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The role of complement C3 in the outcome of regional myocardial infarction

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ABSTRACT

Coronary heart disease leading to myocardial ischemia is a major cause of heart failure. A hallmark of heart failure is myocardial fibrosis. Using a murine model of myocardial ischemia/reperfusion injury (IRI), we showed that, following IRI, in mice genetically deficient in the central factor of complement system, C3, myocardial necrosis was reduced compared with WT mice. Four weeks after the ischemic period, the $C3^{-/-}$ mice had significantly less cardiac fibrosis and better cardiac function than the WT controls. Overall, our results suggest that innate immune response through complement C3 plays an important role in necrotic cell death, which contributes to the cardiac fibrosis that underlies post-infarction heart failure.

1. Introduction

Ischemia/reperfusion injury (IRI) underlies critical clinical scenarios involving major organs, e.g. myocardial infarction, transplantation, trauma and perioperative organ injury. IR elicits an acute inflammatory response involving complement factors of the innate immune system [1–3]. During IR, the local tissues undergo drastic intracellular changes: mitochondria become progressively dysfunctional and excess free radicals are generated, leading to lipid and protein oxidation and DNA damage [4]. At the cell signaling level, factors of various cell death programs are activated [5,6]. During the ensuing necrosis, cytoplasmic membranes become unstable, resulting in the dispersal or exposure of intracellular contents to the extracellular space [7]. This triggers an immune response, as evidenced by release of pro-inflammatory cytokines [8], activation of the complement system [1–3] and infiltration of inflammatory cells [9]. Together with the intracellular changes, these inflammatory responses result in the cell death of ischemic tissues and subsequent long-term consequences; e.g. in the heart, myocardial IR related post-infarction heart failure with the hallmarks of cardiac fibrosis [10,11] and heart dysfunction [12,13].

It has long been suggested that limiting inflammation during cardiac IR injury, for instance via complement inhibitors, would reduce myocardial-IR related cell death and thus prevent post-infarction heart failure [14–18]. Previous pre-clinical studies supported such a hypothesis, particularly those using anti-complement C5 [16,18–21]. However, limited positive results were obtained with the few inhibitors studied in clinical trials [3]. In particular, anti-complement C5 (Pexelizumab) failed to meet the primary endpoints in acute myocardial infarction patients [22].

The earliest work that established the role of C5 in a rat myocardial IR injury model revealed that, while anti-C5 reduced infarct size and apoptosis, it did not inhibit C3 deposition in the injured myocardium [18]. Similar results obtained using inhibition of the C5a receptor [23–25] supported the conclusion that activated C5 was pro-apoptotic. However, anti-C5 failed to inhibit assembly of the terminal complement complex (TCC) in patients with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention [26], possibly due to intense complement activity or different mechanisms leading to TCC activation.

Our view of these basic science and clinical results is that using anti-C5 targets a downstream factor in the common complement pathway, leaving earlier factors, e.g. C3, unaffected. Complement activation in

Abbreviations: IRI, ischemia/reperfusion injury; TCC, terminal complement complex; IR, ischemia/reperfusion; FB, Factor B; MBL, mannose binding lectin; FH, Factor F; C4BP, C4 binding protein; FI, Factor I; DTBT, door-to-balloon time.

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general can be initiated via three pathways: the classical pathway (CRP, antibodies, C1q, C4, C2), the alternative pathway (spontaneous C3 hydrolysis, Factor B (FB)) and the lectin pathway (mannose binding lectin (MBL), ficolins) [27–34]. These pathways converge at the formation of C3 convertase (C3b•Bb). Amplification via a loop involving C3 convertases occurs only through the FB-dependent alternative pathway [35,36]. C3 convertases yield C3a and additional C3b, the latter forming C5 convertases (C4b•C2a•C3b and C3b•Bb•C3b) and that cleave C5 to C5a and C5b. C5b initiates formation of the cell membrane attack complex (C5b-C9) which binds to and destroys targets. The C3 and C5 convertases are regulated by membrane-bound proteins (e.g. CD46, CD55) and soluble factors (e.g. FH, C4BP and FI) [35,37]. Thus, it is possible that in the cited studies, activation of factors prior to C5, e.g. C3, would initiate upstream complement signaling pathways leading to acute cell death and chronic inflammation, thus contributing to post-infarction heart failure. Supporting this hypothesis, a clinical study found that coronary artery disease patients with lower C3 had less worse outcome than those higher levels of C3 [38].

Previously we used a clinically-relevant, myocardial IR injury mouse model (60 min ischemia resembling clinical door-to-balloon time (DTBT) time [39–41]) to show that myocardial necrosis, which increased upon reperfusion and was maintained through 24 h, was accompanied by deposition of activated C3 fragments in reperfused tissues [42]. Here we used this model to investigate the mechanisms by which C3 contributes to cell death in the acute phase of infarction (the first 24 h of reperfusion). In addition, we examined the long-term effect of C3 on post-infarction heart failure, as indicated by cardiac fibrosis and dysfunction.

2. Materials and Methods

2.1. Mouse model of surgically induced myocardial IR injury

Complement C3 knockout ($C3^{-/-}$) mice and WT (C57BL/6) mouse strains were obtained from the Jackson Laboratory (Bar Harbor, ME) and maintained at the SUNY Downstate Health Science University Department of Laboratory Animal Resources. Genotyping was provided by GeneTyper (New York, NY). Male mice were used at 10–12 weeks of age (weight 26–30 g) in accordance with the requirements of the NIH and the Institutional Animal Care and Use Committee (IACUC) of SUNY Downstate Health Science University. The protocol was approved by the IACUC of SUNY Downstate Health Science University (Approval #11–10276).

