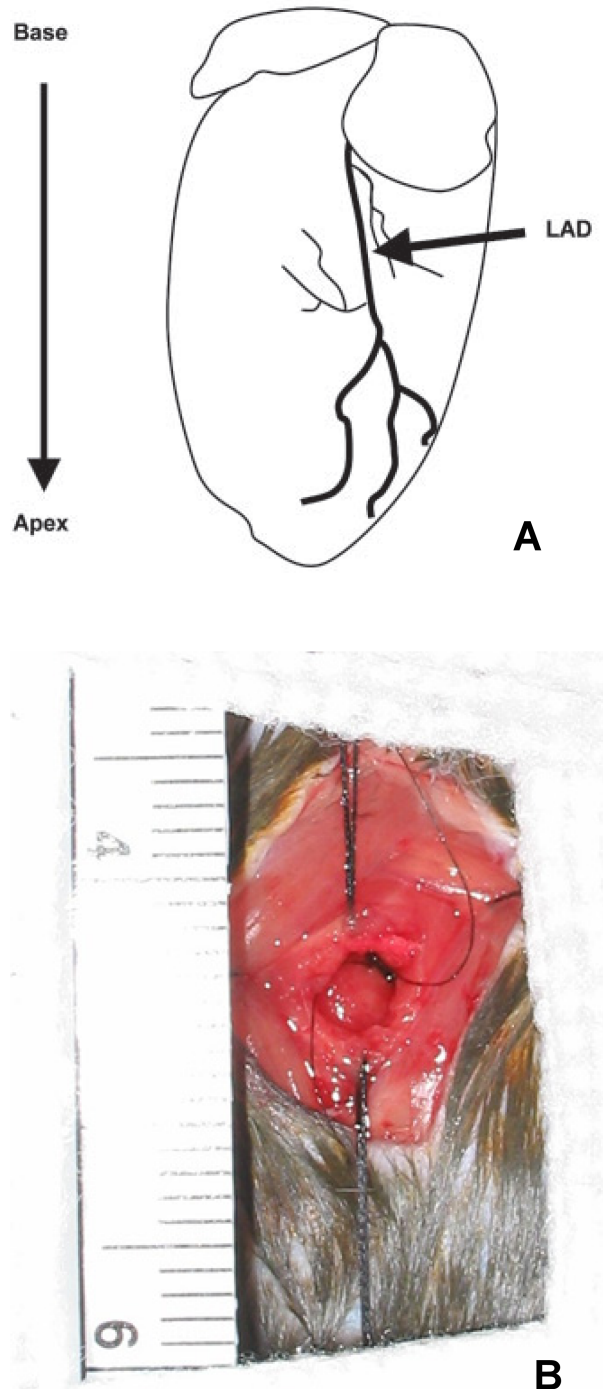
Figure 2: Experimental MI/R
2A: Diagram outlining the location of left anterior descending artery (LAD)-ligation and direction of myocardial sections/slices.
2B: Intraoperative picture of surgical approach through third intercostal space to the heart and LAD-ligation. 5-0 black-braided silk sutures are used to retract third and fourth rib. Myocardial ischemia is induced by LAD-ligation using 8-0 black-braided silk suture over a 1- to 2-mm piece of 0-0 suture placed for protection on the LAD to prevent the 8-0 suture from cutting into the LAD and myocardium.

Echocardiography

Previous work from our laboratory has shown that myocardial damage via infarct analysis is directly correlated with loss of left ventricular function as measured by echocardiography [5]. Therefore, in the present study, echocardiography measurements were used to assess cardiac function. Echocardiography was performed 4 hr after reperfusion using a Philips Sonos 5500 (Philips Medical Systems, Bothell, WA, USA) with a 7–12 MHz animal transducer (Agilent Technologies, Santa Clara, CA, USA), as we described [5], [14], [29]. Ejection fraction (EF) was calculated by left ventricular M-mode measurements and via long axis length and short axis area measurements of the left ventricle (LV) [30], [31], [32]. For EF, only M-mode data are presented, as both methods of EF measurements produced identical results.

