

Acute kidney injury during transplantation and the role of inflammasomes: A brief review

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Abstract

The increasing demand for an effective therapeutic modality in the form of organ transplantation leads to the need to improve the long-term outcomes of the process. Ischemia/reperfusion injuries (IRI) are an integral part of the kidney transplantation process, contributing to inflammation, oxidative stress and activation of the immune system. Inflammasomes, as a component of the immune response, in the form of inflammatory mediators during infection or tissue damage, initiate cell death called pyroptosis. In this context, we have defined the process of inflammasome activation in response to IRI, which is a potential cause of early kidney rejection due to increased susceptibility of the kidneys to ischemia. This review focuses on analyzing the modulation of inflammasome activity in kidney transplants and the activation of the NLRP3 inflammasome as a crucial element of kidney injury during the transplantation procedure, which could be a potential target for future preventive/therapeutic strategies.

Key words: organ transplantation, ischemia/reperfusion injury, inflammatory process, inflammasome, NLRP3

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Introduction

Organ transplants, including kidney transplants, are one of the greatest achievements of modern medicine.¹ The etymology of the term “transplantation” comes from the Latin word *transplantare*, meaning “to graft” or “transfer”. The mentioned concept is defined as “the transplantation of an entire organ or only a specific part, designated tissue, or cells from one body to another or within the same body”.² This dictionary definition is consistent with the one proposed by the World Health Organization (WHO), which adds the therapeutic function of restoring organ function.³ Understanding the concept of transplantation according to both mentioned sources is also accepted in the literature.^{4,5}

The 1st successful kidney transplant was performed in 1954 on identical twins. Currently, there is an increased demand for kidney transplants among patients, resulting in a higher number of transplants performed in Poland and worldwide.¹ According to the latest data from Poltransplant, 2,058 people were enrolled for a transplant procedure in 2021, of whom 749 underwent the procedure.⁶

Types of kidney transplants

The literature divides kidney transplants into 3 main groups: autologous, xenogeneic and allogeneic. Autologous transplantation, also known as autograft, involves the removal of a kidney and its re-implantation.^{4,7} Xenogeneic transplantation is performed between 2 different species. Challenges in xenotransplantation arise from the differences between the 2 species, which are related to the distinct structures of coagulation proteins, the occurrence of inflammatory reactions in the recipient's body and the risk of virus transmission. The utilization of this type of transplantation requires further research before becoming an alternative to kidney allograft.⁸

Allogeneic transplantation is antagonistic to xenogeneic transplantation. This is the most widespread and involves transplantation between a donor and recipient of the same species.⁴

Another criterion for classification is the origin of the organ. Transplantology allows for the transplantation of a kidney from either a living or deceased donor.⁹ Kidneys from living donors can come from related individuals and anonymous individuals. An anonymous donor can donate their organ to a specified recipient or the first recipient on the waiting list for transplantation.¹⁰

A crossmatch transplant is used in cases of immunological mismatch between the donor and recipient. This method involves the exchange of organs between 2 pairs. A specific variation of crossmatch transplant is a chain transplant, which involves more than 2 pairs in the transplant process.^{10,11}

Kidney transplantation as a therapeutic method

Currently, the number of patients suffering from severe kidney diseases is continuously increasing. Lifestyle diseases such as type 2 diabetes (T2D), hypertension and obesity are predisposing factors for the development of chronic kidney disease (CKD). The diagnosis is established based on an estimated glomerular filtration rate (eGFR) value below 60 mL/min/1.73 m², abnormalities detected in urine tests, as well as kidney structure abnormalities observed through imaging and/or histopathological examinations, that persist for more than 3 months.¹² The progressive course of the disease may be associated with a steady decline in glomerular filtration efficiency. Estimated glomerular filtration rate values below 15 mL/min/1.73 m² qualify for the diagnosis of end-stage renal disease (ESRD). This stage, with a range of complications such as metabolic disorders, water-electrolyte disturbances, as well as cardiovascular (CV) disorders, requires renal replacement therapy (RRT). Despite the use of modern hemodialysis techniques, mortality among such patients ranges from 20% to 50% within 24 months.¹³ A significantly longer survival and improved quality of life compared to dialysis therapy are achieved by transplanting a failing organ. The greatest benefits are observed in patients with diabetes, especially young ones. Patients aged 20–39 years and treated with dialysis have an average life expectancy of 8 years, compared to 25 years after transplantation.^{14,15} Preemptive organ transplantation significantly extends the survival time of patients and transplant efficacy while reducing the risks of adverse side effects of dialysis. It also allows avoiding the need to create an arteriovenous fistula (AVF) for hemodialysis or implanting a catheter for peritoneal dialysis.¹⁶