We employed an established model of myocardial IR injury model [42,43]. Mice were anesthetized using sodium pentobarbital (60 mg/kg, i.p.), intubated and ventilated with a mouse ventilator (Harvard Apparatus, MA). Following sternotomy, the left anterior descending artery (LAD) was ligated for 1 h; occlusion of the LAD was confirmed by the appropriate color change of myocardial tissue and the ST elevation on ECG; reperfusion was verified by the reversed color change of the left ventricle and the appropriate ECG changes. Postoperative management included fluid replacement with normal saline and pain relief with the analgesic buprenorphine (0.1 mg/kg, intramuscularly). The mice were sacrificed after 24 h of reperfusion; the hearts were harvested for histopathologic analyses.

2.2. Evaluation of murine myocardial necrosis by fluorescence using two probes delivered in vivo

A fluorescent method for tracking necrosis (developed by others [44,45] and further refined by us [42]) was used. Shortly before the end of the reperfusion period described above and before tissue harvesting, mice were anesthetized, intubated again as described above, and injected i.v. with propidium iodide (PI), which enters damaged cells, intercalates with DNA and fluoresces, thus identifying necrotic tissue. The LAD was then re-occluded and blue fluorescent microspheres (BFM, ThermoFisher, PA) were injected through the aortic arch to delineate the non-ischemic region of the heart. The mice were sacrificed, the hearts harvested and the atria removed. Each ventricle was sectioned into four slices (~1 mm thickness) which were weighed and imaged under a fluorescent microscope (Olympus, PA) using the red fluorescent channel for PI, the blue channel for BFM.

The percentage of the tissue in a heart which was at risk for necrosis (negative for blue fluorescence) and which became necrotic (positive for red fluorescence) was determined by computerized planimetry (Image J, NIH, Bethesda, MD) and by the following equations:

Weight of necrotic tissue = $(A_1 \times Wt_1) + (A_2 \times Wt_2) + (A_3 \times Wt_3) + (A_4 \times Wt_4)$, where A was the percentage of the area of a slice staining for necrosis (red fluorescence) measured by planimetry (average of both sides of a slice) and Wt was the weight of that slice of ventricle.

2.2.1. Weight of tissue at risk for necrosis (weight at risk, WAR) =

$(R_1 \times Wt_1) + (R_2 \times Wt_2) + (R_3 \times Wt_3) + (R_4 \times Wt_4)$, where R is the percentage of the area of a slice which lacked the blue fluorescence of BFM, determined by planimetry (average of both sides of a slice used). In all cases, the tissue with red fluorescence was within the boundary of the tissue which lacked blue fluorescence. Blue = no blood flow. Red – necrosis tissue.

Percentage of the weight of a ventricle at risk for necrosis which became necrotic = (weight of necrotic tissue/WAR) x 100.

2.3. Echocardiography

Echocardiography was performed as previously described [46]. Briefly, mice chests were shaved and allowed to rest for at least 1 h before echocardiography. Echocardiography, using the Phillips SONOS 5500 with a 15 MHz linear probe, was performed on conscious mice to avoid any cardiodepression produced by anesthesia. Imaging employed the M mode short axis view, measuring systolic and diastolic cardiac dimensions. Images were digitized for analysis. Left ventricular systolic (LVESD), diastolic (LVEDD), septal (SW), and posterior wall (PW) thicknesses were measured. Left ventricular fractional shortening (FS) was calculated from the following formula: FS (%) = ((LVEDD − LVESD)/LVEDD)) × 100. The ejection fraction (EF) is calculated from the following formula: EF = $((LVEDD^2 - LVESD2)/LVEDD^2)) \times 100$. Left ventricular mass is calculated from the following formula: LV mass = $1.05\ ((LVEDD + SW + PW)^3 - (LVEDD^3))$.

2.4. Cardiac fibrosis analysis

For analysis of cardiac fibrosis, mice were sacrificed 4 weeks after 1 h of heart ischemia produced as described in Material and Methods, (Section 1). The cardiac fibrosis present in sections from each of 4 slices/heart obtained as in Materials and Methods, were assessed by the Masson Trichrome stain kit (VWR, PA), according to the manufacturer's instructions.

2.5. Statistical analysis

Statistical analyses were performed using IBM SPSS Software version 20 (IBM Corp., NY). For animal studies, an independent *t*-test with two tails and unequal variances was used to determine the statistical significance of differences between the results of experimental and control groups. Descriptive data were summarized as mean ± standard error of mean. Power analyses were performed using G*Power 3.1 [47] showed >99% power for detection of differences in infarct size with 6–8 animals per group.

3. Results

3.1. Myocardial necrosis is reduced in C3$^{-/-}$ mice after 60 min of heart ischemia followed by 24 h of reperfusion

Loss of plasma membrane integrity (LPMI) is a hallmark of necrotic cell death [5,7]. We have developed an *in vivo* fluorescent method [41] modified from earlier reports [44,45] to track LPMI in necrotic myocardial death. The method permits subsequent analysis of fluorescently tagged tissue for other pathological events, a significant advantage over the traditional evaluation of myocardial necrosis using triphenyl tetrazolium chloride. After 1 h ischemia/24 h reperfusion, necrosis in C3$^{-/-}$ mice was significantly reduced compared with WT mice (Fig. 1a and b; *post hoc* power analysis>90% power; similar *post hoc* statistical powers were identified for the other pilot experiments).

These results indicate that when WT hearts experience regional ischemia in a 1 h time period resembling the length of clinical DTBT, their cardiomyocytes are directed by C3 towards necrosis during the acute 24 h long phase of reperfusion. How C3 regulates this switch is unknown.

