Basic Res Cardiol 97: 263 – 267 (2002)
DOI 10.1007/s00395-002-0363-x

David J. Lefer

Do neutrophils contribute to myocardial reperfusion injury?

Introduction

Currently there is a major debate in cardiovascular medicine between both clinicians and scientists regarding the potential involvement of neutrophils in the pathophysiological process of acute myocardial infarction. Experimental studies performed nearly 20 years ago provided very convincing evidence that circulating neutrophils were recruited from the systemic circulation and accumulated within the coronary microcirculation and the myocardium. Based on these early studies it was logical to hypothesize that activated neutrophils releasing an abundance of cytotoxic mediators (i.e., oxidants and proteolytic enzymes) could very readily promote myocardial cell injury or death. For several decades, cardiovascular scientists have attempted to demonstrate the involvement of neutrophils in the process of acute myocardial infarction. However, no specific anti-neutrophil therapy has yet been approved for the treatment of myocardial reperfusion injury in man. Since it is believed that neutrophils primarily promote reperfusion injury, one must first accept the idea that myocardial reperfusion injury does in fact exist in man. This commentary will briefly review the currently available evidence to support a role for neutrophils in myocardial reperfusion injury.

D. J. Lefer, Ph.D. (✉)
Department of Molecular and
Cellular Physiology
Louisiana State University Health
Sciences Center
1501 Kings Highway
Shreveport, Louisiana 71130, USA
Tel.: (318) 675-6974
Fax: (318) 675-4217
E-Mail: dlefer@lsuhsc.edu

Evidence for a role of neutrophils in myocardial reperfusion injury

Experimental evidence

The vast majority of current evidence supporting a role for neutrophils (PMNs) in myocardial reperfusion injury is derived from experimental investigations that were performed in various research laboratories. The first evidence to suggest that neutrophils were involved in the pathophysiology of acute myocardial infarction was provided by seminal studies that clearly demonstrated the accumulation of leukocytes (primarily neutrophils) in the myocardium and coronary microcirculation following acute myocardial ischemia and reperfusion (8–10). These studies suggested that adherent neutrophils were responsible for microvascular "plugging" that contributed to the development of the "no-reflow" phenomenon (8, 10). These early studies provided valuable insights into the potential mechanisms of myocardial reperfusion injury and clearly served as a starting point for subsequent experimental investigations of neutrophil involvement in myocardial reperfusion injury. The results of these very early studies clearly demonstrate that activated neutrophils do accumulate to a very large extent within the ischemic-reperfused myocardium and coronary circulation. In recent years these early studies have been further substantiated by additional investiga-

tions that have observed neutrophil influx into the ischemic/reperfused myocardium (7, 20, 27, 40)

Additional support for the concept that activated PMNs might contribute to the deleterious actions of reperfusion was provided by earlier studies employing leukocyte filters to remove circulating leukocytes (16, 26, 32, 39). The leukocyte filter approach was employed by a number of laboratories in which the coronary circulation was diverted through various types of filters that selectively removed leukocytes at the time of reperfusion. The majority of studies of leukocyte removal demonstrated reductions in myocardial injury, infarct size, and the preservation of coronary endothelial function (16, 26, 32, 39). However, some studies utilizing leukocyte filtration failed to demonstrate significant cardioprotective actions. Leukocyte filtration experiments did provide strong support for a role of neutrophils in terms of myocardial "no-reflow", myocardial "stunning", and myocardial infarction, but the studies did suffer from a number of flaws (32). Leukocyte filters were not a perfect technique for the elimination of neutrophils since these filters could in some cases deplete other forms of leukocytes, did not always remove 100% of circulating neutrophils, and neutrophil-derived inflammatory mediators released by the neutrophils trapped in the filter systems did gain access to the coronary circulation and these mediators alone could produce myocardial injury. Nevertheless, leukocyte filter experiments did extend our knowledge of neutrophils in reperfusion injury and provided an impetus for future studies. In order to circumvent any of the potential limitations of leukocyte filters, scientists began to utilize anti-neutrophil serum preparations as an alternate means for the removal of neutrophils from the systemic circulation of animals (37). The use of anti-neutrophil serum resulted in the complete removal of neutrophils without the removal of any other types of leukocytes. Anti-neutrophil serum attenuated the extent of myocardial ischemia-reperfusion injury and these data provide additional strong support for the concept that neutrophils are mediators of reperfusion injury in the heart (37).