The conditions for achieving the expected results of RRT are the appropriate qualification of the patient for transplantation, done through health assessment and the identification of contraindications.¹⁷

An essential clinical aspect in achieving therapeutic benefits from a transplant is immunologic compatibility. Antibodies directed against human leukocyte antigen (HLA) and red blood cells antigens (ABO) incompatibilities between the donor and recipient initiate rejection of the graft in approx. 30% of cases. For many years, ABO incompatibility was an absolute contraindication for transplantation. The reaction of iso-agglutinins with ABO blood group antigens induces complement system activation.¹⁸ The 2nd important element in donor-recipient selection based on immunological factors is minimal HLA mismatch, absence of panel reactive antibodies (PRA) and negative results in the donor lymphocyte crossmatch with the recipient's serum. These methods provide an opportunity to detect donor-specific antibodies (DSA), allowing for risk stratification of graft rejection.¹⁹ Removal of circulating antibodies or a reduction in their production are known

as desensitization processes, making transplantation acceptable despite partial compatibility. This effect is mainly achieved through plasmapheresis or immunoadsorption (IA). An alternative for immunologically incompatible pairs is the possibility of undergoing crossmatch/chain transplant, as described above.^{18,19}

Appropriate immunosuppression is a key element in maintaining the function of the transplanted organ while preventing rejection. Currently, a treatment regimen combining several medications is used, achieving an appropriate level of immunosuppression while reducing the risk of drug toxicities.²⁰

Transplantation efficacy and complications

In recent years, the effectiveness of transplantation, particularly proper graft function, has improved. However, improvements in long-term outcomes remain a challenge in the field of transplantology.²¹ In Poland, according to data from Poltransplant, the 5-year patient survival rate (2001–2015) was 88%, while the 10-year survival rate was 74%.⁶ The most common factors that negatively affect allografts in the 1st year after transplantation include CV failure, infections and acute rejection (AR). After the 1st year, chronic rejection contributes to the loss of proper kidney function.²² Episodes of organ rejection can be caused by cell-mediated responses involving T lymphocytes or the presence of antibodies, such as ABMR.²³ Delayed graft function (DGF), which lacks a precise definition, is associated with acute kidney injury (AKI) requiring dialysis therapy.²⁴ The incidence of DGF after deceased-donor kidney transplants ranges from 5% to 50%, with an increasing trend.²⁵ Chronic kidney transplant rejection (CKTR) is described by progressive deterioration of graft function, usually emerging 1 year after transplant, along with hypertension and proteinuria. Chronic kidney transplant rejection typically occurs in patients with insufficient immunosuppression; however, the main cause remains the sustained allogeneic immune response.²⁶

Technological advancements and the development of surgical techniques have significantly reduced adverse effects associated with the operation. These mainly involve vascular complications and urological complications.^{27,28}

Acute kidney injuries in the transplant process

The transplanted organ is predisposed to multiple injuries related to the body's immune response, toxicity resulting from pharmacotherapy or ischemia/reperfusion (I/R) injuries (IRI),²⁹ which will be further discussed in the context of this study, given the subject of the research conducted.

Ischemia/reperfusion injuries are defined as a series of cellular processes consisting of 2 distinct stages: ischemia – following the cessation of blood flow to the organ – and the action of restoring the flow of blood to the organ, otherwise known as reperfusion. Paradoxically, reperfusion exacerbates the injury by activating various biochemical mechanisms. These processes lead to endothelial cell damage, contributing, among other things, to the generation of reactive oxygen species (ROS) and activation of neutrophils and platelets, which are significant clinical problems during myocardial infarction (MI), ischemic stroke and AKI.^{30,31} There is a higher frequency of IRI in donors after cardiac death (DCDs) and expanded criteria donors (ECDs), which correlates with a longer warm ischemia time (WIT).³² Modern transplantology defines WIT as the period counted from the time the organ is dissected free from the donor until it is cooled (WIT I). Subsequently, there is a cold ischemia time (CIT) during kidney preservation in a hypothermic preservation solution, followed by the 2nd episode of warm ischemia during organ implantation in the recipient (WIT II).^{33,34} A prolonged CIT contributes to DGF and potentially shortens the long-term survival of the graft. The DCDs are particularly susceptible to the negative consequences of CIT; hence, this period should be minimized.³⁵