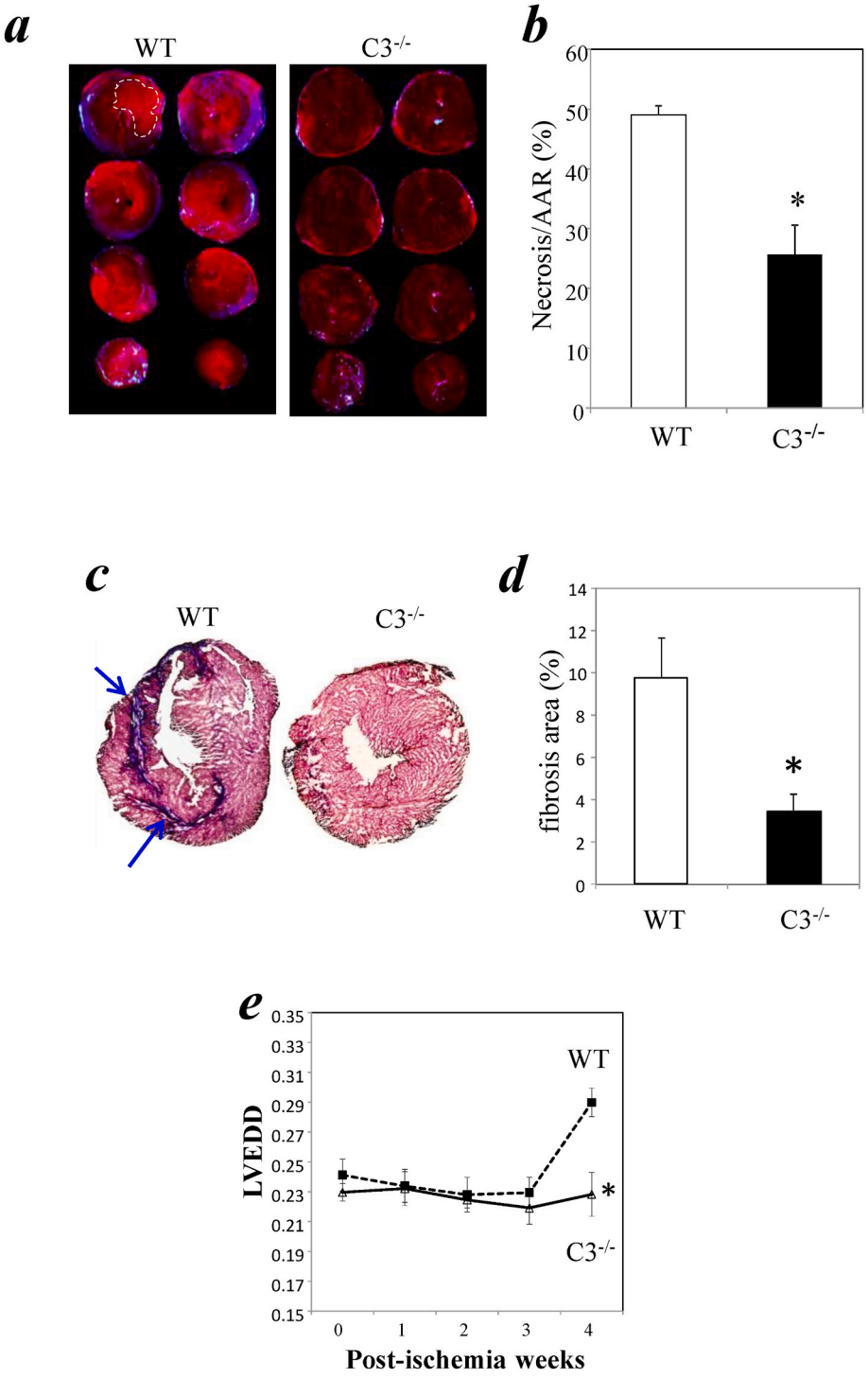

Fig. 1. Necrosis is decreased while apoptosis is increased in the hearts of C3$^{-/-}$ mice after IR.

3.2. Cardiac fibrosis and dysfunction are reduced in $C3^{-/-}$ mice 4 weeks after myocardial IR injury

After a clinically relevant period of myocardial ischemia, $C3^{-/-}$ mice had a significant reduction in necrosis accompanied by an increase in apoptosis during the acute phase of reperfusion. Whether such a change in cell death type could lead to a different long term outcome in heart structure and function is not known. Published work has established that following ischemia, a reparative cardiac fibrosis occurs resulting from cardiomyocyte death, to replace the void left by dead myocytes [9]. Its hallmark is the excessive deposition of extracellular matrix, leading to tissue scarring and organ dysfunction. This is either manifested as myocardial stiffness (diastolic dysfunction) or may impact the entire left ventricle causing dilatation and systolic dysfunction, eventually resulting in heart failure [10,48]. The extent of fibrosis is important for prognosis of heart failure [49,50].

Using $C3^{-/-}$ and WT mice, we examined the long term effect of C3 on post-infarction heart failure, as indicated by cardiac fibrosis and heart dysfunction. Four weeks after the initial 1 h of ischemia, $C3^{-/-}$ mice had significantly less cardiac fibrosis than WT mice (Fig. 1c and d). When cardiac function was evaluated by echocardiography at this time, $C3^{-/-}$ mice showed significantly better LVEDD than WT control mice (Fig. 1 e).