Collection of blood and tissue

Following reperfusion and echocardiography, the chest cavity was opened, the inferior caval vein cut, and blood was collected from the thoracic cavity as described previously [5], [14], [29]. Hearts were excised and fixed in 10% formalin-PBS at 4°C overnight.

Serum troponin I concentrations

Serum troponin I concentrations were measured as an index of myocardial cell death using a commercially available ELISA kit (Life Diagnostics, West Chester, PA, USA) as described previously [5], [14], [29], [33].

Histological sections and staining for PMN

Heart samples were dehydrated, embedded in paraffin and cut cranial to caudal into 7 μm sections as we described [14], [29]. We used comparable sections for each heart, so the staining was performed on similar levels of each heart. Thus, there was no need to compare different areas of each section, as each section represents a cut through the entire heart and each section was evaluated in total by quantitative pixel analysis. To evaluate myocardial PMN infiltration, sections were deparafinized with EZ-DeWax Solution (BioGenex, San Ramon, CA, USA) and incubated with blocking buffer containing 5% normal sheep serum for 1–2 hr at room temperature. Primary antibody incubation was performed with purified rat anti-mouse Ly-6G monoclonal Ab (BD Pharmingen, Franklin Lakes, NJ, USA) for 1–2 hr at room temperature [34]. Following primary antibody incubation, slides were washed four times for 15 min with TBS-Tween and then incubated with a secondary sheep anti-rat IRDye800 Ab (Rockland Immunochemicals, Gilbertsville, PA, USA) for 1–2 hr at room temperature. After washing four times for 15 min each, excess fluid was removed and the slide was covered with Gel Mount (Biomeda, Foster City, CA, USA),

cover slips (Fisher Scientific, Pittsburgh, PA, USA) and sealed after 1 hr with clear nail polish. Ultimately, sections were scanned for PMN infiltration with an Odyssey infrared imaging system (LI-COR, Lincoln, NE, USA) at 800 nm, as we validated and documented previously [14], [29]. Myocardial sections stained for PMN deposition were quantitatively analyzed for 4–6 animals per group by pixel counting using ImageJ software (NIH).

Statistics

All statistical analyses of data were performed using SigmaStat software (SPSS, Chicago, IL, USA). All data were evaluated using one-way ANOVA and post hoc analysis using the Student-Newman-Keuls method. All data are expressed as the mean ± SE and differences were considered significant at $P<0.05$.

Results

Echocardiography

Following 30 min of ischemia and 4 hr of reperfusion, a significant decrease in left ventricular ejection fraction (EF) in non-treated WT mice compared to sham-operated animals was observed (Figure 3 and Figure 4). EF before MI/R was comparable to sham operated animals (data not shown). Mice treated with C5 monoclonal antibody 30 min prior to reperfusion were significantly protected from loss of myocardial function following MI/R compared to non-treated mice (Figure 3 and Figure 4), suggesting a significant role of C5 in MI/R injury. The C3aRA is known to induce neutropenia that resolves within 120 min while its C3a receptor antagonism is still active [27]. MI/R in mice treated 120 min prior to reperfusion, when the neutropenia had resolved, but C3aRA was still active, induced a significant decrease in EF compared to sham-operated mice. In contrast, mice treated with the C3aRA 30 min prior to reperfusion were significantly protected from loss of myocardial function following MI/R, compared to sham-operated mice.

