Review

Innate immunity and cardiac preconditioning: a putative intrinsic cardioprotective program

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Abstract

Ischemic preconditioning is thought to evoke cell survival programs in the heart in large part via the activation of G-protein coupled receptor signal transduction pathways. However, the identification and characterization of G-protein coupled receptor independent pathways would enable researchers to pursue novel cellular events that could direct or promote preconditioning. In this regard recent work has begun to explore the role of the innate immune system in intrinsic cardioprotection against both viral myocarditis and ischemia. Interestingly, cytokines such as TNFα, IL-1β and leukemia inhibitory factor, which are components of innate immunity, have been shown to mimic ischemic preconditioning. Thus as the innate immune system functions via a diverse array of G-protein independent receptors, the study of this immunological system in the heart may provide new insight into mechanisms driving and promoting ischemic preconditioning. We propose that innate immunity is indeed an integral part of ischemic preconditioning. In this review, we provide an overview of the innate immune system, describe the studies whereby cytokines mimic ischemic preconditioning and finally postulate some mechanisms whereby innate immunity may promote cardioprotection as a component of preconditioning.

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1. Introduction

Innate immunity is a preprogrammed, nonspecific first-line of defense that is primarily responsible for eliminating and/or containing microorganisms at the site of entrance into the host (reviewed in Refs. [1,2]). Innate immunity is classically mediated by myeloid-derived cells that produce cytokines and activate the complement system in response to ‘shared molecules’ across different pathogens. For example, lipopolysaccharide (LPS), which is a shared molecule in all gram negative bacteria, is a conserved ‘molecular pattern’ that activates innate immunity. In turn, these pathogen-associated molecular patterns bind to phylogenetically conserved pattern-recognition receptors which initiate downstream signaling events. A prototypic example being the binding of LPS to the opsonic receptor CD14 on monocytes, natural killer cells and neutrophils with the subsequent production of tumor necrosis factor alpha (TNFα) [2,3]. Although these biological responses of innate immunity protect the host from invading pathogens, this response may also have detrimental effects. This is best illustrated where an excessive stimulation of innate immunity by gram-negative bacterial endotoxemia leads to an augmented production of cytokines and other molecules. This in turn, can result in the septic shock syndrome with hypotension, disseminated intravascular coagulation, multi-organ failure and even death. The exquisite regulatory mechanisms that direct these divergent effects of innate immunity are being actively explored, but as yet are not fully characterized [4].

The concept that innate immunity may constitute a component of ‘adaptive cardiac biology’ is being increasingly recognized [5]. This is illustrated whereby components of the innate immune system seem to be
required to resist infectious (myocarditis) and pressure-overload mediated cardiac decompensation and heart failure [6–8]. Moreover, this cytoprotective program appears to be important in protecting the heart against cardiac ischemic injury [9,10]. Interestingly, a biological program termed ischemic preconditioning is proposed to activate innate/intrinsic cytoprotective programs in the heart. Ischemic preconditioning is evoked by a short non-lethal period of ischemia and reperfusion and can result in an acute cardioprotection lasting for 1–2 h following preconditioning (classical preconditioning) and delayed preconditioning which denotes a cardioprotected phenotype from 24 to 72 h following preconditioning. The focus of this review is to evaluate whether a link exists between innate immunity and ischemic preconditioning and to postulate how this first-line of immune activation may orchestrate cytoprotective effects in the heart in response to ischemic preconditioning.