Mechanism of injury

Ischemia/reperfusion injuries are associated with vascular dysfunction, leading to increased vascular permeability and recruitment of inflammatory cells, contributing to multiple injuries.³⁶ Insufficient exposure of cells to oxygen initiates a metabolic shift to anaerobic metabolism, accompanied by tissue acidosis. The resulting acidosis, associated with lactate-dependent adenosine triphosphate (ATP) production, is due to the accumulation of lactic acid that is not removed by the circulation. The change in the direction of ATP synthase, with a noticeable increased hydrolysis, results in a decrease in intracellular ATP levels and the accumulation of ATP metabolites, such as hypoxanthine. These processes lead to negative consequences, including destabilization of lysosomal membranes, leakage of lysosomal enzymes, and subsequent cell structure breakdown.^{31,37} To compensate for the disturbed acid-base and water-mineral balances, the activity of the Na⁺-K⁺-ATPase ion pump is inhibited, with a simultaneous increase in the role of the Na⁺/H⁺ exchanger, contributing to an intracellular increase in sodium ions and water, leading to osmotic swelling. Dysfunction of the Na⁺/Ca²⁺ pump leads to increased Ca²⁺ levels, resulting in the activation of calcium-dependent proteases, such as calpains, upon reperfusion. The resulting calcium overload initiates the synthesis of ROS (Fig. 1).^{37,38} During reperfusion, the mitochondrial burst of ROS is responsible for adverse protein carbonylation and lipid peroxidation.