Increasing interest in inflammatory disease states resulted in the development of novel and exciting reagents that were designed to help elucidate the role of neutrophils in a variety of disease states involving the immune system. Elegant work performed by molecular immunologists revealed that neutrophil trafficking in inflammatory diseases was a very highly orchestrated process that involved a number of specific neutrophil and endothelial cell adhesion glycoproteins. At the same time highly specific monoclonal antibodies directed against various neutrophil adhesion receptors were becoming available and cardiovascular researchers were quick to obtain these highly valuable research tools and investigate them in *in vivo* animal models of myocardial ischemia/reperfusion injury. A landmark study was performed by Simpson and colleagues (43) in which treatment with a novel anti-neutrophil CD11b (Mac-1) antibody was shown to reduce myocardial infarct size in a canine model of myocardial reperfusion injury. A subsequent study (44) demonstrated marked cardioprotective effects with anti-Mac-1 antibody therapy.

A number of anti-CD18 monoclonal antibodies that rendered the neutrophil β-2 integrins (LFA-1, Mac-1, p150,95) nonfunctional were then tested in animal models of myocardial reperfusion injury (1, 2, 22, 28, 35, 46). Studies were performed in a variety of *in vivo* and *in vitro* models employing a number of different species and antibodies with the majority of studies demonstrating beneficial effects of these agents (1, 2, 22, 28, 35). However, there was a negative study in which anti-CD18 therapy did not result in significant reductions in myocardial infarct size or reperfusion injury (46). This negative study only served to add to the controversy surrounding the potential involvement of neutrophils in myocardial reperfusion injury. Concerns have been raised regarding the species cross-reactivity of various antibodies (originally designed for human use) as well as the dosages of antibody, the antibody half-life, duration of myocardial ischemia, and the timing of administration with respect to the negative CD18 antibody studies (35).

A further understanding of endothelial cell activation during inflammation (11, 12) revealed that various adhesion proteins were involved in the early neutrophil rolling response (i.e., P-selectin and E-selectin) and the later neutrophil firm adhesion response (i.e., ICAM-1). Robert Rothlein and colleagues (38) were the first to develop reagent grade anti-ICAM-1 monoclonal antibodies in sufficient quantity to explore the potential role of ICAM-1 in the ischemic-reperfused myocardium. Experimental investigations clearly demonstrated that ICAM-1 expression was upregulated in the coronary circulation *in vivo* (21, 48), cardiac myocytes expressed an abundance of ICAM-1 *in vitro* (45), and immunoneutralization of ICAM-1 did reduce myocardial infarct size and preserved coronary vascular reactivity (25, 29). These experimental investigations strongly support a role for ICAM-1 in the pathogenesis of myocardial reperfusion injury.

Following the studies of ICAM-1, monoclonal antibodies, carbohydrate drugs, and small molecule selectin inhibitors were generated that could specifically inhibit the function of either P-selectin or E-selectin alone or both of these endothelial selectins. Experimental data do demonstrate clear P-selectin expression in the coronary circulation following ischemia and reperfusion (47), but there are no strong data with respect to E-selectin expression in animal models of coronary artery ligation/reperfusion. Thus, a number of experimental investigations employing specific and potent anti-P-selectin antibodies were undertaken (24, 25, 47). The use of anti-P-selectin monoclonal antibodies uniformly demonstrated marked

cardioprotective effects in a variety of animal models of myocardial ischemia/reperfusion (24, 25, 47). Similarly, early studies with the carbohydrate selectin blocker from Cytel Corporation (CY-1503) also demonstrated reductions in infarct size with short duration (5, 23, 41) and prolonged reperfusion (13) as well as strong evidence for endothelial cell protection in the coronary circulation (5, 23). Subsequent studies (4, 14), however, failed to demonstrate any clear benefits of CY-1503 (i.e., Cylexin®) in animal models of myocardial reperfusion injury which only added further controversy to the present debate.

