NECROPTOSIS AND MYOCARDIAL INFARCTION: THERAPEUTIC IMPLICATION

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ABSTRACT

Necroptosis is a form of programmed and controlled cell death that is caspase- and autophagy-independent. Atherosclerosis is a multi-factorial and inflammation-driven disease and the principal cause of myocardial infarction and stroke. The progression of atherosclerosis results from a dynamic interplay involving inflammation, autophagy, apoptosis, and necroptosis in cardiovascular cells. This article summarizes the correlation between the pathway of necroptosis, immune response, and myocardial infarction. Complications resulting from atherosclerosis were the main causal focus for myocardial infarctions. Necroptotic cells have been found in large portions in organs, which have experienced ischemic injuries. Ischemia of the brain, kidneys, and retinas, for example, has led to analyses which have concluded a bigger role of necroptosis in such ailments than previously imagined. Better understanding the specific roles and causes for necroptosis in organ damage can allow for more fine-tuned research in terms of controlling necrosome activation and all correlating consequences of this activation, such as heightened immune responses.

Keywords: Necroptosis; Atherosclerosis; Myocardial infarction.

Necroptosis Pathway

Necroptosis is a form of programmed and controlled cell death that utilizes what appears to be a medium between the workings of apoptosis, in terms of controlled cell death, and necrosis, in terms of uncontrolled cell death due to injury or disease. In contrast with apoptosis, however, necroptosis activation is caspase- and autophagy-independent. All of the stimulating substrates for the formation of the necrosome have not been fully discovered, leaving many questions about the details of different possible pathways in necroptotic activation. In general, it is known that intercellular molecules such as Fas-associated death domain (FADD), TNF-receptor– associated death

domain (TRADD), receptor-interacting protein kinase 1 (RIPK1), receptor-interacting protein homotypic interacting motif (RHIM), mixed lineage kinase domain–like (MLKL), and RIPK3 play important roles in the initial formation of the necrosome (1). The tumor necrosis factor α (TNFα) ligates to the membrane-embedded tumor necrosis factor receptor 1 (TNFR1), causing a conformational change in the receptor, which will reveal a domain in order to recruit TRADD. This begins the assembly of the complex I (Figure 1), comprised of multiple moieties including: TNFR1, TRADD, RIPK1, TNFR-associated factor 2 (TRAF2), and cellular inhibitor of apoptosis 1 and 2 (cIAP 1 and 2).

The cIAP molecule is involved in the modification of RIPK1 that is vital to maintaining the cell on a survival pathway. The cIAPs catalyze the ligation of polyubiquitin chains onto Lys377. These chains recruit and activate transforming growth factor--activated kinase 1 (TAK1)-binding proteins 2 and 3 (TAB2/3), TAK1, and the IκB kinase (IKK) complex, which consequently induces the nuclear factor κB (NF- κB) pathway. This activated pathway now works transcriptionally to promote the production of genes associated with cell survival. Once TNFR1 is internalized, however, the receptor intracellularly dissociates from complex I. This can begin the intracellular formation of complex II (Figure 2) in which FADD is recruited by TRADD via an exposed death domain (2). In this conformation, Fas-associated death domain–like interleukin-1β–converting enzyme [FLICE]–like inhibitory protein (FLIP) will form a heterodimer with caspase-8, controlling for cell death and preventing both caspase dependent apoptosis as well as anti-caspase signaling which could trigger necroptosis (1). The induction of the FLIP protein is dependent on the NF-κB pathway. The heterodimer formed between FLIP and caspase-8 has been referred to as complex II. The structural components of complex II, however, are not universally accepted yet, allowing for some differences in presentation. Research presented by Kung G., et al. also includes TRADD and TRAF2 as part of complex II (2). In the absence of FLIP but presence of caspase-8, the caspase cascade is initiated and necroptotic factors, such as RIPK1 and RIPK3, are cleaved, resulting in cellular activation of apoptosis and inhibition of necroptosis (3). When there is an absence of either FLIP or caspase-8, however, the cell is properly stimulated for necroptosis and TRADD and FADD molecules consequently recruit RIPK1. RIPK1 then goes through a series of transformations and exposes the RHIM domain, which recruits the RIPK3 molecule. RIPK1 and RIPK3 join to form a complex, which is the nascent stage of the assembly of the necrosome and the signal that necroptosis will take place (1). While MLKL was known to have a role downstream of this intracellular complex, the events leading to necroptosis from this point on used to be unknown. Research has now revealed MLKL's role in greater detail. RIPK3 phosphorylates MLKL at T357 and S358 to allow MLKL to form an oligomer, which can now be associated with phosphatidylinositol and cardiolipin. With this association, MLKL mobilizes, moving from the cytosol to the plasma membrane where it can form pores in the membrane and result in cell death (4). The exact mechanism of plasma rupture is controversial and still not universally known.

