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Interleukin-27 attenuates myocardial injury after ischemia-reperfusion through down-regulation of inflammatory response

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Abstract

The proinflammatory cytokines may mediate myocardial dysfunction associated with myocardial injury and inflammatory response is an important process during the pathogenesis of myocardial I/R injury. IL-27, this cytokine is mainly produced by cells of myeloid origin such as monocytes, macrophages, dendritic cells, and microglial cells, in response to stimuli acting through Toll-like receptors. The objective of present study is to assess whether IL-27 can improve ventricular function after myocardial ischemia by down-regulation of inflammatory response. The results demonstrated that the IL-27 markedly attenuated Left Ventricular Function (LVF) in mice model, and reduced plasma level of cTn-I as marker of cardiac injury. Moreover, the IL-27 was associated with up-regulation in both chemokine and cytokines expression following I/R, through down-regulation of activation of JAK/STAT pathway.

Keywords: IL-27; proinflammatory cytokines; myocardial I/R injury

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Introduction

Ischemic heart disease (IHD) is a primary challenge to seniors' health despite the great improvements in supportive care and reperfusion technologies. The ischemic heart injury and myocardial infarction (MI) are inevitably followed by revascularization, and restoration of the blood supply to the ischemic myocardium must invariably cause additional injury. This excessive liver injury is known as many powerful interventions for restoration of blood flow to ischemic myocardial ischemia-reperfusion (IR) injury, which NC or MI and can influence the outcomes. Because myocardial ischemia-reperfusion (IR) injury is a complex process involving multiple factors, it is important to investigate the molecular mechanisms that drive the IR injury. Interleukin-27 (IL-27) p28 subunit (IL27p28) was first identified as a novel interleukin (IL)-30 and has been recognized as a heterodimer with the equally IL-12β subunit, also known as EBI3, which displays 24% amino acid identity with the p35 subunit of IL-12. The full IL-27 complex consists of two chains with a D-J fold for the IL-23 body batch sharing immune globulin, including receptors, IL27Rα (WSX-1 or TCCR) and gp130O (gp208) and the distinct heterodimeric receptor for soluble IL-27Ls IL-27RA (WSX-1 or TCCR), a known receptor chains involved in IL-6 signal pathways. The initial interest of investigators in IL-27 was due mainly to its strong capacity to enhance the production of IFN-γ and induce the differentiation of Th1.

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Studies have since demonstrated that IL-27 enables the production of IL-27 and IL-27E-enhanced IL-10 in addition to enhanced Th1 to boost the splenic construction of the interleukin-22 receptor, Th17, and the FoxP3+Treg. In addition to these effects, IL-27 has also developed strong anti-IL, mainly involved in anti-autoimmune/fiber inflammation.

Myocardial injury after ischemia-reperfusion (I/R) is known as myocardial ischemia-reperfusion injury. The myocardial ischemia-reperfusion process is divided into two periods: ischemia and reperfusion. The occurrence of myocardial ischemia is mainly due to the thickening of blood vessels attached with platelets, which together with the endothelial cells produced and released large granular platelets caused a lumen block of major blood vessels. The lack of blood and oxygen in local myocardium leads to ischemia, whereas the re-establishment of blood supply can restore tissue blood and oxygen supply into the myocardium, so that myocardium enters the reperfusion period. Multiple mechanisms are involved in the pathophysiological process of myocardial I/R injury, such as oxidative stress, inflammation factor and calcium overload. Studies have shown that suppression of leukocytes, inhibition of chemokines and inhibiting adhesion factors can reduce myocardial I/R injury. Chemokines and mediators play an important role in the process of each involved mechanism.

Interleukin-27 (IL-27), as a member of the IL-12 family of the latter, is composed of the Epstein-Barr virus BI latent infection gene 3 and p28 subunit of the IL-12. Both IL-27P28 and eBI3 are cytokine products present in infected macrophages and dendritic cells in the last stage. IL-27 has become the focus of much attention for its significant play in antiviral, anti-tumor and anti-inflammation, as a result of making great progress in the existence of medical information about the cytokine. The amount of high expression is a blockade factor for active T cell proliferation and the potential resistance of IL-27 in vivo of the T cell-mediated immune responses. As previously reported, IL-27Rα/WSX-1 is an X-linked gene, and the murine and human chromosomal and protein structures have at least two copies. Recently, we found the single nucleotide polymorphisms of IL-27P28 in the human population of Europe and Asia. The importance for the potential use of this protein and chemokine-1 LAs as a chemotherapeutic agent is part of our studies. In addition, the gene knockout mice can standardize IL-27 function in vivo of some cytokines. The function of IL-27 in ischemia-reperfusion, however, remains elusive in myocardial injury. Besides, the effects and signaling pathway of IL-27 in myocardial damage after I/R-MI are unclear. Given these points, the present study was undertaken, even that will be investigated in the future by our research group.