Serum troponin I concentrations

Serum troponin I concentrations provided biochemical findings that confirmed and supported the echocardiographic observations. Sham-operated animals did not present detectable serum troponin I concentrations (data not shown). Thirty min of ischemia and 4 hr of reperfusion in vehicle-treated mice significantly increased serum troponin I concentrations. Confirming our echocardiographic findings, mice treated with a C5 monoclonal antibody had significantly reduced serum troponin I concentrations compared to vehicle-treated mice (Figure 5), supporting a significant role for C5 in MI/R injury. Serum troponin I concentrations in mice treated with C3aRA 30 min prior to reperfusion were significantly reduced compared to vehicle-treated mice undergoing MI/R, while administra-
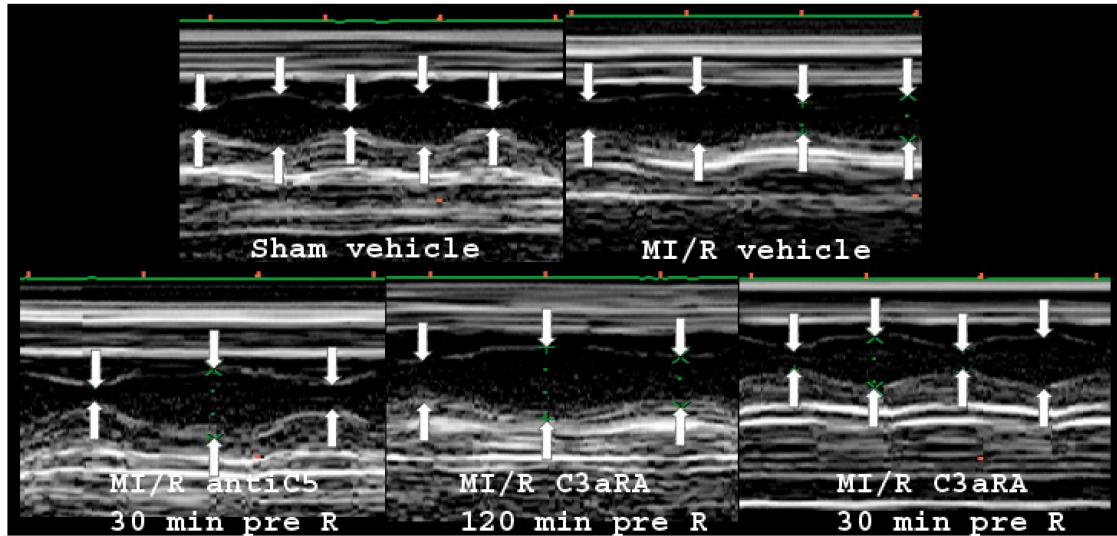
Figure 3: Typical M-mode recordings
M-mode measurements were performed by echocardiography. In the upper row: sham-operated mice (Sham vehicle) and mice that underwent MI/R with 30 of ischemia and 4 hr reperfusion (MI/R vehicle). The lower row represents animals after MI/R treated with C5 monoclonal antibody (antiC5) 30 min prior to reperfusion (R) or with C3a receptor antagonist (C3aRA) 120 min and 30 min prior to R, respectively.