The Role of Necroptosis in the Heart

Results from recent studies suggest that necroptosis and/or autophagy-associated cell death are important in myocardial infarctions. Using a mouse model, Luedde et al. showed a role of necroptosis in the immune response of cells after a myocardial infarction in mouse hearts (5). Through the use of gene knockout strategy, they began by knocking out RIPK1 in the cells and consequently found that RIPK3-dependent necroptosis was not significantly

affected. With this observation, they focused on the consequences of RIPK3 gene knockout specifically as it has a greater effect in cardiomyocytes. RIPK3-/- cells showed differences in multiple different immune responses following infarction. Specifically, these hearts had decreased production of reactive oxygen species (ROS) and inflammatory cell invasion. Additionally, there was less hypertrophy than in the wild type hearts. The activation pathway of the necrosome in cardiomyocytes seems unique in its relative independence from the interactions of RIPK1 (2014). Necroptosis has additionally been found to play a role in the physiological remodeling of the heart following a myocardial infarction (6). As research continues, new and critical associations are being discovered between necroptosis and its role in myocardial infarctions. This suggests great potential for the creation of new target-specific drugs that may aid in preventing myocardial infarctions or in the recovery period following one.

Causes and Implications of Myocardial Infarctions

Myocardial infarction occurs when the blood flow to the heart is interrupted by an arterial blockage or damage and the oxygen supply to the heart is stopped. Complications resulting from chronic atherosclerosis, such as thrombosis, are a common cause of myocardial infarction. The plaque accumulation is not considered to be an acute concern unless it is vulnerable to rupture, which is often the case with deposits that have a necrotic core and thin fibrous cap. The rupture would lead to an acute thrombus and consequent myocardial infarction (7). Atherosclerosis was originally believed to be a disease concerning mainly the path physiological storage of low density lipoproteins (LDL) within the arterial lining of the heart. Research results released by the American Heart Association, however, stressed the role of inflammatory responses as of equal importance in atherosclerosis. The concurrent progression of lipid accumulation, immune responses, and oxidative stress in the disease gives a more inclusive view, relating the response to other ailments, such as diabetes and obesity, and also allows for the use of more biological markers that have successful rates of cardiac episode predictions. While normal functioning endothelial cells of arteries do not promote the adhesion of leukocytes, surface cells of an inflamed endothelium include molecules that have specific adhesive properties to certain leukocytes. Vascular cell adhesion molecule-1 (VCAM-1), for example, is a notable adhesion molecule important in leukocyte interaction in the early stages of the atherosclerotic lesion or atheroma. Selectins and integrins work to further the association and attachment of blood cells. The movement of these certain leukocytes, monocytes, and macrophages into the intima is mediated by cytokines and chemo-attractants within the atheroma. T-lymphocytes may also come into the intima and these immune agents will together create a local inflammatory response. Additionally, this promotes localization and replication of smooth muscle cells. Ultimately, this progression can lead to a rupturing of the endothelium, thrombosis, and myocardial infarction, resulting in cell death with mixed phenotypes of apoptosis, autophagy, necrosis, and necroptosis (8). Patients may also consequently experience a stroke as the brain loses its blood and oxygen supply rapidly following the myocardial infarction.

While apoptosis was previously believed to be the predominant death pathway in advanced atherosclerosis, recent research has revealed that programmed necrosis is the major form of cellular death in this disease and is a result of multiple biological triggers within the cell. High levels of oxidative stress, exhaustion of cellular ATP, decreased efferocytosis ability, and heightened intracellular calcium concentrations are among the identified biological conditions that place preference on necroptotic cell death over apoptosis. High levels of reactive oxygen