2. Evidence that innate immune activation evokes preconditioning-like cardioprotection

Bacterial endotoxin or LPS is the prototypic pathogen-associated molecular pattern that activates innate immunity and has been shown to have profound detrimental effects on cardiac contractile function and on myocardial integrity [11–14]. Interestingly, a preemptive non-lethal exposure to LPS results in a subsequent blunted response to a second LPS challenge [15]. This resilience to the adverse effects of endotoxemia is termed ‘endotoxin tolerance’ and is thought to be an adaptive response providing protection from pathological hyper-activation of the innate immune system during bacterial infection [16]. In parallel to inducing ‘endotoxin tolerance’ pathogen-associated molecular patterns such as endotoxin and lipoteichoic acid induce protection against ischemia–reperfusion injury [17–20]. Here, hearts pre-emptively exposed to LPS have been shown to display increased tolerance against infarction and post-ischemic ventricular arrhythmias [17–20]. These cardioprotective effects of LPS have a similar temporal pattern to that of ischemic preconditioning with acute and delayed temporal phases of cardioprotection. Thus, as primitive pathogen-associated molecular patterns, such as LPS has been shown to promote preconditioning-like cardiac tolerance, the role of innate immunity in the activation of this cardioprotection is worthy of investigation. Innate immunity consists of an exquisitely controlled auto-regulatory network of cytokines that in turn activate numerous putative cell-survival programs. The apical events that activate innate immunity have been reviewed previously [5] and its proposed activation by ischemia and reperfusion is schematized in Fig. 1. The review will discuss the literature regarding activation of the innate immune cytokine network in the context of cardiac ischemia–reperfusion and in ischemic preconditioning with a final section on the putative cardioprotective signaling cascades coupled to this cytokine network.

3. Toll-like receptors in cardiac ischemia

Toll-like receptors (TLR) are the pattern-recognition receptors through which extra-cellular pattern-recognition molecules (e.g., LPS) direct intracellular signaling events and the subsequent activation of the innate immunity
cytokine cascade. The mammalian TLR proteins derive their name from the Drosophila Toll proteins, with which they share sequence similarity. Currently, two TLR subtypes have been described in cardiac tissue and are designated TLR 2 and TLR 4 family members [5,21]. Upregulation of Toll-like receptors in the heart have been associated with oxidative stress [22], endotoxin administration [23], in response to myocardial ischemia and during heart failure [21]. The prototypic activator of innate immunity (LPS) binds to its cognate receptor (CD14) which in turn is thought to interact with TLRs to activate transmembrane signal transduction [2]. Activation of these transmembrane signaling proteins have, in turn, been associated with the activation of the nuclear regulatory factor NFκB. Interestingly, this transcription factor is thought to play a pivotal role in both the activation of innate immunity and in the cardioprotection conferred by ischemic preconditioning [24–26]. However, to date no studies have been published concerning a link between CD14, Toll-like receptors and preconditioning. This putative biological link is attractive as reactive oxygen species are proposed activators of Toll-like receptors and are concurrently recognized as ‘triggers’ of preconditioning cardioprotection [27–30].

4. Activation of the cytokine cascade during cardiac ischemia and reperfusion

As LPS administration can evoke preconditioning-like cardioprotection, the identification of LPS induced cytokines could identify putative cytokines targets that may direct this cardioprotection. In this regard, an in vivo challenge with LPS is known to activate the tumor necrosis factor alpha (TNFα), Interleukin-1 (IL-1), Interleukin-6 (IL-6) and intracellular cytokine mediated signaling molecules and regulatory peptides [31,32]. Similarly, numerous investigators have demonstrated endogenous cardiac and infiltrating inflammatory cell production of similar cytokines, i.e. TNFα, IL-1β and IL-6 in response to cardiac ischemia and reperfusion [33–36]. Here investigators have postulated that this cytokine production may play a role in both reperfusion injury, contractile recovery and in cardiac remodeling post myocardial infarction. However, as will be discussed throughout the duration of this review, these cytokines may have an additional function as integral factors in the intrinsic cardioprotective program that may be activated by ischemic preconditioning.

5. Pro-inflammatory cytokines activate classical and delayed preconditioning

TNFα is an apical pro-inflammatory cytokine in the innate immune system and can be activated in response to both Toll-like receptor activation [3] and via the generation of intracellular reactive oxygen species [37,38]. TNFα is synthesized in myeloid and cardiac tissue in direct response to cardiac ischemia and reperfusion injury–infarction [33–36]. Whether this pro-inflammatory cytokine is produced in response to the non-lethal ischemia and reperfusion that evokes the preconditioning phenotype is less clear. However, suggestive data from Heusch and colleagues have demonstrated that cardiac ischemic preconditioning does result in a modest induction of serum TNFα levels in rabbits [19]. Moreover, in an isolated perfused mouse heart preparation we have recently demonstrated that ischemic preconditioning does evoke an up-regulation of cardiac steady-state TNFα peptide levels [39]. These data demonstrate an association between ischemic preconditioning and TNFα production.