More recently, studies have been performed in gene-targeted mice in which genes encoding various leukocyte and endothelial cell adhesion glycoproteins have been subjected to targeted disruption resulting in "knockout" mice. Studies of P-selectin, CD18, and ICAM-1 deficient animals have revealed cardioprotection in the setting of both acute and chronic models of myocardial ischemia and reperfusion injury (18, 33, 34). However, if the period of myocardial ischemia is increased significantly, the cardioprotection is not observed (18, 33, 34). In addition, diabetic mice are not protected from reperfusion injury with P-selectin immunoneutralization (17). These data support a role for neutrophils in myocardial reperfusion injury, but also reveal limitations to the benefit of inhibition of neutrophil-mediated tissue injury.

Clinical evidence

Despite a wealth of experimental evidence neutrophil-mediated myocardial reperfusion injury there is a relative paucity of evidence provided by clinical studies. The majority of clinical evidence supporting neutrophils in myocardial reperfusion injury is related to the measurement of various leukocyte and endothelial cell adhesion molecules or neutrophil-derived inflammatory mediators. Dinerman et al. (6) clearly demonstrated increased circulating levels of neutrophil-derived elastase in patients with unstable angina and acute myocardial infarction. Another clinical study (30) reported that neutrophil and monocyte adhesion molecule expression was increased in unstable coronary artery disease. This study demonstrated that CD11b/CD18 (Mac-1) expression was increased on the surface of neutrophils and monocytes obtained from the coronary sinus of patients with unstable coronary artery disease (30). Subsequent studies have reported significant elevations in soluble P-selectin in blood obtained from the coronary circulation of patients with unstable angina (15) and coronary artery vasospasm (19). These studies provide strong, but indirect evidence that circulating neutrophils can become activated within the coronary circulation of patients during ischemic episodes and that neutrophils may contribute to myocardial reperfusion injury in man.

Due to the large controversy that exists regarding neutrophils and myocardial reperfusion injury very few clinical trials testing the efficacy specific neutrophil inhibitors in acute myocardial infarction have been performed. One recent double-blinded, randomized, clinical trial termed the LIMIT AMI study (3) employed a recombinant human monoclonal antibody (rhuMAb) directed against CD18 in humans that were treated with recombinant tissue plasminogen activator for acute myocardial infarction. A total of 394 subjects who presented within 12 hours of symptom onset with ST-segment elevation were randomized to receive either placebo or the rhuMAb (0.5 or 2.0 mg/kg). Treatment with the MAb failed to demonstrate any effects on coronary blood flow, infarct size, or resolution of ECG changes. This negative study would suggest that anti-CD18 therapy is not beneficial in patients with acute MI and therefore neutrophils are not important in myocardial reperfusion injury in man. However, the interpretation of these results should be made with caution for several reasons. Patients in this study were included if they presented within 12 hours of symptom onset and this extreme delay in anti-CD18 therapy may have exceeded the time frame in which one can attenuate the potential neutrophil component of reperfusion injury in the heart. Secondly, some of the patients in this study received either heparin or ReoPro, two drugs that have very powerful anti-neutrophil effects (31, 36, 42). A second study of anti-CD18 therapy, the HALT MI study, has been completed, but the results have not been published in full. A major difference in this study is that patients are treated within 6 hours following symptom onset instead of 12 hours and different MAb will be tested.

Summary

Despite a wealth of information regarding the potential role of neutrophils in myocardial reperfusion injury, there is no absolute proof that neutrophils do in fact actively participate in this process. The overwhelming majority of data obtained from experimental studies in animals does suggest that neutrophils do promote myocardial tissue injury and cardiac myocyte death. One must view these data with caution since the current animal models may not accurately depict what happens in humans. A major drawback with most animal models of myocardial reperfusion injury is that they rely on data obtained from otherwise healthy animals. It is well appreciated that humans that suffer an acute myocardial infarction usually exhibit a number of risk factors including hypertension, diabetes mellitus, hypercholesterolemia, etc. Thus, the translation of information obtained from healthy animals to humans with coronary artery disease may not be of the highest fidelity. Furthermore,

neutrophil-mediated myocardial injury has been shown primarily to occur following reperfusion and another major debate still rages regarding the question of reperfusion injury in man.