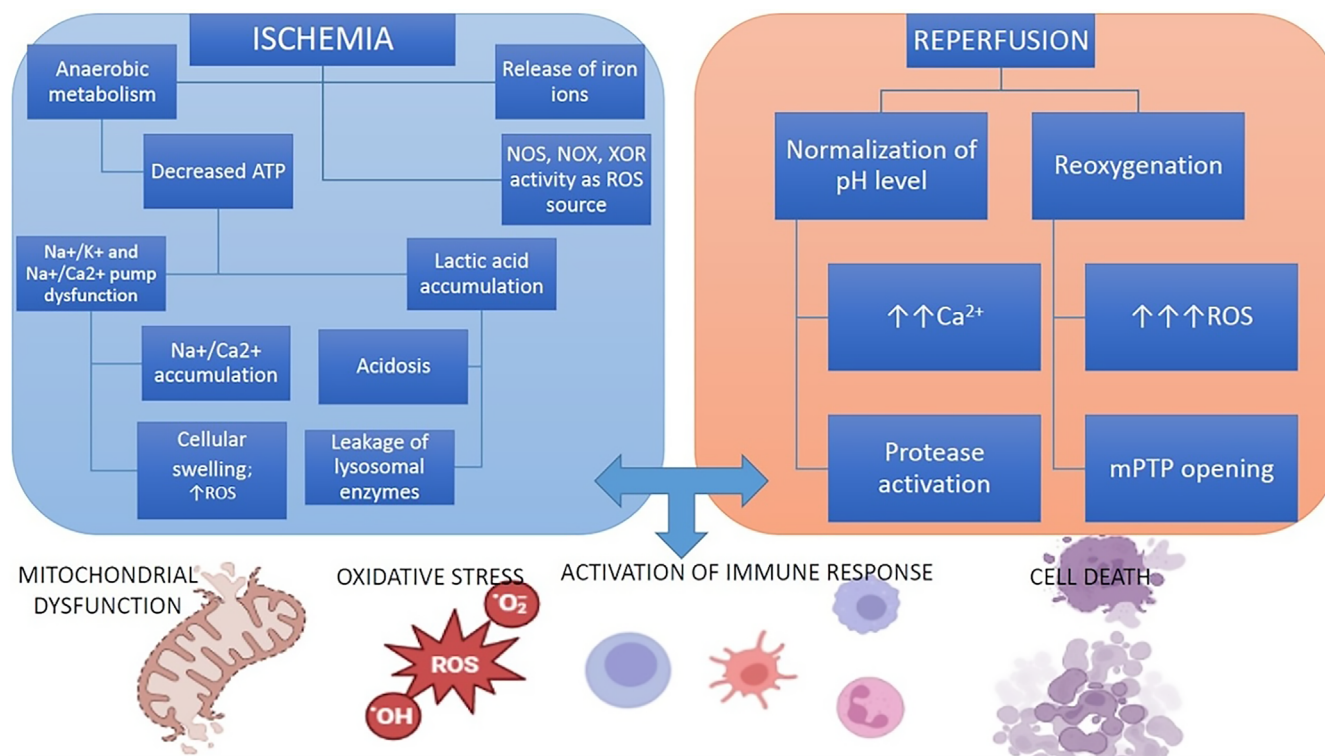


Fig. 1. Simplified diagram of the effect of ischemia/reperfusion on the cell

ROS – reactive oxygen species; NOS – nitric oxide synthase; NOX – NADPH oxidases; XOR – xanthine oxidoreductase; mPTP – mitochondrial mega-channel PTP.³⁹

Simultaneously, an inflammatory response is initiated, activating cell adhesion receptors. Neutrophils migrate through the endothelial wall into the tissue parenchyma, releasing cytotoxic mediators such as tumor necrosis factor (TNF) and interleukins (ILs), and directly or indirectly leading to the production of highly reactive species such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$). Normalization of pH affects calpain activation along with the sustained high calcium levels, contributing to the opening of the mitochondrial permeability transition pore (mPTP) and the release of cytochrome C enzymes, inducing cell death through apoptosis and necrosis.^{39,40}

Mitochondria play a significant role in the cascade of kidney injuries and other organ injuries resulting from I/R. During ischemia, ATP hydrolysis leads to an increase in free inorganic phosphate, which increases membrane permeability. After 60 min of WIT, the electron transport system complexes show reduced activity, resulting in the reduction of iron-sulfur proteins associated with complex I (NADH dehydrogenase) or complex II (succinate dehydrogenase) of the electron transport chain, leading to the release of iron ions (Fe^{2+}), reduction of hydrogen peroxide (H_2O_2) and ROS generation.⁴¹ Further conformational changes in the complexes during acute ischemia lead to their inhibition and arrest of electron flow. During reperfusion, the availability of oxygen and energetic substrates fuels oxidative metabolism, leading to a significant increase in electron leakage through the mitochondria compared to normal function levels.^{42,43} Matrix

metalloproteinases (MMPs) demonstrate a significant impact on kidney injury during transplantation by degrading extracellular matrix (ECM) components, responsible for disrupting the integrity of tissues and facilitating inflammatory cell infiltration. This enzymatic activity intensifies tissue damage and promotes fibrosis, complicating the recovery process post-transplant.⁴⁴ Dendritic cells (DCs) act as a link between innate and adaptive immune responses, directly through antigen presentation to B and T cells or indirectly through cytokine signaling. In the context of kidney transplantation, $CD4^+$ T lymphocytes play a crucial role in the pathophysiology. Normally, T cell activation occurs through antigen binding, but the effects of ROS and cytokines on this process were also demonstrated. The required antigen presentation by DCs or B cells was targeted in therapy, where the blockade of the costimulatory system inhibited T cell activation and alleviated IRI in animal models.⁴⁵

Cellular damage as a result of I/R triggers various types of cell death, including apoptosis, necrosis and autophagy. Renal I/R initiate apoptosis in proximal tubular cells.⁴⁶ Caspases, which are serine proteases, act as the main regulators and molecular effectors activated by pro-apoptotic stimuli (dependent on either the intrinsic mitochondrial pathway or death receptors), leading to the initiation of events that result in the release of mitochondrial cytochrome c into the cytosol, chromosomal DNA fragmentation, and morphological changes that are typical of apoptosis.⁴⁷ Autophagy is a process involving self-degradation and the rebuilding of damaged organelles

and proteins, protecting the cell from apoptosis after AKI and supporting regeneration. Autophagy, involving intracellular lysosomal degradation, is a series of catabolic processes, starting with a small membrane phagophore in the cytoplasm, then elongating to become, at the final stage, a double-membrane structure called an autophagosome, which fuses with lysosomes to form an autophagolysosome. Activated mammalian target of rapamycin (mTOR) signaling leads to the degradation of intracellular components, transporting them back to the cytoplasm for the reuse of macromolecules. Prolonged autophagy activation, however, can have adverse effects after an ischemic incident, triggering pathways leading to kidney cell death and exacerbating kidney injury (KI).⁴⁸ The use of murine models with deficiencies of autophagy-related (ATG) proteins that play a crucial role in autophagosome formation to study the effect of autophagy in I/R helped more definitively demonstrate the protective function of this process in the preservation of tubular integrity.⁴⁹