In our laboratory we have demonstrated that exogenous TNFα administration using a preconditioning-like protocol in the isolated perfused rat heart does mimic classical ischemic preconditioning [40]. Moreover, neutralizing antibodies directed against both TNFα and the cytokine IL-1β abrogate exercise induced preconditioning-like cardioprotection in the rat [41]. Finally, in mice genetically depleted of TNFα, we have recently demonstrated that TNFα is necessary for ischemic preconditioning in the isolated perfused heart and that this cytokine is sufficient to activate this acute cardioprotective phenotype [39].

The role of TNFα in delayed preconditioning has also been firmly established. Here Nelson et al. [42] demonstrated that pretreatment of rabbits with intravenous TNFα 24 h before simulated ischemia and reperfusion, resulted in improved cardiac contractile functional recovery and reduced lactate dehydrogenase release. These investigators noted that TNFα pretreatment with the resultant reduction in ischemia–reperfusion injury, correlated with an increase in myocardial manganese superoxide dismutase (Mn–SOD) activity. The upregulation of the free radical scavenger is, at least in part, a plausible mechanism whereby TNFα can protect against ischemia and reperfusion injury. A role for TNFα in delayed preconditioning has now been confirmed by numerous investigators [41,43,44]. Interestingly, TNFα mediated upregulation of Mn–SOD is thought to be, in part via transactivation of NFκB [45], and the activation of NFκB is thought to be essential in the activation of the late preconditioning program [25].

Of note, in classic ischemic preconditioning, the initial ischemic ‘trigger’ or administration of the preconditioning-mimetic adenosine has been shown to attenuate subsequent ischemia–reperfusion associated TNFα production [19,46]. These experiments suggest that a component of the preconditioning ‘trigger’ attenuate post ischemia–reperfusion-induced TNFα production. This putative biphasic regulation of TNFα is consistent with the preconditioning paradigm, as it is thought that elevated TNFα levels at reperfusion would be pro-inflammatory and could hence augment reperfusion damage [47,48]. Thus a hypothesis
that requires testing states that the preconditioning trigger activates TNFα to putatively promote innate cell survival. In parallel, a putative negative autoregulatory effect of this TNFα production could attenuate the subsequent production of this proinflammatory cytokine during post-ischemic reperfusion. This hypothesis, if proven, would support the concept that transient production of TNFα may promote innate cellular survival pathways. Moreover, this temporal regulation of TNFα biosynthesis would be consistent with the exquisite autoregulatory role of the cytokine network in directing the appropriate physiologic response to a given biomechanical stress [4,49].

IL-1β and TNFα are generally thought to be regulated in parallel and have synergistic pro-inflammatory and chemokine activating effects in myeloid cells. This coactivation is mirrored in cardiac ischemia–reperfusion where IL-1β is co-expressed with TNFα. However, the role of IL-1β in activating preconditioning has not been as extensively studied as that of TNFα. However, as was alluded to previously, a combination of neutralizing antibodies to both IL-1β and to TNFα were required to attenuate the preconditioning phenotype evoked by exercise in rats [41]. Moreover, numerous studies have demonstrated that the administration or activation of IL-1β does evoke the delayed preconditioning phenotype in the rat heart [20,50,51].

Collectively, these data support the role of these two apical pro-inflammatory pleiotropic cytokines in the activation of the cell survival program mediated by ischemic preconditioning. Recognition that preconditioning can arise from the activation of pro-inflammatory cytokines should now allow molecular and cellular analysis of the specific intracellular signaling cascades downstream of cytokine–receptor interaction that may play a critical role in the development of this tolerant cardiac phenotype.

6. Glycoprotein-130 receptor mediated cardioprotection

In addition to the direct activation of cytoprotective signaling molecules (discussed in Section 8), the proinflammatory cytokines TNFα and IL-1β are known to activate numerous mediator cytokines, in part via the regulatory kinase TAK1 [52,53]. The glycoprotein 130 (gp130) receptor family of cytokines is known to be activated by the apical pro-inflammatory cytokines [9,54] and three members of this family, i.e., leukemia inhibitory factor (LIF), IL-6 and cardiotrophin-1 (CT-1) are endogenously synthesized by the heart [55,56].