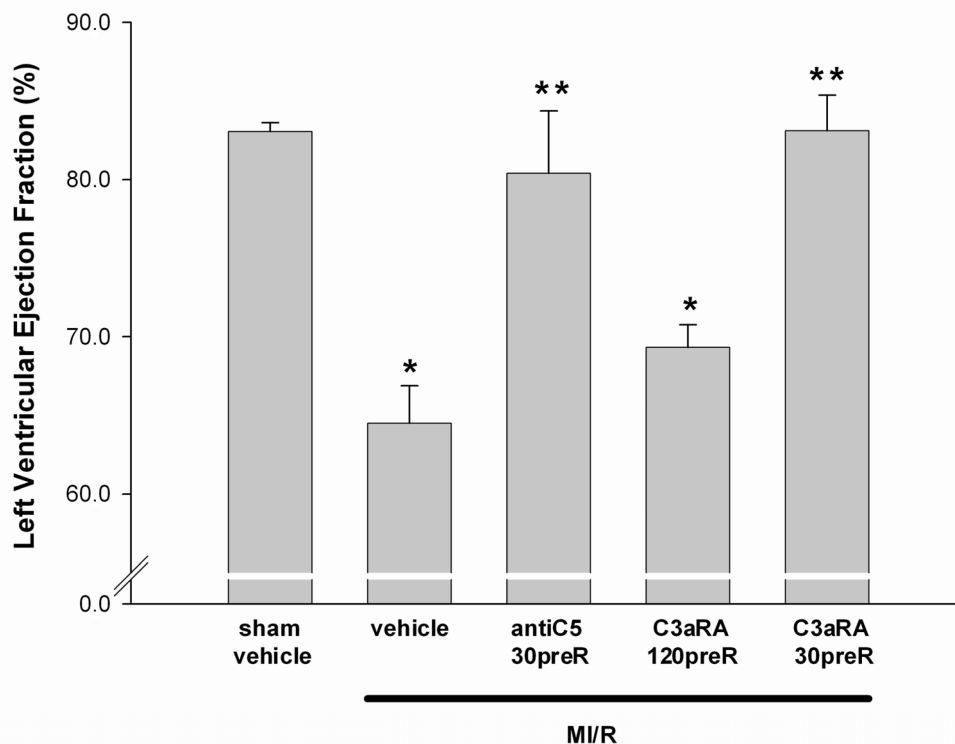
Figure 4: Summary of ejection fraction data
Echocardiography was performed and ejection fraction (%) was calculated from the M-mode measurements as described in *Methods*. Groups: vehicle-treated sham-operated mice (sham vehicle), vehicle-treated mice following 30 min of ischemia and 4 hr of reperfusion (vehicle) and mice undergoing MI/R that were treated with C5 monoclonal antibody (antiC5) 30 min prior to reperfusion (30preR) or with C3a receptor antagonist (C3aRA) 120 min and 30 min prior to R, respectively (120preR or 30preR). All data are mean ± SE of 4–6 animals per group. * $p < 0.05$ compared to sham-operated animals (sham). ** $p < 0.05$ compared to untreated mice undergoing MI/R with 30 min of ischemia and 4 hr of reperfusion.
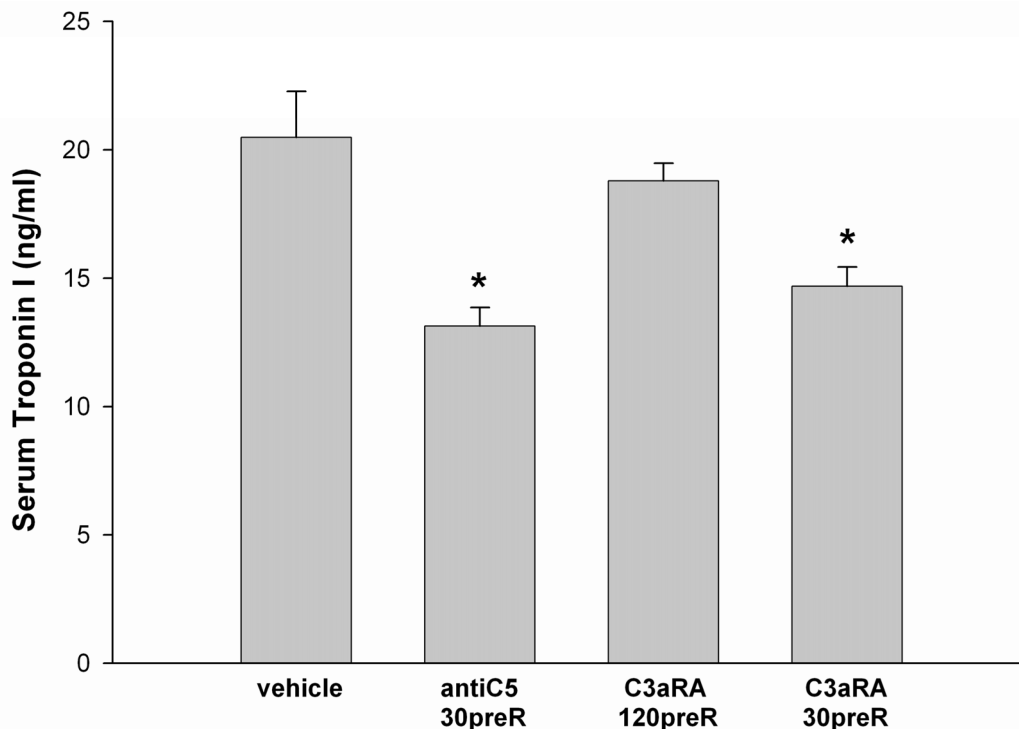
Figure 5: Serum troponin I concentrations after MI/R
Serum troponin I concentrations after MI/R were measured as described in *Methods*. The columns represent mice that were vehicle-treated, mice injected with C5 monoclonal antibody (antiC5) 30 min prior to reperfusion (30preR) or with C3a receptor antagonist (C3aRA) 120 min and 30 min prior to R, respectively (120preR or 30preR). All data are mean ± SE of 4–6 animals per group. * $p<0.05$ compared to untreated mice following MI/R.