Markers of kidney injury during transplantation

There is a continuous need to define new markers used in routine monitoring of patients to serve as warning signals at different time points during transplantation.⁵⁰ Currently, the gold standard for assessing kidney function is eGFR, which is calculated based on serum creatinine levels. However, due to the influence of various additional factors, such as diet, muscle mass, metabolism, and sex, serum creatinine measurements have limited utility. In addition, creatinine levels will increase in 50% of renal failure and AKI and may not reflect renal tubular damage.⁵¹ Cystatin C is another commonly used functional biomarker of glomerular filtration, with increased sensitivity in detecting early renal dysfunction (within 24 h) compared to serum creatinine.⁵² The most promising new biomarkers, as reflected in the literature, with the clinical application are neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule-1 (KIM-1).⁵⁰

Neutrophil gelatinase-associated lipocalin is a protein produced by cells of the gastrointestinal, respiratory and immune systems, hepatocytes, and also by renal distal tubular epithelial cells. Toxic KI and I/R lead to the increased urinary excretion of NGAL, making it one of the earliest markers of KI. During acute tubular injury, NGAL also plays a protective role by enhancing autophagy in distal tubular cells, inhibiting apoptosis and inducing regeneration. Recently, attention has been drawn to the use of NGAL as a marker for CKD, including diabetic nephropathy, as well as a marker for CV risk. In kidney transplantation, NGAL, measured in the early post-transplant period, is considered a predictor of DGF.^{53,54} Studies revealed lower urine NGAL (uNGAL) levels in kidney transplant recipients in the early days after transplantation, which,

in combination with other parameters, serves as a helpful marker in the early assessment of kidney function.^{55,56} Moreover, both uNGAL levels and serum NGAL levels were found to be useful in determining and predicting early-stage KI.^{57,58} The lack of standardization of the measurements, the excretion of a large amount of urine and its dilution, as well as the induction of NGAL by some drugs such as cephalosporins, cisplatin and bisphosphonates limit its clinical application.^{53,59}

The 2nd widely documented marker is KIM-1, a transmembrane type I glycoprotein, a sensitive and reproducible biomarker of AKI with nephrotoxic and ischemic origins. It is expressed on the surface of the renal proximal tubular epithelial cells (PTECs). Its extracellular domain is cleaved by metalloproteinases and secreted into the urine in response to hypoxia or damage to the renal tubules.⁵⁹ The use of KIM-1 levels is currently being investigated for monitoring kidney transplant recipients, contributing to the early detection of organ rejection.^{59,60}

Interleukin 18, a pro-inflammatory cytokine, is activated by caspase-1. In healthy individuals, serum and urine levels remain generally low; however, these levels significantly increase in damaged kidney tissues. Interleukin-18 is often measured as a mediator and biomarker of ischemic tissue damage causing AKI.⁵¹

YKL-40 is a glycoprotein that plays an important role in tissue damage, inflammation, tissue remodeling, and protection against apoptosis. According to studies, YKL-40 influences organ repair mechanisms after ischemic injury in mice, making it a potentially useful marker for injury in transplants and as a prognostic marker for assessing the suitability of donor kidneys for transplantation.^{61,62}

Fatty acid-binding protein (FABP) is a cytoplasmic protein involved in fatty acid metabolism, contributing to the reduction of oxidative stress during I/R. Its highest expression is found in the distal tubular cells, making it a major marker of injury in the transplanted organ. Its levels in perfusion solutions were found to correlate with high vascular resistance and early graft dysfunction; however, their sensitivity and specificity are insufficient to make decisions about graft rejection.⁶³

In several experimental and clinical models, the utility of urine biomarkers such as uNGAL, urinary KIM-1 (uKIM-1), urinary IL-18 (uIL-18), and urinary FABP (uFABP) was identified in AKI and/or IRI.⁶⁴

A promising kidney-related biomarker is the Klotho protein. It exhibits diverse physiological actions, such as reducing oxidative stress and inhibiting the apoptosis and fibrotic processes. During I/R, Klotho is an early biomarker of AKI and shows decreased levels in urine and blood, preceding the rise in creatinine levels. Furthermore, in studies on its expression, Klotho correlated with eGFR 1 week after transplantation. Albuminuria, certain medications and local or systemic inflammation can reduce its expression. However, the standardization of soluble Klotho measurement methods remains an unresolved issue, necessitating

further research on the potential use of Klotho as a marker for AKI.^{65,66}

A major challenge still lies in implementing discovered biomarkers into clinical practice due to the lack of standardized guidelines for method validation and the complexity and multifactorial nature of injuries. Larger, multicenter validation studies are necessary before new solutions can be widely used in practice.⁶⁷