species (ROS) can cause irreversible damage to cellular components, triggering necrosis. A depletion of cellular NAD+ and ATP promotes necrosis since the cell is suffering from energy depletion and apoptosis has ATP dependence. Since atherosclerosis presents with arterial blockage, glycolysis is impaired due to an insufficient flow of oxygen and reduced access to glucose. Severely reduced efferocytosis ability suggests that apoptotic debris is not cleared as efficiently, which leads to secondary necrosis of the debris. In terms of calcium levels, an increased influx of calcium into the cell gives cellular preference to necrosis (Martinet et al. 2011). Additional research concluded that the necroptotic pathway is associated with the development and vulnerability of the plaque deposit to rupturing, or what is dubbed as unstable atherosclerotic plaques. Gene expression of RIP3 and MLKL, both vital proteins in necroptosis, were significantly increased in patients who suffered acute symptoms of their disease (such as transient ischemic attack or stroke). Positive phosphorylated MLKL stains, which indicate one of the final markers of the committed necroptotic pathway, were found close to the necrotic core of the plaque in arteries that have advanced atheroma with histochemical staining and analysis. No positive phosphorylated MLKL stains were detected in those classified as early lesions, suggesting that necroptosis is associated with advanced atherosclerosis and is also contributing to the vulnerability of the lesion to rupture (7). Given these cues, programmed necrosis, or necroptosis, is stimulated in advanced atherosclerosis. Early research does propose that control or modification of necroptosis and its biological triggers could impact the severity of the disease (9). Therefore, it is critically important to continue to investigate and understand the molecular basis of cell death triggered by atherosclerotic lesions and to then use the gained knowledge to effectively and specifically target the culprits of this complex disease.

However, it is important to note that myocardial infarctions can result from factors besides atherosclerotic diseases. Identifying and addressing the causation of a specific cardiac incident is necessary to provide the most effective treatment for the patient. While research has shown that necrosis does play a large role in cell death associated with atherosclerosis, it also contributes in myocardial infarctions that are caused by other cardiac, ischemic, or illness-related conditions. In the article written by Alpert et al., researchers classified the causation of myocardial infarctions into three broad categories: type 1, type 2, or nonischemic myonecrosis. A type 1 myocardial infarction is categorized as a result of some form of atherosclerotic plaque disturbance, resulting in thrombosis and, consequently, arterial blockage leading to ischemia and necrosis, as discussed previously. A type 2 myocardial infarction is categorized as a result from changes in supply and demand of oxygen to the heart, which results in secondary necrosis. This characterization typically follows an increased demand in myocardial oxygen, such as in someone experiencing atrial fibrillation or tachycardia. Lastly, Alpert et al. discuss nonischemic myonecrosis, which suggests myocardial infarction as proceeding implications of a serious illness or disease. In this case, cells are stimulated towards a necroptotic pathway as a result of heightened levels of inflammatory cytokines and catecholamine circulating in the biological system. Necrosis is a common factor throughout these three characterizations of myocardial infarctions; however, therapeutic implications are argued to be dependent on the correct diagnosing and understanding of the specific role of the pathway in each case (2014).

Treatment of Myocardial Infarctions and Therapeutic Implications

Yet with this recommendation, more research still needs to be conducted to fully understand how to target specific cases of cardiac incidents. An experiment conducted by Adamek et al. focuses on the inhibition of immune responses in order to observe any consequences in remodeling of the left ventricle following an induced myocardial infarction in mice (10). This induction of a cardiac event would most closely fall into the characterization of a type 1 myocardial infarction, as defined by Alpert et al. (11). Proinflammatory proteins have shown a correlation with myocardial depression and physiological remodeling following a myocardial infarction (12). Proinflammatory cytokines are associated with necroptosis of cardiomyocytes in addition to other responses. Based on this premise and with the understanding that non-steroidal anti-inflammatory drugs (NSAID), such as aspirin, can decrease this immune response, researchers induced myocardial infarctions in 78 mice and directly followed this with regulated administration of either a placebo or high-dose aspirin for four weeks. Researchers did not find evidence that the high-dose aspirin treatment improved remodeling and dysfunction of the left coronary artery following the induced myocardial infarction. As expected, however, the aspirin treatment did significantly reduce production of cytokines TNF and IL-1β. Considering that increased immune responses can contribute to heart failure, aspirin treatments post-myocardial infarction can still be beneficial (10). Contrary to this study, researchers have found that induced necroptosis following myocardial infarctions in elderly rats actually activated endogenous cardiac stem cells, promoted cardiomyocyte proliferation, and improved cardiac remodeling (6). As the role of necroptosis becomes clearer in myocardial infarctions, the use of modern medicines, such as aspirin, have the potential to be more personalized for specific variables concerning each patient. Additionally, current reports suggest that concurrent administration of aspirin with thrombolytics should occur in all patients who report with ST-segment elevation myocardial infarction (STEMI) and are not capable of receiving primary percutaneous coronary intervention (PCI). Aspirin increases the efficacy of the administered thrombolytic drug, aiding in the reperfusion of blood, which is necessary to minimize cardiac damage following an ischemic episode. Thrombolytic drugs are important to administrate when PCI is not an option and have also shown to be more effective with concurrent administration of anticoagulation agents or clopidogrel (8, 11).