tion 120 min prior to reperfusion was not protective (Figure 5).

Histological Sections and Staining for PMN

PMN infiltration into the cardiac tissue as a measure of MI/R related inflammation mirrored our echocardiographic and serum troponin I measurements (Figure 6). In tissue samples from sham-operated animals, no significant PMN infiltration was observed. In contrast, 30 min of ischemia and 4 hr of reperfusion induced a significant inflammatory response in vehicle-treated mice (Figure 6 A and B). Treatment with C5 monoclonal antibody attenuated the inflammatory response significantly compared to vehicle treated mice, while administration of C3aRA attenuated PMN infiltration significantly only in the group treated 30 min prior to reperfusion in the neutropenic time frame and not 120 min prior to reperfusion after the neutropenic effect had resolved (Figure 6 A and B).

Discussion

Using a C5 monoclonal antibody in a mouse MI/R model, we confirmed a significant contribution of C5 in MI/R-induced injury, an observation already reported in rats [18]. Mice treated with C5 monoclonal antibody were significantly protected from loss of myocardial function following MI/R compared to vehicle-treated mice. Since the role of early complement components upstream from C5 cleavage in MI/R has not been elucidated, we investigated the effect of C3aR antagonism in MI/R.

Interestingly, our results indicate that the anaphylatoxin C3a plays only a minimal role in MI/R-induced injury. In 2001, Ames et al. identified a selective non-peptide antagonist of the C3a receptor [35]. Proctor et al. showed that the C3aRA has a strong neutropenic side effect that resolves within 120 min, while its C3aR antagonism remains active [27]. While both groups carried out in depth pharmacological examinations of this antagonist in models of complement activation, both *in vitro* and *in vivo*, the mechanism of C3aRA mediated neutropenia was not elucidated. It was speculated that this effect was secondary and not related to receptor antagonism, as strategies to deplete neutrophils and/or inhibit their adhesion to local endothelium also ameliorated subsequent inflammatory effects [27], [35].