Methods of injury prevention

Reducing the effect of IRI during the transplantation process involves several approaches: proper preparation of the donor and recipient, minimizing CIT and optimizing perfusion of the transplanted organ. Recommended supplementation for the recipient includes Thymoglobulin (Sanofi, Paris, France), whose short-term effectiveness in preventing the risk of AR is widely documented in the literature.^{31,68}

Protein Klotho and its role in reducing the expression of MMPs provide an alternative for kidney protection during transplantation. Matrix metalloproteinases, as proteolytic enzymes, can degrade extracellular proteins and play a crucial role in the remodeling of the ECM.⁶⁹ Therefore, the use of MMP inhibitors may demonstrate a protective effect on kidney grafts, making it a subject for further research.^{70,71} Klotho can protect against both AKI and CKD through gene therapy or administration of its soluble form, reducing unfavorable treatment outcomes regardless of the etiology, as shown in preclinical studies. Research findings support the protective protein concept; however, its clinical application in humans remains uncertain and clinical efficacy needs confirmation.⁶⁵

Innovative techniques continue to be a challenge and a focus for researchers in the field of graft protection. In one of the latest studies, an electric field was used to activate Na⁺/K⁺ pumps, thus maintaining optimal ATP levels. In murine AKI models during WIT and CIT, this technique reduced KI, suggesting its potential usefulness in minimizing allograft dysfunction.⁷²

Inflammasome

In response to molecular patterns, a protein complex called the inflammasome coordinates the body's immune response to engage protective mechanisms against infectious agents and initiates them during physiological disturbances. Inflammasome activation is part of the innate immune process, leading to the secretion of pro-inflammatory cytokines such as IL-1 β and IL-18, thus participating in the inflammatory process.⁷³ Mature IL-1 β is a potent mediator in various immune reactions, recruiting and shaping immune system cells to the site of infection, while IL-18 is responsible for producing interferon gamma

(IFN- γ) and enhancing the cytolytic activity of NK cells and T lymphocytes. Active caspase, which is a product of inflammasome assembly, contributes to the secretion of interleukins and cell death, known as pyroptosis, exerting a significant effect on the defense against bacteria and viruses.⁷⁴

Various stimuli, including pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), activate inflammasome formation processes through pattern recognition receptors (PRRs), which are regarded as sensors. In most cases, each inflammasome contains an adaptor protein called an apoptosis-associated speck-like protein containing a CARD (ASC) and a procaspase enzyme that acts as an effector. Currently, 5 main representatives of PRRs have been identified: NLRP1, NOD-like receptor family pyrin domain-containing 3 (NLRP3), NLRC4 (belonging to the nucleotide oligomerization domain (NOD)-like receptor family – NLR), pyrin, and absent in melanoma 2 (AIM2), which serve as the basis for inflammasome assembly.^{75,76} Inflammasomes represented by these 5 sensors, known as canonical inflammasomes, can directly activate caspase-1 and are found in many tissues. Their presence is increased in bone marrow, lymphoid tissues and immune cells such as neutrophils, monocytes and macrophages. Furthermore, NLRP3 inflammasomes are activated in endothelial, epithelial and mesenchymal cells. Non-canonical inflammasomes participate in the activation of caspase-4/5 (in humans) and -11 (in mice) in response to lipopolysaccharides (LPSs) of Gram-negative bacteria. Recently, several new intracellular complexes have been discovered: NLRP6, NLRP7, NLRP12, and interferon gamma-inducible protein 16 (IFI16), whose activation mechanisms require further research.^{77,78}

Structurally, members of the NLR family consist of an N-terminal pyrin domain – PYD (for NLRPs) or a caspase recruitment domain – CARD (for NLRCs), a central nucleotide-binding oligomerization domain called NACHT, and C-terminal leucine-rich-repeats (LRRs). The NLR activation triggers a rapid oligomerization process, leading to the recruitment and binding of inactive pro-caspase-1, directly (NLRC4) or via the adaptor protein ASC, which is typical of NLRP3.⁷⁹ Different inflammasomes may exhibit differences in overall structure. Unlike NLRP3 and AIM2, NLRC4 representatives may contain only a CARD domain, not requiring the ASC domain for procaspase recruitment. On the other hand, AIM2 belongs to the AIM2-like receptor family (ALR) possessing 2 main components – HIN200 and PYD.⁸⁰