Finally, the role of neutrophils in myocardial reperfusion injury in man will only be fully determined if a very well designed clinical trial of anti-neutrophil therapy is performed. This study must include a sufficient number of patients, must only include patients who receive the therapy in a rapid manner, receive an adequate dose of the agent, and allows for interpretation of various complicating factors (i.e., heparin, lidocaine, and IIb/IIIa therapy).

In conclusion, we still do not really know if neutrophils are important mediators of cardiac tissue injury in the setting of acute myocardial infarction.

References

1. Arai M, Lefer DJ, So T, DiPaula T, Aversano T, Becker LC (1996) An anti-CD18 antibody limits infarct size and preserves left ventricular function in dogs with ischemia and 48-hour reperfusion. J Am Coll Cardiol 27: 1278–1285
2. Aversano T, Zhou W, Nedelman M, Nadada M, Weisman H (1995) A chimeric IgG4 monoclonal antibody directed against CD18 reduces infarct size in a primate model of myocardial ischemia and reperfusion. J Am Coll Cardiol 25: 781–788
3. Baran KW, Nguyen M, McKendall GR, Lambrew CT, Dykstra G, Palmeri ST, Gibbons RJ, Borzak S, Sobel BE, Gourlay SG, Rundle AC, Gibson CM, Barron HV (2001) Double-blind randomized trial of an anti-CD18 antibody in conjunction with recombinant tissue plasminogen activator for acute myocardial infarction: limitation of myocardial infarction following thrombolysis in acute myocardial infarction (LIMIT AMI) study. Circulation 104: 2778–2783
4. Birnbaum Y, Patterson M, Kloner RA (1997) The effect of CY1503, a sialyl lewis^x analog blocker of the selectin adhesion molecules, on infarct size and "no reflow" in the rabbit model of acute myocardial infarction/reperfusion. J Mol Cell Cardiol 29: 2013–2025
5. Buerke M, Weyrich AS, Zheng Z, Gaeta FCA, Forrest MJ, Lefer AM (1994) Sialyl Lewis^x-containing oligosaccharide attenuates myocardial reperfusion injury in cats. J Clin Invest 93: 1140–1148
6. Dinerman JL, Mehta JL, Saldeen TGP, Emerson S, Wallin R, Davida R, Davidson A (1990) Increased neutrophil elastase release in unstable angina pectoris and acute myocardial infarction. J Am Coll Cardiol 15: 1559–1563
7. Dreyer WJ, Michael LH, West MS, Smith CW, Rothlein R, Rossen RD, Anderson DC, Entman ML (1991) Neutrophil accumulation in ischemic canine myocardium. Circulation 84: 400–411
8. Engler RL, Schmid-Schonbein GW, Pavelec RS (1983) Leukocyte capillary plugging in myocardial ischemia and reperfusion in the dog. Am J Pathol 111: 98–111
9. Engler RA, Dahlgren MD, Peterson MA, Dobbs A, Schmid-Schonbein GW (1986) Accumulation of polymorphonuclear leukocytes during 3-h experimental myocardial ischemia. Am J Physiol 251: H93–H100
10. Engler RL, Dahlgren MD, Morris DD, Peterson MA, Schmid-Schonbein GW (1986) Role of leukocytes in response to acute myocardial ischemia and reflow in dogs. Am J Physiol 251: H314–H322