Due to the temporary neutropenic side effect, we administered the C3aRA at two different time points in two groups of mice. Mice were only protected from MI/R induced cardiac injury when the C3aRA was administered during its neutropenic time frame. Administration 120 min prior to reperfusion, when the neutropenia was resolved, but C3aRA is still active [27], did not prevent mice from MI/R associated injury. These data indicate that a benefit of the C3aRA in MI/R is based on its neutropenic side effect only, and not a result of specific C3aR antagonism. Troponin I concentrations and neutrophil infiltra-
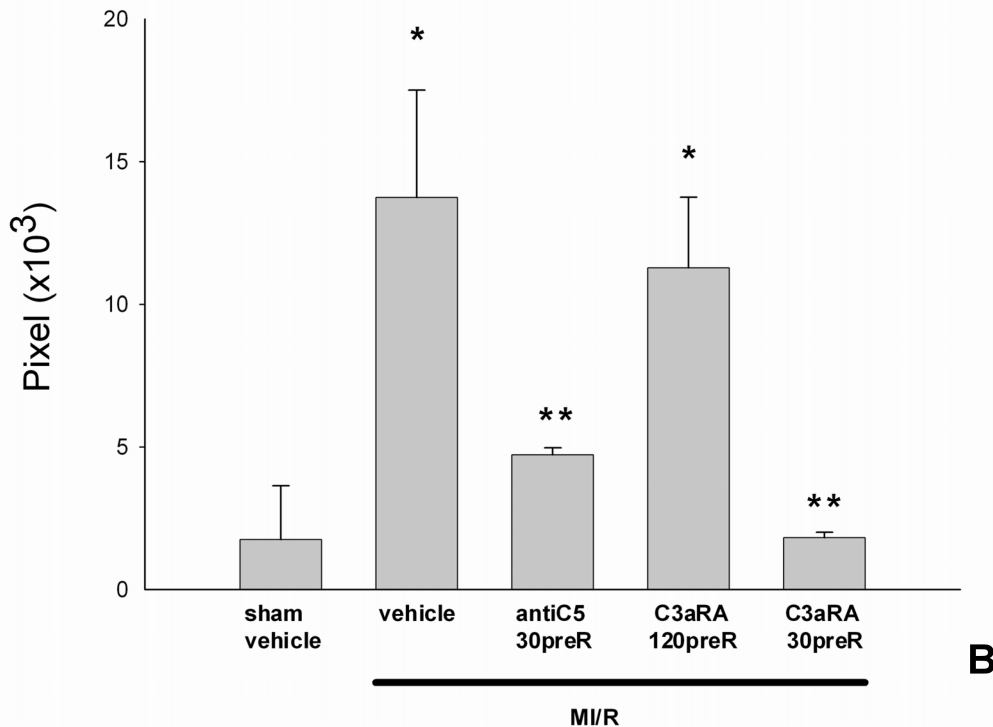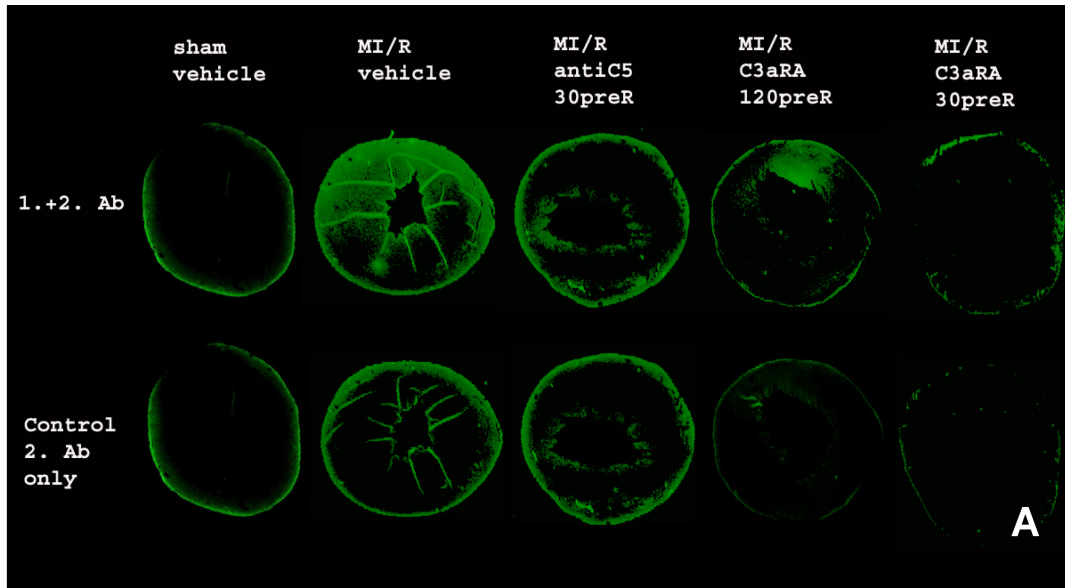
Figure 6: PMN infiltration into the myocardium following MI/R

6A: Representative sections. Heart sections were stained and PMN infiltration into the myocardium was measured using an infrared imaging system as described in *Methods*. Representative sections are shown from 4–6 animals per group. Upper row: 1.+2. Ab (primary and secondary antibody). Lower row: control group with 2. Ab only (secondary only antibody). Groups: vehicle-treated sham-operated mice (sham vehicle), vehicle-treated mice that underwent MI/R with 30 min of I and 4 hr of R (vehicle), mice undergoing MI/R and treated with C5 monoclonal antibody (antiC5) 30 min prior to R (30preR) or with C3a receptor antagonist (C3aRA) 120 min and 30 min prior to R respectively (120preR or 30preR). **6B:** Quantitatvie analysis of sections by pixel count. Pictures from heart sections stained for PMNs from 4-6 animals per group were quantitatively analyzed by pixel counting using ImageJ software. * p<0.05 compared to sham-operated animals (sham). ** p<0.05 compared to untreated mice undergoing MI/R (MI/R vehicle).

tion into the myocardium further supported this observation.