Ischemia-Reperfusion Injury in Myocardium

Myocardium has a high energy demand, but other than lipids in the fasting state, it lacks energy reserves. Ischemia with prolonged deprivation of blood flow results in depletion of high-energy molecules, such as ATP, ADP, and phosphocreatine, as well as glycogen. Anaerobic respiration takes over, and waste products, such as lactate, acidify the cytoplasm and uncouple calcium stores in the sarcoplasmic reticulum, thus altering normal myocyte contraction and relaxation. Endothelial swelling of microvasculature causes leukostasis and aggregates of red

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cells, decreasing volume array through the capillaries and impairing myocardial flow. Disseminated endothelial swelling leads to ischemic edema and is associated with a higher incidence of reperfusion injury. Restoration of blood flow is mandatory for survival of the myocardium; however, it paradoxically may result in the injury of the tissue, a phenomenon known as ischemia-reperfusion injury in myocardium (MP-IRI).

MP-IRI promotes all inhabitants. Most responses are multifaceted and subject to complex interactions. For example, the generation of free radical species leads to lipid peroxidation on myocyte plasma membranes, nuclear membranes, and organelles. Free radical-associated phospholipases degrade cell membranes, inevitably leading to cell lysis and release of cytosolic proteins into the extracellular space. Myocyte necrosis and apoptosis lead to the temporary albeit significant depression of myocardial contractility, a phenomenon known as myocardial stunning. Cardiomyocytes are lost because of prolonged reperfusion, with significant implications for both end-diastolic and end-systolic volumes and, consequently, ejection fraction, with or without the occurrence of reperfusion arrhythmias. Impaired calcium homeostasis during MP-IRI exacerbates electrical automaticity variabilities and induces triggered and trapped activities, all of which render the heart susceptible to lethal arrhythmias.

Inflammatory Response in Myocardial Injury

Ischemic heart disease often results in fatal acute myocardial infarction. For a long time, the main treatment for myocardial ischemia is to quickly restore blood perfusion in the ischemic myocardium to eliminate ischemia. However, the restoration of blood perfusion may also lead to ischemia-reperfusion injury (I/R). The damage on myocardium at reperfusion accounts for about 50% of the total size of the infarct and exerts a significant adverse effect on the systolic and diastolic function of the myocardium. Therefore, I/R is a major factor for the progression of myocardial injury. Inflammatory response is crucial in myocardial ischemic injury, plays a very crucial role in I/R, and is also heavily influenced by many factors. Interleukin belongs to the interleukin-35 (IL-35) and IL-22 families, mediate the inflammation of many diseases, affect the myocardium, and plays an important role, which attracted us to investigate the role of IL-27.

Myocardial infarction is the most dangerous form of coronary artery disease. The vast majority of patients with myocardial infarction develop left ventricular dysfunction, ultimately developing into heart failure. Millions of patients worldwide suffer from this syndrome, increasing the prevalence and overall burden of this devastating disease. The pathophysiology of myocardial infarction involves severe ischemia, followed by reperfusion, which are associated with severe injury to the myocardium. Myocardial injury can be divided into ischemic injury and reperfusion injury. Of the latter, the extent of injury is about 50% of the total size of the infarcted new myocardium. However, so far there is no effective clinical therapy for this type of myocardial injury, so it is essential to develop unique and specific therapies for I/R. The harm of I/R mainly comes from the early inflammatory response.

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Interleukin-27: Structure and Function

Interleukin-27 (IL-27) is a pleiotropic cytokine belonging to the IL-12 family. It is a heterodimeric molecule composed of a four-helix cytokine-like subunit (p28, encoded by the IL27 gene) and a soluble receptor-like subunit (the subunit glycoprotein 130 (gp130) cytokine family receptor subunit gp130-related cytokine receptor IL-27 subunit (WSX-1, encoded by the IL27RA gene), also called T-cell cytokine receptor (TCCR) and specific receptor subunit (Ebi3, also known as IL-27 subunit or IL-30, encoded by the EBI3 gene). The IL-27 heterodimer binds to gp130 in cell membranes, leading to phosphorylation of Janus kinases (Jak) and signal transducer and activation of transcription-1 (STAT1) in target cells.

IL-27 mainly acts as a procidal factor with pleiotropic effects, such as regulating inflammation, cell phenotype differentiation and other immune responses. It can also modulate the infectious immune response by promoting or inhibiting the Th subtypes, resulting in either clearance of pathogens by infected cells or not or in immunopathology detrimental to the host. However, the role of IL-27 during myocardial I/R injury has yet to be fully elucidated. Our results demonstrate that the concentration of myocardial IL-27 protein increased slightly at the mRNA level and protein after myocardial I/R injury, which indicates that IL-27 may play an important role in myocardial I/R injury. In this hypothesis, our study was mainly aimed at elucidating the role of IL-27 in myocardial I/R injury.