Another implication of current studies is to inhibit or defer the cell from necroptosis through the use of necrostatins. The controlled inhibition of RIPK1 through the use of necrostatins effectively disrupts necroptosis. Currently, necrostatins have been shown to decrease the oxidative damage due to ischemic injuries of the brain (1). Necrostatins stabilize the inactive form of RIPK1 when attached to the hydrophobic kinase domain of the molecule. The interaction of RIPK1 and RIPK3 has a downstream effect on receptor molecules necessary for signal transduction. The interaction between these kinases is largely dependent on hydrophobic interactions. Since necrostatins bind to the hydrophobic region of RIPK1, its interaction with RIPK3 is compromised and the formulation of the necrosome halts (13). A study conducted on isolated rat hearts researched the implications of necrostatin 1 (NCS-1) and necrostatin 5 (NCS5) on cardio protection when administered prior to a myocardial infarction, as compared to DMSO. Researchers considered infarction size and intracardiac hemodynamic parameters when looking for cardioprotective capabilities of the necrostatins. The results showed a significantly smaller infarction zone after intraperitoneal injection of NCS-1 and NCS-5 compared to DMSO. Intracoronary NCS-1

administration resulted in heightened systolic IVP while intraperitoneal injection of NCS-1 resulted in a positive inotropic and infarction-limiting effect (14). The use of NCS-1 has specifically been shown to reduce lesion size, prevent further progression of lesions, and reduce the necrotic core area in mice with advanced atherosclerosis. Additional markers of plaque instability, such as positive phosphorylated MLKL stains, were reduced in mice that received the necrostatin (7). Administration of NCS-1 has also been shown to have cardioprotective properties in terms of improved ventricular function following reperfusion (15). Further research into the utilization of necrostatins as a means of interrupting necroptosis could reveal forms of targeting and specification of the causes, results, and secondary effects of factors leading up to myocardial infarction.

CONCLUSION

Recent research has highlighted the role of necroptosis in diseases and injuries and their correlated ramifications in multiple organ systems, such as the brain, kidneys, retinas, and heart. Necroptosis has shown to have specific roles in myocardial infarction, leading to multiple therapeutic implications concerning the manipulation of the cell's use of this death pathway. Current treatments for myocardial infarction, such as the use of aspirin as a form of immunosuppressant to reduce further cardiac damage, may already be manipulating necroptosis as studies have shown that necroptosis has implications in cardiac immune responses. Certain aspects of the pathway are still not completely clear, as this is a relatively new topic being discussed. Further research focused on understanding the detailed workings of this type of cell death can be beneficial in discovering the most effective forms of treatment for myocardial infarction. Gaining this important knowledge would increase the understanding of different treatments, as well as the specificity of different causations of the cardiovascular event. This could minimize harmful side effects and increase effectiveness, potentially saving lives of some patients. Therefore, future research concerning this relatively new concept in the context of myocardial infarction could reveal important forms of management and treatment of fatal cardiac episodes.

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Compliance with Ethical Standards

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Current Forms of Treatment	Purpose of Treatment
Aspirin	Reduce production of cytokines, should always be administered with thrombolytic drugs
Percutaneous Coronary Intervention (PCI)	Most effective form of reperfusion following STEMI
Thrombolytic Drugs	Activate plasminogen which promotes plasmin enzyme, breaks down fibrin, removes arterial blockage
Clopidogrel	Blood thinner, administered with thrombolytic drugs

Potential Form of Treatment	Purpose of Treatment
Necrostatins	Inhibit necroptosis within cardiomyocytes, stabilize atherosclerotic lesions

Table 1: A summary of current treatments for myocardial infarction, as well as possible treatments, which would manipulate cellular regulation of necroptosis.

Figure 1: This figure shows the formation of complex I following the ligation of substrate TNFα to cellular membrane embedded receptor TNF1. This conformation activates the NF- κB pathway, transcriptionally promoting cell survival.