The role of gp130 receptor mediated cytokine signaling in ischemic preconditioning has not been extensively investigated. However, LIF when administered as a preconditioning mimetic has been shown to confer delayed protection against post-ischemic contractile dysfunction and cardiac damage in rabbits [42]. Here the mechanism of protection was thought to be due to the upregulation of the reactive oxygen species (ROS) scavenger manganese superoxide dismutase. In addition, the three cardiac enriched gp130 receptor cytokine ligands have been shown to promote cardiac protection against toxic insults including ischemia–reperfusion injury. In brief, pre-incubation of rat neonatal cardiomyocytes with LIF conferred protection against hypoxia–reoxygenation injury via attenuation of ROS production [57]. IL-6, in turn promotes cytoprotection of rat neonatal cardiomyocytes against sphingosine mediated apoptosis [9]. Furthermore, CT-1 when administered as either a pre-treatment or at the time of reoxygenation has been shown to be cardioprotective against simulated ischemia–reoxygenation injury in rat cardiomyocytes [58].

The gp130-receptor signaling is usually mediated via JAK–STAT transduction pathways [59]. These pathways have not been extensively explored in the context of ischemic preconditioning; however, Bolli and colleagues recently established that functional JAK–STAT signaling is essential to induce the delayed preconditioning phenotype [60]. Further data implicate the involvement of the JAK–STAT system in classical preconditioning, but requires additional studies to establish a mechanism [61,62]. In short, the role of gp130-receptor signaling in preconditioning has not been extensively investigated. However, the data presented above suggest that this cytokine-receptor linked regulatory system may play a role in preconditioning and may provide insight into the activation of the JAK–STAT signal transduction in ischemic preconditioning.

7. The role of anti-inflammatory cytokines in cardiac protection

The innate immune system is usually tightly controlled by comprehensive autoregulatory mechanisms [63–65]. For example, the immunosuppressive and anti-inflammatory cytokines such as transforming growth factor β and interleukin 10 (IL-10) are thought to have evolved to control immune reactions and to prevent over-robust reactions that become destructive to the host. A role for immunosuppressive or anti-inflammatory cytokines in the attenuation of the pro-inflammatory cytokines during post-ischemic reperfusion would be an equivalent scenario that could result in an adaptive modulation of cytokines during cardiac ischemia and reperfusion. To date, this concept has not been explored in the context of preconditioning. However, the role of IL-10 has been investigated in cardiac ischemia and reperfusion. As background, IL-10 which was first identified as a cytokine synthesis inhibiting factor [66], is a homodimeric cytokine and plays a key role in the regulation of inflammatory and immune responses. The anti-inflammatory properties of IL-10 are partially mediated by inhibition of the production of pro-inflammatory
cytokines such as IL-1α, IL-1β, IL-6, IL-8 and TNFα from the monocytes, macrophages [67,68] and neutrophils [69]. Multiple studies have demonstrated IL-10 in the plasma from patients following acute myocardial infarction or during cardiopulmonary bypass [70–72]. In mice IL-10 levels increase 2–6 h after myocardial reperfusion [73]. This endogenous production of IL-10 appears to be critical in diminishing myocardial injuries after myocardial ischemia–reperfusion. Using an IL-10 deficient mice model, the same investigators confirmed that the absence of IL-10 was associated with a large mortality following ischemia–reperfusion [47]. Here, it was suggested that endogenous IL-10 inhibits the production of TNFα and nitric oxide, and hence serves to protect the ischemia–reperfusion exposed myocardium through the suppression of neutrophil recruitment. Moreover, recently Jones et al. confirmed that deficiency of IL-10 exacerbated myocardial injury after ischemia–reperfusion via an infiltration of neutrophils and postulated that this cardioprotective effects of IL-10 was independent of the presence or absence of iNOS [74].

While a cardioprotective effect of IL-10 has been described in acute myocardial infarction, a role of IL-10 in ischemic preconditioning has not yet been reported. However, indirect data to support the hypothesis that IL-10 may participate in ischemic preconditioning includes the fact that numerous cellular systems that modulate preconditioning such as adenosine [75], heat shock proteins [76] and ROS [77] augment the production of IL-10. Thus, although not explored, the anti-inflammatory and immunosuppressive regulatory events that control immune activation may play a role in ischemic preconditioning.