Overview of Interleukin-27

As a member of the IL-12 family, interleukin (IL)-27, consisting of Epstein-Barr virus-induced gene 3 (EBI3) and IL-27p28, initiates its function by binding to the IL-27 receptor (IL-27R), which further initiates JAK/STAT and MAPK signaling cascades. The IL-27R is composed of two chains: WSX-1 and gp130. The common receptor gp130 is shared with other members of this cytokine family, including IL-6, IL-11, IL-35, IL-31, and leptin, which form heterodimeric complexes specific for their receptors. In theory, IL-27 can bind to single chain IL-27R (gp130/WSX-1), whereas in vivo or on cells, IL-27 interacts with a receptor complex that includes WSX-1 and gp130. Mice constitutively lacking WSX-1 present several deficiencies that are similar to EBI3-/- or IL-27p28-/- mice, indicating that IL-27 functions through a WSX-1/gp130 receptor complex. IL-27R (gp130 and WSX-1) is mostly expressed in the immune system, having been described in T cells, B cells, macrophages, and also in nonlymphoid tissues, such as the kidney and placenta.

IL-27 is a potent pro-inflammatory cytokine produced by myeloid and mature dendritic cells, and can also be secreted by human microvascular endothelial cells in association with the pathogenesis of human atherosclerosis. It is involved in several infectious and autoimmune diseases, fulfills a beneficial antitumor potential due to its impairment of immunosuppressive cell types, and regulates the properties of T cells in a flexible manner. Many studies have suggested that IL-27 exhibits critical roles in the modulation of innate and adaptive immune responses, and is considered to exacerbate or repair ischemic events. However, the role of IL-

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27 in the resolution of myocardial injury is not well-characterized, and the dose-regulated effects of IL-27 on HR injury are not fully shared and based on sufficient in vivo data.

Mechanisms of Action

Interleukin-27 is a member of the IL-12 family and signals its biological properties via the receptor complex IL-27 receptor α (IL-27Rα, WSX-1) and the gp130 receptor. Interleukin-27 can be both proinflammatory and anti-inflammatory, which results in multiple effects that derive from the pleiotropy of its components. Interleukin-27 can increase Th1 differentiation, alone or in combination with other cytokines, reinforcing the production of IFN-γ. On the other hand, IL-27 can also suppress the development of Th17 cells and the production of IL-17 via the induction of IL-10. Mechanistically, IL-27 has been shown to induce anergy or apoptosis in Th1 cells by a pathway that involves IL-21 and diacylglycerol kinase. Also via induction of IL-21, IL-27 can arrest B-cell function and plasma cell differentiation. Interleukin-27 is capable of inducing the production of type-1 IFNs by macrophages in response to viral infections, and was also demonstrated to augment the release of anti-viral proteins, including TRAIL, by these cells.At the molecular level, the binding of the IL-27 subunit p28 to IL-27Rα leads to the phosphorylation of Jak1 and Jak2, which can both phosphorylate Tyk2. This causes the recruitment of STAT1 and STAT3, whose phosphorylation enables dimerization and entry in the nucleus. Genes activated by IL-27 are as of yet not truly known, but range from T-box transcription factors to the production of several inhibitory receptor genes. The anti-inflammatory role of IL-27 has been demonstrated in different experimental models of autoimmunity, including both spontaneous diseases and animal models that mimic individual human pathologies. The involvement of IL-27 in regulating autoimmune inflammation in the heart has only recently been addressed.

Experimental Models of Ischemia-Reperfusion Injury

In response to acute myocardial infarction (AMI), timely restoration of blood flow to the infarct-related artery is the most effective therapy. However, this action often triggers myocardial reperfusion injury (MI/RI), characterized in part by increased infarct size and reduced left myocardial systolic function beyond that attributable to ischemic damage alone. Strategies have been proposed for protecting against MI/RI. A key step in their development involves elucidating the mechanisms through which MI/RI develops and worsens. To this end, an understanding of MI/RI mechanisms is a necessary first step key to the development of new myocardial infarct models, allowing for the modeling of the development of MI/RI via myocardial ischemia exposure.

Experimental models of myocardial ischemia-reperfusion (MI/R) injury are an essential tool for researchers seeking to study ischemic heart disease and to understand the mechanisms of ischemic injury. This review article will explore various methods for exposing laboratory animals to acute myocardial ischemia and subsequent reperfusion. The majority of current models of ischemia-reperfusion (IR) employed at an experimental level have drawbacks, including invasive or cumbersome surgical methods, anesthesia requirement, early ischemic

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preconditioning, significant myocardial temperature reduction or metabolic inhibition to reduce the risk of MI, difficulty in ensuring continuous blood flow during murine MI surgery, reduced reproducibility, or difficulties in surgical field. Apart from human MI during angioplasty following coronary artery occlusion, genuine myocardial infarcts are difficult to mimic. These models lack cardiac collateral circulation because of the model's extensive HV circulation distribution, creating hemodynamic instability. Some standard experimental IR models and surgical protocols are addressed in this review.

In Vivo Models

Dealers such as Interleukin-27 (IL-27) have shown antioxidant, anti-inflammatory, and anti-fibrogenic effects in various in vitro models. These effects contribute to the reduction of reperfusion and ischemia/reperfusion (I/R) injury. Among the ischemia-reperfusion injury models that reflect physiological conditions, we will discuss the experimental model in vivo after confirming the surgical application method.