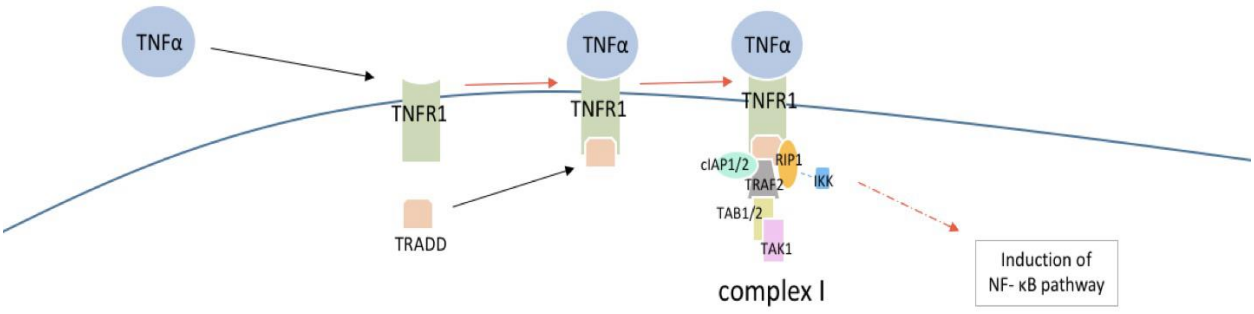
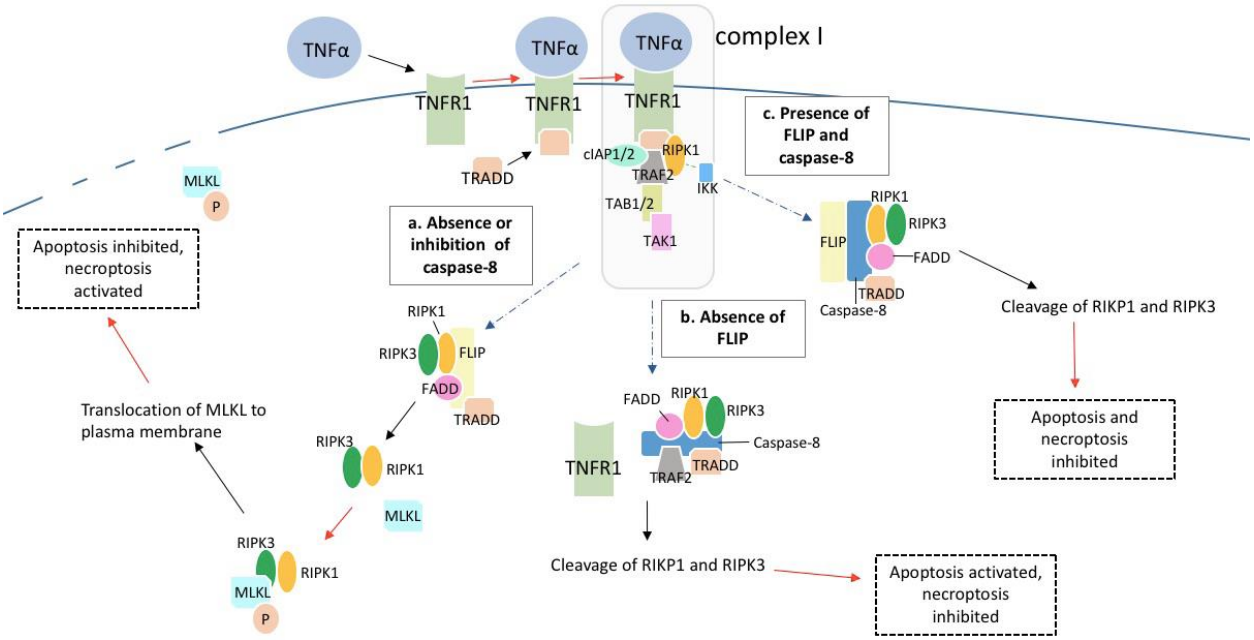


Figure 2: Complex I is internalized into the cell. Receptor TNFR1 intracellularly dissociates from the remaining moiety. (**a.)** In the absence or inhibition of caspase-8, the cell is geared towards necroptosis. The complex including RIPK1 and RIPK3 is the beginning of the formation of a necrosome. MLKL is recruited by this complex, and is then phosphorylated by RIPK3. Phosphorylated MLKL can now form an oligomer, allowing for association with phosphatidylinositol and cardiolipin. Phosphorylated MLKL can now translocate to the plasma membrane where it will rupture the membrane through an unknown process. **(b.)** In the absence of FLIP, the cell is geared towards apoptosis. This formation initiates a downstream caspase cascade. It also cleaves important necroptotic mediators, such as RIPK1 and RIPK3. **(c.)** In the presence of FLIP and caspase-8, the cell is geared towards survival.



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