11. Entman ML, Michael LH, Rossen RD, Dryer WJ, Anderson DC, Smith CW (1991) Inflammation in the course of early myocardial ischemia. FASEB J 5: 2529–2537
12. Entman ML, Ballantyne CB (1993) Inflammation in acute coronary syndromes. Circulation 88: 800–803
13. Flynn DM, Buda AJ, Jeffords PR, Lefer DJ (1996) A sialyl Lewis^x-containg carbohydrate reduces infarct size: Role of selectins in myocardial reperfusion injury. Am J Physiol 271: H2086–H2096
14. Gill EA, Kong Y, Horwitz LD (1996) An oligosaccharide sialyl-lewis^x analogue does not reduce myocardial infarct size after ischemia and reperfusion. Circulation 94: 542–546
15. Ikeda H, Takajo Y, Ichiki K, Ueno T, Maki S, Noda T, Sugi K, Imaizumi T (1995) Increased soluble form of P-selectin in patients with unstable angina. Circulation 92: 1693–1696
16. Jolly SR, Kane WJ, Hook BG, Abrams GD, Kunkel SL, Lucchesi BR (1986) Reduction of myocardial infarct size by neutrophil depletion: effects of duration of occlusion. Am Heart J 112: 682–690
17. Jones SP, Girod WG, Granger DN, Palazzo AJ, Lefer DJ (1999) Reperfusion injury is not affected by blockade of P-selectin in the diabetic mouse heart. Am J Physiol 277: H763–H769
18. Jones SP, Trocha SD, Strange MB, Granger DN, Kevil CG, Bullard DC, Lefer DJ (2000) Leukocyte and endothelial cell adhesion molecules in a chronic murine model of myocardial reperfusion injury. Am J Physiol 279: H2196–H2201
19. Kaikita K, Ogawa H, Yasue H, Sakamoto T, Hisakazu, Sumida H, Okumura K (1995) Soluble P-selectin is released into the coronary circulation after coronary spasm. Circulation 92: 1726–1730
20. Kloner RA, Giacomelli F, Alker KJ, Hale SL, Matthews R, Bellows S (1991) Influx of neutrophils into the walls of large epicardial coronary arteries in response to ischemia/reperfusion. Circulation 84: 1758–1772
21. Kukielka GL, Hawkins HK, Michael L, Manning AM, Youker K, Lane C, Entman ML, Smith CW, Anderson DC (1993) Regulatin of intercellular adhesion molecule-1 (ICAM-1) in ischemic and reperfused canine myocardium. J Clin Invest 92: 1504–1516
22. Lefer DJ, Shandelya SM, Serrano CV, Becker LC, Kuppusamy P, Zweier JL (1993) Cardioprotective actions of a monoclonal antibody against CD-18 in myocardial ischemia-reperfusion injury. Circulation 88: 1779–1787
23. Lefer DJ, Flynn DM, Phillips ML, Ratcliffe M, Buda AJ (1994) A novel sialyl Lewis^x analog attenuates neutrophil accumulation and myocardial necrosis after ischemia and reperfusion. Circulation 90: 2390–2401
24. Lefer DJ, Flynn DM, Buda AJ (1996) Effects of a monoclonal antibody directed against P-selectin after myocardial ischemia and reperfusion. Am J Physiol 270: H88–H98
25. Lefer DJ, Flynn DM, Anderson DC, Buda AJ (1996) Combined inhibition of P-selectin and ICAM-1 reduces myocardial injury following ischemia and reperfusion. Am J Physiol 271: H2421–H2429