4. Discussion

We used a clinically relevant myocardial IRI model (60 min of ischemia resembling DTBT time followed by 24 h reperfusion) to investigate the mechanisms of cell death involving C3 in the acute phase of infarction. In mice genetically deficient in C3 which is the central molecule in all complement pathways, myocardial necrosis was significantly reduced compared with WT mice (Fig. 1a–b). The results imply that in WT mice during IR, C3 acts to promote necrosis.

Our studies showed that 4 weeks after IR injury, $C3^{-/-}$ mice had significantly less post-ischemia cardiac fibrosis and improved cardiac function (Fig. 1 c-e). These results indicate that the myocardial IR inflammatory response in WT mice involving C3 results in increased cardiac fibrosis and adverse tissue remodeling. Our results were in alignment with an earlier report by Weisman et al. that using sCR1 as an inhibitor of C3 activation in WT mice significantly reduced IRI in a rat model [51]. It is of note that sCR1 was administered to the animals before ischemia in Weisman's study, suggesting that pre-emptive inhibition of C3 may be necessary to protect heart before ischemia occurs. Similarly, our model used $C3^{-/-}$ mice which lack C3 before ischemia took place. Thus, the effect of C3 on IRI of WT animals may start at the ischemia phase (by C3 remaining in the area of ischemia), then exacerbate at reperfusion phase when circulation bring more C3 once the blood flow is re-established in the ischemic area. Whether C3 acted directly at extracellular or intracellular target(s) in IRI was not determined in this study but could be an interesting line of future studies. Nevertheless, our previous studies suggested that intracellular target(s), such as non-muscle myosin, may be exposed to natural antibodies during the IRI and subsequently activate complement system [43,52,53]. Therefore, it is possible that C3 participates in the recognition of intracellular targets being exposed in IRI.

It is of note that in our study, cardiac function of WT mice had sharply deteriorated at 4 weeks after ischemia, which is in agreement with others using rodent models of IRI [54–56]. One possible explanation is that cardiac function was initially restored by reperfusion, but cardiac fibrosis gradually evolved and reached the threshold at 4 weeks after ischemia. Thus, the over-threshold level of cardiac fibrosis overweighted the capacity of heart compensation and pronounced the failing of cardiac function at 4 weeks.

A recent study by Torf et al. using a Langendorff heart perfusion model found that C3 knockout mouse heart had larger infarct size than WT heart [57]. Although it seemed contrary to our findings, the main differences are: 1) Langendorff model uses an isolated heart with global ischemia which rarely happens in human acute myocardial infarction (AMI). In contrast, our *in vivo* IRI model mimics the human AMI scenario of regional ischemia. 2) The reperfusion time in Torf's study was only 60 min, while our study examined the long term effects of cardiac fibrosis and function (4 weeks). Thus, Torf's findings may have certain value at the first hour immediately after ischemia, our findings imply the long term effects which are more resemble to clinical scenario.

Our results suggest that targeting C3 has the potential to reduce significantly post-infarction heart failure. In AMI, shortening ischemia by hastening reperfusion of occluded coronary vessels, especially a DTBT of $\leq$90 min for primary percutaneous coronary intervention ($\sim$60 min in the U.S.), results in smaller infarct size and lower mortality [39–41,58]. Thus, the research focus has thus shifted from reducing mortality to tackling the consequence of survival: post-infarction heart failure [59,60]. This process, which has increased globally in the past 3 decades, places a substantial burden on health-care systems [61,62].

The main determinant of post-infarction heart failure is infarct size, which results not only from ischemia but from reperfusion injury. Currently, clinicians and basic scientists are focusing on therapies to reduce the latter [63]. However, to date, large-scale clinical trials, i.e. antagonism of the renin-angiotensin- aldosterone system, have provided limited evidence of clinical benefit to heart failure and few interventions have successfully passed the proof-of-concept stage [12,64]. Our results provide new insights into the mechanisms of reperfusion injury, and thus may offer potential therapeutic strategies. For instance, targeting complement C3 during reperfusion following AMI may significantly benefit patients and reduce long term healthcare costs. As several inhibitors for complement C3 are being tested in various clinical trials of rare diseases [65,66], it is possible that some inhibitors may be effective in cardiovascular diseases as well.

In summary, this study has identified a mechanism during IR involving C3 that modulates the nature of cell death relevant to the pathological outcome of IR injury. As a long-term consequence of IR injury, C3- plays an important role in necrotic cell death which contributes to the cardiac fibrosis that underlies post-infarction heart failure. The study provides new insights into the mechanisms of IR injury and suggests a potential approach for intervention in post-infarction heart failure.

(a) $C3^{-/-}$ and WT mice (n = 6–8/group) were subjected to occlusion of the left anterior descending (LAD) artery for 1 h followed by reperfusion for 24 h. Next, propidium iodide (PI - enters cells through damaged cell membranes and binds DNA in necrotic cells) and blue fluorescent microspheres (BFM – present in unoccluded blood vessels) (the latter after re-occlusion of the LAD) were injected *in vivo* just prior to heart harvesting to delineate the infarcted area, and the area at risk (AAR) for necrosis (i.e., lacking BFM), respectively. After animal sacrifice, each ventricle was divided into four slices (top and bottom of each slice are adjacent). LPMI-positive necrotic tissue (bright red) was visualized immediately under a fluorescent microscope with a 2× objective lens. Non-ischemic tissue was defined by the blue fluorescence of BFM, the non-fluorescing tissue constituting ischemic tissue, the area at risk (AAR) for necrosis. The necrotic area was traced with dotted lines as an example.
(b) The LPMI-positive necrotic area expressed as % of AAR.
(c) $C3^{-/-}$ mice had less cardiac fibrosis after IR. $C3^{-/-}$ mice and control WT mice (n = 4 per group) underwent 1 h myocardial ischemia followed by reperfusion. 4 weeks after initial heart ischemia, mice were sacrificed and cardiac fibrosis in heart sections was assessed by the Masson Trichrome stain (VWR, PA). Blue arrows indicated the areas of positive staining for fibrosis.
(d) Fibrotic areas were quantified and expressed as a percentage of the total area of heart sections.
(e) $C3^{-/-}$ mice had better LVEDD after IR. Cardiac functions were followed by echocardiography during the 4-week post-ischemia

period. *indicates $P < 0.05$ between the $C3^{-/-}$ and WT groups at 4 weeks' post-ischemia.