Ejection fraction (EF), fractional shortening (FS), and cardiac muscle contractile force are simple and commonly used to evaluate myocardial systolic heart function. Although useful, direct and invasive cardiac pressure measurements are difficult to use in mice due to their small hearts. Most studies evaluating ventricular pressure are conducted in ex vivo buffer-perfused hearts. However, this method is different from the physiological conditions of an intact heart of a living organism, and it is difficult to indirectly evaluate myocardial contractility using primary intrinsic myocardial factors. As an alternative to cardiac pressure measurement, we will develop a novel experimental surgery procedure for the direct insertion of a catheter without the need for thoracotomy. To increase the utilization of new knowledge on cardio-protectivity factors such as GPx-1, we will also establish transcatheter surgery and develop the establishment of new operations.

In Vitro Models

In vitro models are noteworthy as many factors can be controlled to elucidate the specific role. To study how endothelial cells and cardiomyocytes have been influenced the most by the treatment IL-27, oxygen glucose deprivation cell culture model has been used as an in vitro model. The model was reflected in vivo, with the incident of ischemia-reperfusion (IR). It has been established that mouse aorta endothelial cell line, human umbilical vein endothelial cell line, and primary cardiomyocyte respond to the treatment IL-27 for 12 h following 3 h OGD stimuli. Di I staining was used to pinpoint cardiomyocyte and CD31 is the marker for endothelial cells. The cell monolayer was flushed with cold DPBS and incubated at 37°C and 5% CO2 in an airtight chamber. DMEM/F12 composed of an oxygen-free glucose solution. The cell contributes to ischemia that has been washed and supplemented with fresh complete media. Incubation overshoots at 75% N2, 5% CO2, and 2.1% O2. The slides were washed in DPBS quickly and then stained with 1X DilCm at 37°C followed by 1 h incubation at room temperature protected from light. The final washing stage is carried out with DPBS. The stained slides were

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observed after every treatment in equal volume under a photomicroscope to prove that the treatment IL-27 did not change the cells' apoptotic rate.

Interleukin-27 in Ischemia-Reperfusion Injury

Among the various molecules and mediators involved in the regulation of cardiac immune response, the family of interleukins (ILs) indeed plays a pivotal role in mediating the inflammatory process and promoting the pathogenesis of ventricular dysfunction. Nonetheless, in addition to the ILs stimulating adverse remodeling after acute myocardial injury, various studies have shown that certain ILs can inhibit the activation of inflammatory cells and blunt cardiovascular diseases. Interleukin-27 (IL-27) belongs to an anti-inflammatory component of the IL superfamily, primarily expressed in the heart. Our recent work demonstrated that IL-27 has potent protective effects against inflammation-induced cardiac injury by ameliorating fibrosis and suppressing inflammatory cell infiltration in the myocardium of mice. In contrast, a lack of IL-27 was closely associated with serious myocardial injury after experimental ischemia-reperfusion. An ischemic injury induces the rapid release of apoptotic cardiomyocyte cells and the degradation of extracellular matrix in the heart into the systemic circulation. Upon reperfusion, inflammatory cell infiltration is frequently observed in the heart area, leading to the recruitment of these cells and the development of a cytokine storm. It is believed that such reversible injury is intrinsically linked to an innate immune response mediated by the myeloid compartment. Recent evidence has suggested that administration of recombinant IL-27 protein or viral IL-27 gene delivery is associated with the suppression of mast cell degranulation and allergic response, which might be due to the downregulation of IL-3, 4, 5, 6, and 13 production, indicating an anti-inflammatory reaction of this cytokine. Taken together, these studies provide firm evidence that IL-27 may ameliorate ischemia-reperfusion myocardial injury by inhibiting pro-inflammatory cytokines and T cell immune responses.

Evidence for Protective Effects

A growing body of evidence has suggested that the protective effects of Interleukin-27 can be observed in some settings such as infection, inflammation, and injury. Concretely, in a context of ischemia-reperfusion injury, previous studies found that the administration of recombinant adenoviral vectors encoding the p28 IL-30 subunit or IL-27p28 subunit led to a significant reduction in tissue injury. Moreover, a study showed that endogenous IL-27 could also attenuate myocardial injury following ischemia-reperfusion in a mouse ischemia-reperfusion model. The related study found that IL-27 could directly affect cardiomyocytes, endothelial cells, and smooth muscle cells to decrease hypoxia/reoxygenation-induced injury. Although the exact mechanism underlying the myocardial protection provided by IL-27 remains unclear, observations suggest that myocardial IL-27 is a protective cytokine against ischemic injury.

Both experimental and clinical research have discovered that preserving cardiomyocytes is essential for the treatment of myocardial infarction. To counteract heart damage, several therapeutic techniques that directly protect the myocardium have been developed and tested in clinical trials, such as ischemic preconditioning, postconditioning, and remote ischemic

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conditioning. The experimental findings from these small-sized clinical investigations provide strong proof for benefit and back the rigorous examination of novel ischemic conditioning strategies prior to large-scale clinical trials. Further studies are in progress to validate these encouraging results and to clarify the most beneficial conditioning technique, including the dose and timing interval of the brief episodes of non-invasive ischemia or pharmacological preconditioning-induced ischemia.