A peptidoglycan fragment derived from both Gram-positive and Gram-negative bacteria directly binds to human NLRP1, causing its structural change. It plays a significant role in human keratinocytes, where UVB radiation induces activation, contributing to skin inflammation and increasing susceptibility to cancer development.⁸¹ The NLRC4 inflammasome responds to bacterial infections, during which, after ligand binding by the NAIP part, it initiates

the assembly of the NAIP/NLRC4 inflammasome. Due to its ability to recognize host DNA, AIM2 is crucial for protecting the body against DNA viruses, other pathogens and the abnormal accumulation of self-DNA in the cytosol. Its involvement in the pathogenesis of systemic lupus erythematosus (SLE) and tumors was also found.^{82,83}

The NLRP3 inflammasome is the most widely described in the literature and serves as a key focus of research. It consists of 3 essential components typical of the NLR group: NLRP3 receptor, ASC adaptor and pro-caspase-1. The receptor has a NACHT domain (named after NAIP, CIITA, HET-E, and TEP-1 components) that is capable of oligomerization and functions as an ATPase; a PYD domain that enables interaction with ASC (forming protein-protein interactions); and leucine-rich repeat domains (LRRs) at the C-terminal end, recognizing microbial signals and microbial ligands (Fig. 2). In the ASC protein, there is also a distinguishable part responsible for the binding of procaspase – the CARD domain.^{84,85} The molecular signal associated with danger is received by the LRR domain, leading to the oligomerization of NLRP3 monomers through their NACHT domains. This is followed by interaction between the NLRP3 PYD and ASC domains, resulting in the assembly of many ASC filaments into a single aggregate of macromolecules, forming a “speck” within the activated cell. Finally, pro-caspase-1 is recruited to the complex through interactions between the CARD domains (CARD–CARD interactions), facilitated by ASC. Full-length pro-caspase-1 has an N-terminal CARD, a central large catalytic domain (p20) and a C-terminal small catalytic subunit domain (p10). This unit is subsequently cleaved at the p20–p10 junction, and further processed between the CARD and p20, ultimately releasing the active form, the p20–p10 heterotetramer, from the inflammasome.^{84,86,87} NEK7, a serine/threonine kinase, is often cited as a key NLRP3-specific component required for activation. However, recent research shows that in various cell systems, the polymerization of the ASC domain is independent of NEK7.^{88,89}

Activation of the NLRP3 inflammasome is crucial for host defenses against pathogen invasion and maintaining homeostasis. However, excessive response additionally

contributes to the progression of various inflammatory diseases, such as arthritis, atherosclerosis, T2D, and cancers. Many studies imply that neuroinflammatory and neurodegenerative diseases, such as Alzheimer’s disease and Parkinson’s disease, are also linked to the NLRP3 inflammasome. Dysregulation of the activation of this multidimensional complex can also lead to several autoimmune diseases, including multiple sclerosis; thus, activation must be tightly regulated, and the molecular mechanisms of this process and their connection to disease pathogenesis are becoming an increasingly popular subject for researchers.⁹⁰

Inflammasome activation

Inflammasome activation, with particular emphasis on NLRP3, in response to the release of molecules (from infected cells and damaged tissues), through PRR, is tightly regulated in resting cells. Canonical activation follows a standard 2-step model in which 2 signals are required for optimal activation of the NLRP3 inflammasome. Cytokine receptors, toll-like receptors (TLRs) and tumor necrosis factor receptors (TNFR) are stimulated by molecules such as IL-1 β , LPS and TNF. This leads to the initiation of a cell pathway involving nuclear factor kappa B (NF- κ B), triggering the transcription of NLRP3 components.⁹¹ This stage, referred to as the priming or initiating signal, is 1 of 2 necessary factors for initiating the complex process of inflammasome activation and assembly. Some studies indicate that the transcription process is unnecessary during priming, as evidenced by time observations between NLRP3 inflammasome activation and the increase in its expression. Furthermore, while TLR-related signaling is essential for rapid NLRP3 activation, current attention is primarily focused on the dependence of this receptor on the MyD88 adaptor protein and IL-1 receptor-associated kinases, IRAK-1 and IRAK-4.⁷⁷ Increasing evidence implies that the initiating signal, additionally serving as an inducer of posttranslational modifications (PTMs), enables NLRP3 activation.⁹¹