In clinical studies, the benefit of pexelizumab, a recombinant humanized single-chain antibody fragment inhibiting C5, has been controversial. In the "Phase 2 Complement Inhibition in Myocardial Infarction Treated with Angioplasty" (COMMA) trial, a greater than 50% reduction in mortality at 90 days among ST-elevation MI (STEMI) patients treated with pexelizumab, was reported [19]. Additionally, prexelizumab was beneficial in patients un-

dergoing coronary artery bypass graft surgery [20], [21]. In contrast, prexelizumab administration did not reduce adverse clinical outcomes in STEMI patients treated for fibrinolysis [36]. These aforementioned results in early clinical studies resulted in the design of 2 large Phase 3 studies: the "Assessment of Pexelizumab in Acute Myocardial Infarction" (APEX AMI) trial [22] and the "Pexelizumab for Reduction in Myocardial Infarction and Mortality in Coronary Artery Bypass Graft Surgery" (PRIMO CABG-II) study [24]. The APEX AMI trial was terminated early due to a lower than expected mortality rate in the placebo group. Thus, an increase in the required sample size to maintain study power to more than 11,000 patients, compared to the previously estimated 8,500 patients would be necessary [23]. A second reason for the premature termination, were results from the parallel PRIMO-CABG II trial, where the primary objective was not achieved [23], [37]. As a result, the use of pexelizumab in clinical cardiovascular disease settings is unlikely to be advanced.

The anaphylatoxins C3a and C5a are not initiating factors of the complement system, but they appear to be responsible for promoting, amplifying and perpetuating inflammatory reactions [3], [4], [38]. Interestingly, while the anaphylatoxin C5a has been shown to be a potent activator of inflammation [3], [4], there are only a few studies investigating the role of C3a in I/R injury and none in MI/R injury. Application of the C3aRA in a murine stroke model resulted in significant neurological improvement and stroke volume reduction [25]. Thus, a C3a anaphylatoxin-mediated mechanism in cerebral I/R injury was assumed. However, since the same C3aRA was used as in our study and was administered 45 min before ischemia in the neutropenic time frame of the C3aRA, it is questionable, if the observed benefit in cerebral I/R was primarily a result of C3aR antagonism [25], [27]. In contrast to myocardial I/R, C3a appears to play a significant role in I/R injury of the kidneys. In a mouse model of renal I/R, C3a generated by activation of the alternative complement pathway appears to plays a key role in mediating I/R injury through subsequent activation of the NF-κB system [26].

Conclusion

In conclusion, our study confirms a pathophysiologic role for C5 in MI/R injury and indicates that the anaphylatoxin C3a plays only a minimal role in MI/R-induced injury. While C5 inhibition in the clinical setting of MI/R does not appear to be therapeutic, our results raise the possibility that inhibition of either C5a or C5b-9 may be more advantageous than inhibition of C3a or complete inhibition of C5 in humans to attenuate complement related inflammation and subsequent myocardial injury.

List of abbreviations

- Ab(s) – antibody(ies)
- Ag(s) – antigen(s)
- Ag-Ab – antigen-antibody
- APEX AMI trial – "Assessment of Pexelizumab in Acute Myocardial Infarction" trial
- COMMA trial – "Phase 2 Complement Inhibition in Myocardial Infarction treated with Angioplasty" trial
- C3aRA – C3a receptor antagonist
- EF – ejection fraction
- GI/R – gastrointestinal ischemia and reperfusion
- IACUC – Institute's Animal Care and Use Committee
- I/R – ischemia and reperfusion
- LAD – left anterior descending coronary artery
- LV – left ventricle
- MAC – membrane attack complex
- MASP – MBL-associated serine protease
- MBL – mannose binding lectin
- MI – myocardial infarction
- MI/R – myocardial ischemia and reperfusion
- NIH – National Institutes of Health
- PRIMO CABG-II trial – "Pexelizumab for Reduction in Myocardial Infarction and Mortality in Coronary Artery Bypass Graft Surgery"
- STEMI – ST-elevation MI
- TBS – Tris-buffered saline
- TCC – terminal complement complex
- WT – wild type

Notes

Conflict of interest

None declared.

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Corresponding author:

Marc N. Busche, MD
Department of Plastic, Hand and Reconstructive Surgery, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
marcbusche@web.de