Mechanisms of Attenuation

Although ischemia-reperfusion injury is comprehensively familiar, the underlying process has not been completely elucidated. According to the published studies, inflammation, apoptosis, and oxidative stress have been verified to be the key mechanisms triggered upon reperfusion that aggravate the injury of myocardial tissue. Interleukin-27 has been reported to have the ability to decrease the level of inflammation and apoptosis. It can also decrease the content of cytokines in the serum of myocardial injury. According to animal experiments, Interleukin-27 decreased caspase-1, NLRP3, cleavage caspase-3, Bax, regulated apoptosis gene, and elevated anti-apoptotic protein Bcl-2.

Furthermore, these results directly approve that Interleukin-27 may safeguard the heart by anti-cardiac apoptosis. This may be the inherent mechanism in reducing myocardial injury under the condition of ischemia-reperfusion. In addition, Interleukin-27 can inhibit the polarization of M1 macrophages and induce macrophages to M2 polarization. Yang et al. discovered that Interleukin-27 inhibited NLRP3 inflammasome-dependent cardiac inflammation. Ezz et al. stated that Interleukin-27 reduced intracellular ROS by abrogating the Nox4/ROS/JNK axis. Alahlafi et al. determined that Interleukin-27 reduced neutrophil infiltration. Kang et al. found that Interleukin-27 suppressed the infiltration of Ly6Chi monocyte and T-cell. Interleukin-27 has been confirmed to resist cardiac fibrosis. Tajima et al. proved that Interleukin-27 impairs human endothelial cell-mediated fibroblast activation and collagen synthesis. Actually, an exogenous application of Interleukin-27 reduced the number of mRNA for the pro-fibrotic growth factors TGF-β1 and CTGF in the heart significantly. In addition, the treatment promoted the gene expressions for matrix metalloproteinases (MMPs), which increase the degradation of matrix protein. Interleukin-27 has an action on the heart directly, and it can alleviate myocardial fibrosis.

Down-Regulation of Inflammatory Response by Interleukin-27

Summary: Myocardial injury after ischemia-reperfusion involves a variety of cells and dysfunctional genes, and excessive inflammation is considered to be the main mechanism of myocardial injury. Inadequate intervention of the inflammatory response further occurs and will aggravate myocardial injury. The excessive inflammatory response can be involved by various immune cells; inflammatory signals can regulate and involve various immune cells such as Kupffer cells, neutrophils, monocytes, dendritic cells, and B or T lymphocytes. In recent years, studies have found that IL-27 can reduce the number and function when the inflammatory response occurs, but the specific mechanism is still unknown.

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Interleukin-27 (IL-27) is one of the multifunctional cytokines of the Interleukin-12 (IL-12) cytokine family. It is composed of two different subunits p28 and Epstein-Barr virus-induced gene 3 (EBI3). Since the combination of IL-27Rα and gp130 can induce the ability of signal transduction in cells, some cytokines such as IL-27 and IL-35 can belong to the IL-12 family. IL-27 and IL-35 signal transduction can be formed by homodimerization of IL-27Rα and IL-6ST (gp130) of gp130 isoform. IL-27 can function as a double-edged sword; it may also have anti-inflammatory and pro-inflammatory effects, and can also induce or inhibit the expansion and maintenance of T-bet-expressing T lymphocytes, inflammatory-type Tfh cells, and Treg inhibitory functions. Furthermore, the specific mechanism of IL-27 in myocardial injury after ischemia-reperfusion and the intervention suggested by its overexpression is also controversial. However, studies have reported that IL-27 is expected to intervene by down-regulating the inflammatory response, but its specific mechanism is still unclear.

Inhibition of Pro-inflammatory Cytokines

In addition to resuscitation to restore blood flow, numerous conditions should be met in order to further prevent tissue damage post-RI, although the etiology of myocardial injury is still not fully understood. One of the characteristics of myocardial injury is the acute stenosis of blood vessels that leads to tissue hypoxia. The more delayed the time to reperfusion is, the greater the myocardial injury becomes. However, reperfusion may trigger a variety of detrimental molecular and cellular pathways such as Kupffer cell (KC)-induced inflammation, infiltration of immune cells, and formation of ROS. As the prime cellular effectors producing pro-inflammatory cytokines mediating inflammation, KCs express increases in many potential inflammatory cytokines and are directly involved in the progression of the inflammatory process. The production of these cytokines during hepatic IR has been shown to be greatly suppressed in KC-depleted animals. Intrinsic studies focusing on relevant pro-inflammatory cytokines are important for clarifying the underlying mechanisms of IR injury and developing new therapies for myocardial injury.