26. Litt MR, Jeremy RW, Weisman HF, Winkelstein JA, Becker LC (1989) Neutrophil depletion limited to reperfusion reduces myocardial infarct size after 90 minutes of ischemia: evidence for neutrophil-mediated reperfusion injury. Circulation 80: 1816–1827
27. Lucchesi BR (1990) Modulation of leukocyte-mediated myocardial reperfusion injury. Annu Rev Physiol 52: 561–576
28. Ma XL, Tsao PS, Lefer AM (1991) Antibody to CD18 exerts endothelial and cardiac protective effects in myocardial ischemia and reperfusion. J Clin Invest 88: 1237–1243
29. Ma XL, Lefer DJ, Lefer AM, Rothlein R (1992) Coronary endothelial and cardiac protective effects of a monoclonal antibody to intercellular adhesion molecule-1 in myocardial ischemia and reperfusion. Circulation 86: 937–946
30. Mazzone A, Servi SD, Ricevuti G, Mazzucchelli I, Fossati G, Pasotti D, Bramucci E, Angoli L, Marsico F, Specchia G, Notario A (1993) Increased expression of neutrophil and monocyte adhesion molecules in unstable coronary artery disease. Circulation 88: 358–363
31. Mickelson JK, Nadir AM, Kleiman NS (1999) Chimeric 7E3 Fab (ReoPro) decreases detectable CD11b on neutrophils from patients undergoing coronary angioplasty. J Am Coll Cardiol 33: 97–106
32. O'Neill PG, Charlat ML, Michael LH, Roberts R, Bolli R (1989) Influence of neutrophil depletion on myocardial function and flow after reversible ischemia. Am J Physiol 25: H341–H351
33. Palazzo AJ, Jones SP, Anderson DC, Granger DN, Lefer DJ (1998) Coronary endothelial P-selectin in pathogenesis of myocardial ischemia-reperfusion injury. Am J Physiol 275: H1865–H1872
34. Palazzo AJ, Jones SP, Girod WG, Granger DN, Anderson DC, Lefer DJ (1998) Myocardial ischemia-reperfusion injury in CD18- and ICAM-1 deficient mice. Am J Physiol 275: H2300–H2307
35. Perez RG, Arai M, Richardson C, DiPaula A, Siu C, Matsumoto N, Hildreth JEK, Mariscalco MM, Smith CW, Becker LC (1996) Factors modifying protective effect of anti-CD18 antibodies on myocardial reperfusion injury in dogs. Am J Physiol 270: H53–H64
36. Peter K, Schwarz M, Conradt C (1999) Heparin inhibits ligand binding to the leukcoyte integrin MAC-1 (CD11b/CD18). Circulation 100: 1533–1539
37. Romson JL, Hook BG, Kunkel SL, Arams GD, Schork MA, Lucchesi BR (1983) Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. Criculaton 67: 1016–1023
38. Rothlein R, Dustin ML, Marlin SD, Springer TA (1986) A human intracellular adhesion molecule (ICAM-1) distinct from LFA-1. J Immunol 137: 1270–1274
39. Sheridan FM, Dauber IM, McMurtry IF, Lesnefsky EJ, Horwitz LD (1991) Role of leukocytes in coronary vascular endothelial injury due to ischemia and reperfusion. Circ Res 69: 1566–1574
40. Sheridan FM, Cole PG, Ramage D (1996) Leukocyte adhesion to the coronary microvasculature during ischemia and reperfusion in an in vivo canine model. Circulation 93: 1784–1787
41. Silver MJ, Sutton JM, Hook S, Lee P, Malycky JL, Phillips ML, Ellis SG, Topol EJ, Nicolini FA (1995) Adjunctive selectin blockade successfully reduces infarct size beyond thrombolysis in the electrolytic canine coronary artery model. Circulation 92: 492–499
42. Simon D, Xu H, Ortlepp S (1997) 7E3 monoclonal antibody directed against the platelet glycoprotein IIb/IIIa cross-reacts with the leukocyte integrin Mac-1 and blocks adhesion to fibrinogen and ICAM-1. Arterioscler. Thromb Vasc Biol 17: 528–535
43. Simpson PJ, Todd RFI, Fantone JC, Mickelson JK, Griffin JD, Lucchesi BR (1988) Reduction of experimental canine myocardial reperfusion injury by a monoclonal antibody (Anti-Mo1, Anti-CD11b) that inhibits leukocyte adhesion. J Clin Invest 81: 624–629
44. Simpson PJ, Todd RF, Mickelson JK, Fantone JC, Gallagher KP, Lee KA, Tamura Y, Cronin M, Lucchesi BR (1990) Sustained limitation of myocardial reperfusion injury by a monoclonal antibody that alters leukocyte function. Circulation 81: 226–237
45. Smith CW, Entman ML, Lane CL, Beaudet AL, Ty TI, Youker K, Hawkins HK, Anderson DC (1991) Adherence of neutrophils to canine cardiac myocytes in vitro is dependent on intercellular adhesion molecule-1. J Clin Invest 88: 1216–1233
46. Tanaka M, Brooks SE, Richard VJ, FitzHarris GP, Stoler RC, Jennings RB, Arfors KE, Reimer KA: Effect of anti-CD18 antibody on myocardial neutrophil accumulation and infarct size after ischemia and reperfusion in dogs. Circulation 87: 526–535
47. Weyrich AS, Ma XL, Lefer DJ, Albertine KH, Lefer AM (1993) In vivo neutralization of P-selectin protects feline heart and endothelium in myocardial ischemia and reperfusion injury. J Clin Invest 91: 2620–2629
48. Youker KA, Hawkins HK, Kukielka GL, Perrard JL, Michael LH, Ballantyne CM, Smith CW, Entman ML (1994) Molecular evidence for induction of intracellular adhesion molecule-1 in the viable border zone associated with ischemia-reperfusion injury of the dog heart. Circulation 89: 2736–2746