8. Cardioprotective cell signaling downstream of cytokine–receptor interaction

Ischemic preconditioning is classically known to invoke the production of G-protein coupled receptor ligands, such as adenosine, opioids and bradykinin [78]. The signaling intermediates activated by these ligands have been extensively investigated in the characterization of cardioprotective cellular events induced by preconditioning (reviewed in Refs. [78,79]). The hypothesis that the innate immune system may contribute to the cardioprotective programs activated by ischemic preconditioning could identify putative novel signaling events that may be important in this cell-survival promoting biological phenomenon. The remainder of this review will focus on describing the known cardioprotective programs activated by innate immunity.

The signaling cascades driven by the prototypic proinflammatory cytokines TNFα and IL-1β are remarkably similar, despite being initiated via distinct cell surface receptors. These signaling cascades have been reviewed previously [49,80–83] and are not discussed extensively in this review. However, cardioprotective pathways downstream of the TNFα receptors that may be relevant to the promotion of cardioprotective programs against ischemia–reperfusion injury are proposed to be mediated via PKC [84], NfκB [85] and SAPK [86]. Although the data supporting a role for IL-1β in preconditioning is less extensive than for TNFα seemingly parallel signaling can promote the cell survival effects of these two cytokines. Hence in the context of preconditioning, we will briefly review the IL-1β activated cell survival signaling, although we should caution that delineation of IL-1β signaling has been less extensively characterized in the heart. In diverse tissue types IL-1β has been shown to activate classical pro-survival signaling kinases including the phosphatidylinositol 3-kinase–Akt pathway [87] and tyrosine kinase signaling [88]. Moreover, similarly to TNFα, IL-1β activates cytoprotective signaling cascades and upregulates mediator cytokines such as IL-6 via the activation of protein kinase TAK1 [89,90]. Finally, numerous cytoprotective molecules that have been implicated in preconditioning-induced cell survival, such as Bcl2, iNOS and Mn–SOD, have been shown to be activated by IL-1β and TNFα [41,88,91].

The IL-6 family of cytokines may bind to their own ligand specific cognate receptor. The ligand–receptor complex then interacts with gp130, a cell surface signaling receptor, which then goes on to signal a host of intracellular pathways [92]. Following the activation of the ubiquitously expressed gp130, by the cytokine–receptor complex, the gp130 undergoes homo- or hetero-dimerization. Although gp130 and its dimerizing partner possess no intrinsic tyrosine kinase domain, associated cytoplasmic tyrosine kinases are activated, allowing subsequent modification of transcription factors [92]. The downstream pathways activated by the gp130 complex include Janus kinase (JAKs) and several members of the signal transducer and activator of transcription (STAT) family [93,94]. In addition, gp130 signaling is known to activate signaling intermediates that promote cell survival such as the src family of tyrosine kinases, RAS, mitogen activated protein kinases (MAPK) and phosphatidylinositol 3-kinase (PI-3 kinase) [57].

Finally, downstream cytoprotective targets of innate immune activation in the heart probably overlap considerably with the signaling cascades activated by G-protein-coupled receptor signaling in ischemic preconditioning. However, novel putative G-protein independent signaling events could be identified in the context of innate immunity in ischemic preconditioning. Fig. 2 is a schematic model of putative overlapping and novel signaling between classical ischemic preconditioning activated signaling and those more unique to innate immune mediated events.

9. Conclusions

Scientific evidence supports a role for at least three distinct cytokines, i.e., TNFα, IL-1β and leukemia inhib-
Fig. 2. Proposed model of the role of innate immunity in the activation of ischemic preconditioning. The signaling molecules that probably represent activation of classical preconditioning (acute-induction) are represented in the square box. Alternatively, the activation of Gp-130 signaling and JAK–STAT activation would be more consistent with delayed preconditioning. The putative negative feedback loop with modulation of pro-inflammatory cytokines is illustrated by the induction of the anti-inflammatory cytokine IL-10 by TNFα. Abbreviations: PKC=Protein kinase C, MAPK=represents the mitogen activated protein kinase cascade, PI3K-Akt=phosphatidylinositol 3-kinase-Akt cell survival signaling cascade, ROS=reactive oxygen species, IL=interleukin, JAK=Janus activated kinase, STAT=signal transduction and activation of transcription factors.

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