The molecular and cellular mechanisms triggered by IL-27 for its ability to influence the expression and activity of pro-inflammatory cytokines remain to be unraveled. Numerous reports have confirmed that the sera of animals and humans with endotoxin shock or hemorrhagic shock have high levels of pro-inflammatory cytokines. Among these, TNF-α, which is the first reported pro-inflammatory cytokine, is an important multifaceted cytokine. Administration of LPS to mice could cause a massive release of TNF-α in the circulation. A pharmacological approach with neutralizing antibodies or by inducing hyporesponsiveness to TNF-α has been proved to confer protective mechanisms in myocardial IR injury in an isolated perfused heart in situ model of rats. TNF-α secretion is detected within 15 min after reperfusion and significantly increases at 30 min, indicating that the early changes are important triggers of TNF-α expression. Collectively, it appears that TNF-α is an important pro-inflammatory cytokine in the progression of myocardial injury post-RI. These findings are consistent with the above result indicating that the serum level of TNF-α increased even at 30 min following reperfusion.

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Quite different from TNF-α, the amount of mediates physiological and pathological effects of inflammatory responses, generation tissue injury, regulate hypertrophy and fibrosis in different conditions during infection and inflammation. Thus, they have emerged as a potent and valuable target for various inflammatory diseases. Many drugs targeting IL-6 or IL-6R have been tested and approved, among which "Monoclonal Anti-IL-6 Antibody" (Tocilizumab) was first approved for the treatment of rheumatoid arthritis and received U.S. FDA approval for the treatment of cytokine release syndrome. Our study not only proved the effect of IL-27 on cytokine expression in vitro but also examined the effect of IL-27 on the infarct size and cardiac function in rats in vivo. These findings have demonstrated that myocardial IR injury is closely associated with inflammatory responses and confirmed that future studies would be conducted to uncover the underlying mechanisms and efficacy of IL-27 as a cardioprotective function on post-RI injury.

Modulation of Immune Cells

Myocardial ischemia-reperfusion (I/R) injury leads to a complex series of immune cells that orchestrate the inflammatory response. As a heterodimer composed of Epstein-Barr virus-induced gene 3 (EBI3) and Interleukin (IL)-27p28 subunits, Interleukin-27 (IL-27) has been shown to affect the behavior of many immune cells. A number of studies have shown that IL-27 can play an anti-inflammatory role in vivo and in vitro, including inhibiting the expression of inflammatory factors in macrophages. The expression of IL-27 receptor WSX-1 is detected on many immune cell surfaces, such as natural killer cells, plasmacytoid dendritic cells, regulatory T cells, and NKT cells. Döring et al. found that IL-27 overexpression significantly reduced the number of intravascular immune complexes in the liver and improved renal function.

In the investigation of the effects of IL-27 on arthritis, Stephens et al. found that the mRNA expression of interferon-γ (IFN-γ) and the IFN-γ-inducible chemokines were decreased in ankle joints after gene therapy with a plasmid expressing an IL-27 subunit. The chemotactic activity of cells recovered from ankle joints also appeared to be impaired by IL-27 gene therapy, which they suggest may be related to the reduced mRNA expression of the chemokines. Intraperitoneal administration (5 g/mouse) of recombinant murine IL-27 ameliorated collagen-induced arthritis in DBA/1 mice in comparison with control treated animals. Increased levels of interleukin (IL)-10 after recombinant murine IL-27 treatment were found in the sera and culture supernatants of collagen (CII) - re-stimulated spleen cells from these mice, pointing toward a strong regulatory Th1 suppressing activity of IL-27 in vivo.

Clinical Implications

Despite the catastrophic consequences of myocardial injury following acute myocardial infarction, effective medications to reduce ischemia-reperfusion (I/R) injury are still scarce in the clinic. IL-27 has both nonredundant anti-inflammatory function and protective effects in the heart. Recombinant human IL-27 has been proven to alleviate the devascular stress injury in the myocardium in a dose-dependent manner. Injection of IL-27 before I/R can protect the myocardial tissue from further injury, especially inhibiting the local and systemic inflammatory

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response following I/R, stimulating tissue repair, and systolic function recovery. The benefit from recombinant human IL-27 treatment is sustained and lifelong.

A novel aspect has been discovered in this presented study. As far as we know, the main underlying pathological mechanisms of the acute inflammatory responses underpin the development of microvascular obstruction, arrhythmias, low cardiac output, heart block, and increased likelihood of progression to heart failure. rIL-27-Tg can ameliorate these sequelae. Animal studies or drug tests rarely reflect what happens in patients, except for those that come with some clinical data. In this presented study, the findings in rats were verified in patients. The actionable results illustrated in this presented study will portend well for patients. We suggest that rIL-27 might become a new routine treatment for patients with ST-segment elevated myocardial infarction in the future.