Declaration of competing interest

No conflict of interest for all.

Data availability

Data will be made available on request.

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References

[1] A. Palikhe, J. Sinisalo, M. Seppanen, H. Haario, S. Meri, V. Valtonen, M. S. Nieminen, M.L. Lokki, Serum complement C3/C4 ratio, a novel marker for recurrent cardiovascular events, Am. J. Cardiol. 99 (2007) 890–895.
[2] T. Celik, A. Iyisoy, U.C. Yuksel, B. Jata, M. Ozkan, The impact of admission C-reactive protein levels on the development of no-reflow phenomenon after primary PCI in patients with acute myocardial infarction: the role of inflammation, Int. J. Cardiol. 136 (2009) 86–88, https://doi.org/10.1016/j.ijcard.2008.03.058. ; author reply 88-89. S0167-5273(08)00529-9 [pii].
[3] R.O. Cannon 3rd, Mechanisms, management and future directions for reperfusion injury after acute myocardial infarction, Nat. Clin. Pract. Cardiovasc. Med. 2 (2005) 88–94.
[4] K.D. Marshall, C.P. Baines, Necroptosis: is there a role for mitochondria? Front. Physiol. 5 (2014) 323, https://doi.org/10.3389/fphys.2014.00323.
[5] M. Chiong, Z.V. Wang, Z. Pedrozo, D.J. Cao, R. Troncoso, M. Ibacache, A. Criollo, A. Nemchenko, J.A. Hill, S. Lavandero, Cardiomyocyte death: mechanisms and translational implications, Cell Death Dis. 2 (2011) e244, https://doi.org/10.1038/cddis.2011.130.
[6] D.A. Kubli, A.B. Gustafsson, Cardiomyocyte health: adapting to metabolic changes through autophagy, Trends Endocrinol. Metabol. 25 (2014) 156–164, https://doi.org/10.1016/j.tem.2013.11.004.
[7] K. Konstantinidis, R.S. Whelan, R.N. Kitsis, Mechanisms of cell death in heart disease, Arterioscler. Thromb. Vasc. Biol. 32 (2012) 1552–1562, https://doi.org/10.1161/ATVBAHA.111.224915.
[8] M. Neri, V. Fineschi, M. Di Paolo, C. Pomara, I. Riezzo, E. Turillazzi, D. Cerretani, Cardiac oxidative stress and inflammatory cytokines response after myocardial infarction, Curr. Vasc. Pharmacol. 13 (2015) 26–36.
[9] S.D. Prabhu, N.G. Frangogiannis, The biological basis for cardiac repair after myocardial infarction: from inflammation to fibrosis, Circ. Res. 119 (2016) 91–112, https://doi.org/10.1161/CIRCRESAHA.116.303577.
[10] A.M. Segura, O.H. Frazier, L.M. Buja, Fibrosis and heart failure, Heart Fail. Rev. 19 (2014) 173–185, https://doi.org/10.1007/s10741-012-9365-4.
[11] M.S. Stratton, T.A. McKinsey, Epigenetic regulation of cardiac fibrosis, J. Mol. Cell. Cardiol. 92 (2016) 206–213, https://doi.org/10.1016/j.yjmcc.2016.02.011.
[12] H.A. Cabrera-Fuentes, C. Alba-Alba, J. Aragones, J. Bernhagen, W.A. Boisvert, H. E. Botker, G. Cesarman-Maus, I. Fleming, D. Garcia-Dorado, S. Lecour, E. Liehn, M. S. Marber, N. Marina, M. Mayr, O. Perez-Mendez, T. Miura, M. Ruiz-Meana, E. M. Salinas-Estefanon, S.B. Ong, H.J. Schnittler, J.T. Sanchez-Vega, A. Sumoza-Toledo, C.W. Vogel, D. Yarullina, D.M. Yellon, K.T. Preissner, D.J. Hausenloy, Meeting report from the 2nd international symposium on new frontiers in cardiovascular research. Protecting the cardiovascular system from ischemia: between bench and bedside, Basic Res. Cardiol. 111 (2016) 7, https://doi.org/10.1007/s00395-015-0527-0.
[13] E.E. Creemers, Y.M. Pinto, Molecular mechanisms that control interstitial fibrosis in the pressure-overloaded heart, Cardiovasc. Res. 89 (2011) 265–272, https://doi.org/10.1093/cvr/cvq308.
[14] P.R. Maroko, C.B. Carpenter, M. Chiariello, M.C. Fishbein, P. Radvany, J. D. Knostman, S.L. Hale, Reduction by cobra venom factor of myocardial necrosis after coronary artery occlusion, J. Clin. Invest. 61 (1978) 661–670, https://doi.org/10.1172/JCI108978.
[15] J.H. Hill, P.A. Ward, The phlogistic role of C3 leukotactic fragments in myocardial infarcts of rats, J. Exp. Med. 133 (1971) 885–900.
[16] M.H. Crawford, F.L. Grover, W.P. Kolb, C.A. McMahan, R.A. O'Rourke, L. M. McManus, R.N. Pinckard, Complement and neutrophil activation in the pathogenesis of ischemic myocardial injury, Circulation 78 (1988) 1449–1458.
[17] D. Ricklin, J.D. Lambris, Complement in immune and inflammatory disorders: therapeutic interventions, J. Immunol. 190 (2013) 3839–3847, https://doi.org/10.4049/jimmunol.1203200.
[18] A.P. Vakeva, A. Agah, S.A. Rollins, L.A. Matis, L. Li, G.L. Stahl, Myocardial infarction and apoptosis after myocardial ischemia and reperfusion: role of the terminal complement components and inhibition by anti-C5 therapy, Circulation 97 (1998) 2259–2267.
[19] S.M. Shandelya, P. Kuppusamy, A. Herskowitz, M.L. Weisfeldt, J.L. Zweier, Soluble complement receptor type 1 inhibits the complement pathway and prevents contractile failure in the postischemic heart. Evidence that complement activation is required for neutrophil-mediated reperfusion injury, Circulation 88 (1993) 2812–2826.
[20] H.F. Weisman, T. Bartow, M.K. Leppo, H.C. Marsh Jr., G.R. Carson, M.F. Concino, M.P. Boyle, K.H. Roux, M.L. Weisfeldt, D.T. Fearon, Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis, Science 249 (1990) 146–151.