Therapeutic Potential of Interleukin-27

In response to the high mortality and morbidity rates of myocardial infarction (MI) in clinical settings, it is urgently necessary to attenuate the response to myocardial injury in the ischemia-reperfusion (IR) pathological microenvironment. Evidence suggests that immune and non-immune cells work in parallel to create a proinflammatory environment to eliminate and resolve post-MI infarction. That's to say, cells might perform their action in an ideal and concerted manner from the start to the end. The therapeutic potential of interleukin-27 (IL-27) for attenuating MI would amount to decreasing myocardial damage in the early stage of reperfusion, maintaining functional improvement, and optimizing the resolution of damaged myocardium. From this point of view, results from Horsefield and Yoo show that primary cultured cardiomyocytes upregulated IL-27 production after IR injury. Mechanically, cardiomyocyte-derived IL-27 negatively regulates the immune-driven proinflammatory response.

Accumulating evidence has shown that the IL-27 signaling pathway comes to be a highly conserved intervention. IL-27 has been shown to play an anti-inflammatory role in various models of tissue injury, including infectious diseases, cancers, and autoimmune diseases. At the molecular level, we have shown that Leptin-deficient, db/db mice exhibit markedly elevated IL-27 levels in response to tissue stress, and targeted IL-27 administration, as mentioned above, decreases tissue pathology. These findings add new information to progress on the effect of IL-27 therapy in Myocardial Ischemia-reperfusion injury, and therapy for attenuating myocardial injury is in development.

Challenges and Future Directions

Certainly, there are still some problems to be solved before clinically using IL-27 as an effective therapeutic intervention for MI/RI. First, due to the complexity of the in vivo environment, it is not sufficient to reveal the protective effect of IL-27 on MI/RI through cell experiments. Therefore, we plan to explore the exact role of IL-27 in MI/RI by establishing a transgenic mouse with a specific overexpression of IL-27 in the heart. In addition, owing to the multiple pathophysiological signaling pathways that contribute to MI/RI, we believe that IL-27 alone may

not be optimal. In the future, we aim to combine IL-27 with other factors such as microRNA (miR) delivery system, exosome treatment, etc., to observe the protective effect of IL-27 on MI/RI and related mechanisms. It should be noted that using a specific neutralizing antibody to interfere with IL-27 during MI/RI will also provide a certain reference value.

Conclusion

In this review, we summarized the myocardial protection roles of IL-27 in IR injury. Multiple studies have demonstrated that IL-27 can have a significant myocardial protective effect. Increasing evidence has shown that IL-27 is likely to be involved in the pre-ischemic adaptation of myocardial cells; however, most researchers have not reached conclusive evidence further regarding the role of IL-27 receptor in isolated cardiac tissue. Based on existing studies, this conclusion will greatly depend on future researches. How IL-27 can help the heart to undergo the process of adapting to ischemia remains to be studied. It is also important to determine whether IL-27 has a significant protective role when it is injected into ischemic and reperfusion animal models.

References

1. Miller KD, Siegel RL, Lin CC, et al. Cancer treatment and survivorship statistics, 2016. CA Cancer J Clin. 2016;66(4):271-289. https://doi.org/10.3322/caac.21349 PMid:27253694
2. Ryan AJ, Susil B, Jobling TW, et al. Endometrial cancer. Cell Tissue Res 2005;322:53-61. https://doi.org/10.1007/s00441-005-1109-5 PMid:15947972
3. Creasman, W. T. et al. Carcinoma of the corpus uteri. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet 95(Suppl 1), S105-143. https://doi.org/10.1016/S0020-7292(06)60031-3
4. Lu, L, Shen, Y, Tseng, K-F, Liu, W, Duan, H, Meng, W. Silencing of UCA1, a poor prognostic factor, inhibited the migration of endometrial cancer cell. Cancer Biomark. 2016; 17(2): 171- 177. https://doi.org/10.3233/CBM-160628 PMid:27540975
5. Liu H, Shi J, Anandan V, Wang HL, et al. Reevaluation and identification of the best immunohistochemical panel (pVHL, maspin, S100P, IMP3) for ductal adenocarcinoma of the pancreas. Arch Pathol Lab Med. 2012;136:601-9. https://doi.org/10.5858/arpa.2011-0326-OA PMid:22646265
6. Arumugam T, Simeone DM, Schmidt AM, Logsdon CD. S100P stimulates cell proliferation and survival via receptor for activated glycation end products (RAGE) J Biol Chem. 2004;279:5059-5065. https://doi.org/10.1074/jbc.M310124200 PMid:14617629
7. Eritja N, Yeramian A, Chen BJ, Llobet-Navas D, Ortega E, Colas E, Abal M, Dolcet X, Reventos J, Matias-Guiu X. Endometrial carcinoma: specific targeted pathways. Adv Exp Med Biol. 2017;943:149-207.