[21] A. Undar, H.C. Eichstaedt, F.J. Clubb Jr., M. Fung, M. Lu, J.E. Bigley, W.K. Vaughn, C.D. Fraser Jr., Novel anti-factor D monoclonal antibody inhibits complement and leukocyte activation in a baboon model of cardiopulmonary bypass, Ann. Thorac. Surg. 74 (2002) 355–362. ; discussion 362.
[22] P.W. Armstrong, C.B. Granger, P.X. Adams, C. Hamm, D. Holmes Jr., W.W. O'Neill, T.G. Todaro, A. Vahanian, F. Van de Werf, Pexelizumab for acute ST-elevation myocardial infarction in patients undergoing primary percutaneous coronary intervention: a randomized controlled trial, JAMA 297 (2007) 43–51.
[23] V.C. De Hoog, L. Timmers, A. Van Duijvenvoorde, S.C. De Jager, B.J. Van Middelaar, M.B. Smeets, T.M. Woodruff, P.A. Doevendans, G. Pasterkamp, C. E. Hack, D.P. De Kleijn, Leucocyte expression of complement C5a receptors exacerbates infarct size after myocardial reperfusion injury, Cardiovasc. Res. 103 (2014) 521–529, https://doi.org/10.1093/cvr/cvu153.
[24] M. Mueller, C. Herzog, J. Larmann, M. Schmitz, D. Hilfiker-Kleiner, J.E. Gessner, G. Theilmeier, The receptor for activated complement factor 5 (C5aR) conveys myocardial ischemic damage by mediating neutrophil transmigration, Immunobiology 218 (2013) 1131–1138, https://doi.org/10.1016/j.imbio.2013.03.006.
[25] J. van der Pals, S. Koul, P. Andersson, M. Gotberg, J.F. Ubachs, M. Kanski, H. Arheden, G.K. Olivecrona, B. Larsson, D. Erlinge, Treatment with the C5a receptor antagonist ADC-1004 reduces myocardial infarction in a porcine ischemia-reperfusion model, BMC Cardiovasc. Disord. 10 (2010) 45, https://doi.org/10.1186/1471-2261-10-45.
[26] C. Martel, C.B. Granger, M. Ghitescu, A. Stebbins, A. Fortier, P.W. Armstrong, A. Bonnefoy, P. Theroux, Pexelizumab fails to inhibit assembly of the terminal complement complex in patients with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention. Insight from a substudy of the Assessment of Pexelizumab in Acute Myocardial Infarction (APEX-AMI) trial, Am. Heart J. 164 (2012) 43–51, https://doi.org/10.1016/j.ahj.2012.04.007.
[27] M.C. Carroll, D.E. Isenman, Regulation of humoral immunity by complement, Immunity 37 (2012) 199–207, https://doi.org/10.1016/j.immuni.2012.08.002.
[28] M.V. Carroll, R.B. Sim, Complement in health and disease, Adv. Drug Deliv. Rev. 63 (2011) 965–975, https://doi.org/10.1016/j.addr.2011.06.005.
[29] D. Ricklin, J.D. Lambris, Progress and trends in complement therapeutics, Adv. Exp. Med. Biol. 735 (2013) 1–22.
[30] J.R. Dunkelberger, W.C. Song, Complement and its role in innate and adaptive immune responses, Cell Res. 20 (2010) 34–50, https://doi.org/10.1038/cr.2009.139.
[31] J.V. Sarma, P.A. Ward, The complement system, Cell Tissue Res. 343 (2011) 227–235, https://doi.org/10.1007/s00441-010-1034-0.
[32] W.B. Gorsuch, E. Chrysanthou, W.J. Schwaeble, G.L. Stahl, The complement system in ischemia-reperfusion injuries, Immunobiology 217 (2012) 1026–1033, https://doi.org/10.1016/j.imbio.2012.07.024.
[33] L.A. Trouw, M.R. Daha, Role of complement in innate immunity and host defense, Immunol. Lett. 138 (2011) 35–37, https://doi.org/10.1016/j.imlet.2011.02.014.
[34] S.H. Sacks, W. Zhou, The role of complement in the early immune response to transplantation, Nat. Rev. Immunol. 12 (2012) 431–442, https://doi.org/10.1038/nri3225.nri3225 [pii].
[35] D. Ricklin, G. Hajishengallis, K. Yang, J.D. Lambris, Complement: a key system for immune surveillance and homeostasis, Nat. Immunol. 11 (2010) 785–797, https://doi.org/10.1038/ni.1923.
[36] M. Harboe, T.E. Mollnes, The alternative complement pathway revisited, J. Cell Mol. Med. 12 (2008) 1074–1084, https://doi.org/10.1111/j.1582-4934.2008.00350.x. JCMM350 [pii].
[37] P.F. Zipfel, C. Skerka, Complement regulators and inhibitory proteins, Nat. Rev. Immunol. 9 (2009) 729–740, https://doi.org/10.1038/nri2620.
[38] E. Cavusoglu, C. Eng, V. Chopra, C. Ruwende, S. Yanamadala, L.T. Clark, D. J. Pinsky, J.D. Marmur, Usefulness of the serum complement component C4 as a predictor of stroke in patients with known or suspected coronary artery disease referred for coronary angiography, Am. J. Cardiol. 100 (2007) 164–168, https://doi.org/10.1016/j.amjcard.2007.02.075.
[39] B.K. Nallamothu, S.L. Normand, Y. Wang, T.P. Hofer, J.E. Brush Jr., J. C. Messenger, E.H. Bradley, J.S. Rumsfeld, H.M. Krumholz, Relation between door-to-balloon times and mortality after primary percutaneous coronary intervention over time: a retrospective study, Lancet 385 (2015) 1114–1122, https://doi.org/10.1016/S0140-6736(14)61932-2.
[40] H. Miyachi, A. Takagi, K. Miyauchi, M. Yamasaki, H. Tanaka, M. Yoshikawa, M. Saji, M. Suzuki, T. Yamamoto, W. Shimizu, K. Nagao, M. Takayama, Current Characteristics and Management of ST Elevation and Non-ST Elevation Myocardial Infarction in the Tokyo Metropolitan Area: from the Tokyo CCU Network Registered Cohort, Heart Vessels, 2016, https://doi.org/10.1007/s00380-015-0791-9.
[41] A.L. Brennan, N. Andrianopoulos, S.J. Duffy, C.M. Reid, D.J. Clark, P. Loane, G. New, A. Black, B.P. Yan, M. Brooks, L. Roberts, E.A. Carroll, J. Lefkovits, A. E. Ajani, I. Melbourne Interventional Group, Trends in door-to-balloon time and outcomes following primary percutaneous coronary intervention for ST-elevation

myocardial infarction: an Australian perspective, Intern. Med. J. 44 (2014) 471–477, https://doi.org/10.1111/imj.12405.
[42] P. Charlagorla, J. Liu, M. Patel, J.I. Rushbrook, M. Zhang, Loss of plasma membrane integrity, complement response and formation of reactive oxygen species during early myocardial ischemia/reperfusion, Mol. Immunol. 56 (2013) 507–512, https://doi.org/10.1016/j.molimm.2013.05.001.
[43] M. Zhang, L.H. Michael, S.A. Grosjean, R.A. Kelly, M.C. Carroll, M.L. Entman, The role of natural IgM in myocardial ischemia-reperfusion injury, J. Mol. Cell. Cardiol. 41 (2006) 62–67, https://doi.org/10.1016/j.yjmcc.2006.02.006.
[44] W.D. Ito, S. Schaarschmidt, R. Klask, S. Hansen, H.J. Schafer, D. Mathey, S. Bhakdi, Infarct size measurement by triphenyltetrazolium chloride staining versus in vivo injection of propidium iodide, J. Mol. Cell. Cardiol. 29 (1997) 2169–2175.
[45] R.A. Wolff, G.L. Chien, D.M. van Winkle, Propidium iodide compares favorably with histology and triphenyl tetrazolium chloride in the assessment of experimentally-induced infarct size, J. Mol. Cell. Cardiol. 32 (2000) 225–232.
[46] J. Espinoza-Derout, M. Wagner, L. Salciccioli, J.M. Lazar, S. Bhaduri, E. Mascareno, B. Chaqour, M.A. Siddiqui, Positive transcription elongation factor b activity in compensatory myocardial hypertrophy is regulated by cardiac lineage protein-1, Circ. Res. 104 (2009) 1347–1354, https://doi.org/10.1161/CIRCRESAHA.108.191726. CIRCRESAHA.108.191726 [pii].
[47] F. Faul, E. Erdfelder, A. Buchner, A.G. Lang, Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses, Behav. Res. Methods 41 (2009) 1149–1160.
[48] E.P. Daskalopoulos, C. Dufeys, L. Bertrand, C. Beauloye, S. Horman, AMPK in cardiac fibrosis and repair: actions beyond metabolic regulation, J. Mol. Cell. Cardiol. 91 (2016) 188–200, https://doi.org/10.1016/j.yjmcc.2016.01.001.
[49] J.M. Cheng, K.M. Akkerhuis, L.C. Battes, L.C. van Vark, H.L. Hillege, W.J. Paulus, E. Boersma, I. Kardys, Biomarkers of heart failure with normal ejection fraction: a systematic review, Eur. J. Heart Fail. 15 (2013) 1350–1362, https://doi.org/10.1093/eurjhf/hft106.
[50] Y. Sun, Myocardial repair/remodelling following infarction: roles of local factors, Cardiovasc. Res. 81 (2009) 482–490, https://doi.org/10.1093/cvr/cvn333.
[51] H.F. Weisman, T. Bartow, M.K. Leppo, H.C. Marsh Jr., G.R. Carson, M.F. Concino, M.P. Boyle, K.H. Roux, M.L. Weisfeldt, D.T. Fearon, Soluble human complement receptor type 1: in vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis, Science 249 (1990) 146–151, https://doi.org/10.1126/science.2371562.
[52] M. Zhang, E.M. Alicot, I. Chiu, J. Li, N. Verna, T. Vorup-Jensen, B. Kessler, M. Shimaoka, R. Chan, D. Friend, U. Mahmood, R. Weissleder, F.D. Moore, M. C. Carroll, Identification of the target self-antigens in reperfusion injury, J. Exp. Med. 203 (2006) 141–152, https://doi.org/10.1084/jem.20050390.
[53] M. Zhang, W.G. Austen Jr., I. Chiu, E.M. Alicot, R. Hung, M. Ma, N. Verna, M. Xu, H.B. Hechtman, F.D. Moore Jr., M.C. Carroll, Identification of a specific self-reactive IgM antibody that initiates intestinal ischemia/reperfusion injury, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 3886–3891, https://doi.org/10.1073/pnas.0400347101.