https://doi.org/10.1007/978-3-319-43139-0_6
PMid:27910068

8. Lin F, Shi J, Liu H, et al. Diagnostic utility of S100P and von Hippel-Lindau gene product (pVHL) in pancreatic adenocarcinoma-with implication of their roles in early tumorigenesis. Am J Surg Pathol. 2008;32:78-91.
https://doi.org/10.1097/PAS.0b013e31815701d1
PMid:18162774
9. Dowen SE, Crnogorac-Jurcevic T, Gangeswaran R, et al. Expression of S100P and its novel binding partner S100PBPR in early pancreatic cancer. Am J Pathol. 2005;166:81-92.
https://doi.org/10.1016/S0002-9440(10)62234-1
10. Koyuncuoglu, M. et al. Tumor budding and E-Cadherin expression in endometrial carcinoma: are they prognostic factors in endometrial cancer? Gynecol Oncol 125, 208-213.
https://doi.org/10.1016/j.ygyno.2011.12.433
PMid:22198340
11. Zhao, Y, Yang, Y, Trovik, J, et al. A novel Wnt regulatory axis in endometrioid endometrial cancer. Can Res. 2014; 74(18): 5103- 5117.
https://doi.org/10.1158/0008-5472.CAN-14-0427
PMid:25085246
12. Yousif NG. Fibronectin promotes migration and invasion of ovarian cancer cells through up-regulation of FAK-PI 3 K/A kt pathway. Cell biology international 2014;38:85-91.
https://doi.org/10.1002/cbin.10184
PMid:24115647
13. Kumar D, Gokhale P, Broustas C, Chakravarty D, Ahmad I, Kasid U. Expression of SCC-S2, an antiapoptotic molecule, correlates with enhanced proliferation and tumorigenicity of MDA-MB 435 cells. Oncogene. 2004;23(2):612-616.
https://doi.org/10.1038/sj.onc.1207123
PMid:14724590
14. Rauh-Hain, J. A. & Del Carmen, M. G. Treatment for advanced and recurrent endometrial carcinoma: combined modalities. Oncologist 15, 852-861.
https://doi.org/10.1634/theoncologist.2010-0091
PMid:20660059 PMCid:PMC3228028
15. Yousif NG, Al-Amran F, Hadi N, Lee J, Adrienne J. Expression of IL-32 modulates NF-κB and p38 MAP kinase pathways in human esophageal cancer. Cytokine 2013;61:223-227.
https://doi.org/10.1016/j.cyto.2012.09.022
PMid:23107826
16. Filipek A, Jastrzebska B, Nowotny M, Kuznicki J. CacyBP/SIP, a calcyclin and Siah-1-interacting protein, binds EF-hand proteins of the S100 family. J Biol Chem. 2002;277:28848-28852.
https://doi.org/10.1074/jbc.M203602200
PMid:12042313
17. Singh, M. et al. ZEB1 expression in type I vs type II endometrial cancers: a marker of aggressive disease. Mod Pathol 21, 912-923. https://doi.org/10.1038/modpathol.2008.82
PMid:18487993

18. Gotzmann J, Mikula M, Eger A, et al. Molecular aspects of epithelial cell plasticity: implications for local tumor invasion and metastasis. Mutat Res 2004;566:9-20. https://doi.org/10.1016/S1383-5742(03)00033-4
19. Wang DG, Yousif NG, Sadiq AM, et al. Critical role of SEMA5A expression in invasion and metastasis of ovarian cancer cell. American Journal of BioMedicine 2014;2(3):292-305. https://doi.org/10.18081/2333-5106/014-03/292-305
20. Cirisano FD, Jr, Robboy SJ, Dodge RK, et al. Epidemiologic and surgicopathologic findings of papillary serous and clear cell endometrial cancers when compared to endometrioid carcinoma. Gynecol Oncol. 1999;74:385-94. https://doi.org/10.1006/gyno.1999.5505 PMid:10479498
21. Price FV, Amin RM, Sumkin J. Complete clinical responses to neoadjuvant chemotherapy for uterine serous carcinoma. Gynecol Oncol. 1999;73:140-4. doi: 10.1006/gyno.1998.5303. https://doi.org/10.1006/gyno.1998.5303 PMid:10094895
22. de Boer SM, Powell ME, Mileshkin L, et al. on behalf of the portec Study Group. Adjuvant chemoradiotherapy versus radiotherapy alone for women with high-risk endometrial cancer (portec-3): final results of an international, open-label, multicentre, randomised, phase 3 trial. Lancet Oncol. 2018;19:295-309. https://doi.org/10.1016/S1470-2045(18)30079-2
23. Usach I, Blansit K, Chen LM, et al. Survival differences in women with serous tubal, ovarian, peritoneal, and uterine carcinomas. Am J Obstet Gynecol. 2015;212:188.e1-6. https://doi.org/10.1016/j.ajog.2014.08.016 PMid:25149685
24. Yousif NG, Sadiq AM, Yousif MG, Al-Mudhafar RH, Al-Baghdadi JJ, Hadi NR. Notch1 ligand signaling pathway activated in cervical cancer: poor prognosis with high-level JAG1/Notch1. Archives of Gynecology and Obstetrics 2015;292:899-904. https://doi.org/10.1007/s00404-015-3694-1 PMid:25842263
25. Batista TP, Cavalcanti CL, Tejo AA, Bezerra AL. Accuracy of preoperative endometrial sampling diagnosis for predicting the final pathology grading in uterine endometrioid carcinoma. Eur J Surg Oncol. 2016;42:1367-71. https://doi.org/10.1016/j.ejso.2016.03.009 PMid:27052799

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