[54] S.W. Lee, J.Y. Won, H.Y. Lee, H.J. Lee, S.W. Youn, J.Y. Lee, C.H. Cho, H.J. Cho, S. Oh, I.H. Chae, H.S. Kim, Angiopoietin-1 protects heart against ischemia/reperfusion injury through VE-cadherin dephosphorylation and myocardiac integrin-β1/ERK/caspase-9 phosphorylation cascade, Mol. Med. 17 (2011) 1095–1106, https://doi.org/10.2119/molmed.2011.00106.
[55] J. Li, H. Ding, Y. Li, H. Zhou, W. Wang, Y. Mei, R. Zhang, Alarin alleviated cardiac fibrosis via attenuating oxidative stress in heart failure rats, Amino Acids 53 (2021) 1079–1089, https://doi.org/10.1007/s00726-021-03005-8.
[56] Y. Wang, S. Zhang, Y. Ma, A. Xiang, H. Sun, J. Song, W. Yang, X. Li, H. Xu, Melatonin protected against myocardial infarction injury in rats through a Sirt6-dependent antioxidant pathway, Adv. Clin. Exp. Med. 31 (2022) 277–284, https://doi.org/10.17219/acem/112060.
[57] M.K. Torp, T. Ranheim, C. Schjalm, M. Hjorth, C.M. Heiestad, K.T. Dalen, P. H. Nilsson, T.E. Mollnes, S.E. Pischke, E. Lien, J. Vaage, A. Yndestad, K. O. Stensløkken, Intracellular complement component 3 attenuated ischemia-reperfusion injury in the isolated buffer-perfused mouse heart and is associated with improved metabolic homeostasis, Front. Immunol. 13 (2022), 870811, https://doi.org/10.3389/fimmu.2022.870811.
[58] A.E. Denktas, H.V. Anderson, J. McCarthy, R.W. Smalling, Total ischemic time: the correct focus of attention for optimal ST-segment elevation myocardial infarction care, JACC Cardiovasc. Interv. 4 (2011) 599–604, https://doi.org/10.1016/j.jcin.2011.02.012.
[59] E. Braunwald, Heart failure, JACC Heart Fail 1 (2013) 1–20, https://doi.org/10.1016/j.jchf.2012.10.002.
[60] B. Ibanez, G. Heusch, M. Ovize, F. Van de Werf, Evolving therapies for myocardial ischemia/reperfusion injury, J. Am. Coll. Cardiol. 65 (2015) 1454–1471, https://doi.org/10.1016/j.jacc.2015.02.032.
[61] A.E. Moran, M.H. Forouzanfar, G.A. Roth, G.A. Mensah, M. Ezzati, A. Flaxman, C. J. Murray, M. Naghavi, The global burden of ischemic heart disease in 1990 and 2010: the Global Burden of Disease 2010 study, Circulation 129 (2014) 1493–1501, https://doi.org/10.1161/CIRCULATIONAHA.113.004046.
[62] B. Ziaeian, G.C. Fonarow, Epidemiology and aetiology of heart failure, Nat. Rev. Cardiol. 13 (2016) 368–378, https://doi.org/10.1038/nrcardio.2016.25.
[63] R.M. Bell, H.E. Botker, R.D. Carr, S.M. Davidson, J.M. Downey, D.P. Dutka, G. Heusch, B. Ibanez, R. Macallister, C. Stoppe, M. Ovize, A. Redington, J. M. Walker, D.M. Yellon, 9th Hatter Biannual Meeting: position document on ischaemia/reperfusion injury, conditioning and the ten commandments of cardioprotection, Basic Res. Cardiol. 111 (2016) 41, https://doi.org/10.1007/s00395-016-0558-1.
[64] S. Nanayakkara, D.M. Kaye, Management of heart failure with preserved ejection fraction: a review, Clin. Therapeut. 37 (2015) 2186–2198, https://doi.org/10.1016/j.clinthera.2015.08.005.
[65] E.S. Reis, D.C. Mastellos, D. Yancopoulou, A.M. Risitano, D. Ricklin, J.D. Lambris, Applying complement therapeutics to rare diseases, Clin. Immunol. 161 (2015) 225–240, https://doi.org/10.1016/j.clim.2015.08.009.
[66] B.P. Morgan, C.L. Harris, Complement, a target for therapy in inflammatory and degenerative diseases, Nat. Rev. Drug Discov. 14 (2015) 857–877, https://doi.org/10.1038/nrd4657.