However, to initiate functional inflammasome assembly, a 2nd stimulus is required, such as ATP, ROS, viral proteins,

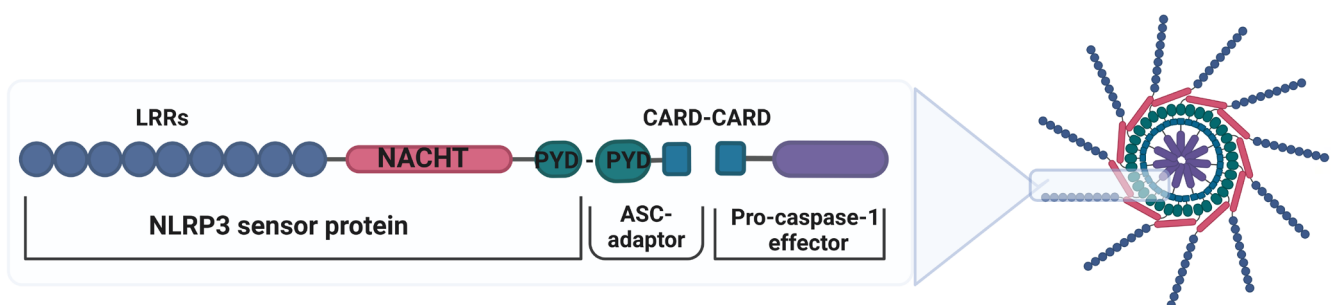


Fig. 2. The general structure of the NLRP3 inflammasome.

NLRP3 – NOD-like receptor 3, containing the pyrin domain; ASC – adaptor protein; LRRs – leucine-rich repeats; NACHT – oligomerization domain (components: NAIP, CIITA, HET-E, and TEP1); PYD – pyrin domain; CARD – caspase activation and recruitment domain.

toxins, or uric acid released by neighboring necrotic cells or damaged tissues.⁹¹

An essential element in the activation pathways is ion flow within the cell, particularly involving potassium, calcium, sodium, and chloride ions. A decrease in intracellular potassium concentration occurs, in part, through the P2X7R receptor and TWEAK2 in response to the accumulated extracellular reservoir of ATP, indirectly triggering inflammasome activation. Nigericin, a commonly used NLRP3 inflammasome agonist, correlates with potassium efflux.⁹² Consequently, high cytoplasmic K⁺ levels inhibit inflammasomes. However, it has not been determined how K⁺ efflux is detected by NLRP3 and enables the assembly and activation of the inflammasome. The role of potassium ion flow from the cytosol to the extracellular compartment seems to be associated with other types of triggers, such as lysosomal destabilization and the action of its enzymes. Despite its significant role in NLRP3 activation, the exact nature underlying potassium flow remains to be fully understood.⁹³

Signaling associated with calcium flow is often emphasized as critical for the activation of the NLRP3 inflammasome. It is hypothesized that calcium influx into mitochondria enhances mitochondrial stress and intensifies its activation. However, contrary to earlier data, recent systematic studies have revealed that calcium flux does not occur in response to most NLRP3 activators. Moreover, it is neither necessary nor sufficient for its activation; thus, the interconnections in the cell pathway remain a subject of ongoing research. The flow of chloride ions and the associated intracellular channel proteins, such as chloride intracellular channel 1 (CLIC1) and 4 (CLIC4), are required for NLRP3 activation.^{94,95}

The release of both mitochondrial DNA (mtDNA) and mitochondrial ROS (mtROS) from damaged mitochondria is a process also associated with the NLRP3 activation pathway. Reactive oxygen species generation, especially from mitochondria, was one of the first identified factors that could contribute to inflammasome activation. However, recent studies imply that mtROS might be released by NLRP3 activators but is not the cause of its activation. In another study concerning mitochondria, it was found that mtDNA must first be oxidized (ox-mtDNA) to activate NLRP3. Furthermore, many scientific reports suggest a hypothesis that preventing the removal of damaged mitochondria initiates NLRP3 activity, implying that these organelles play an important role in the activation pathway.^{95,96} Cardiolipin is a highly negatively charged lipid located on the inner side of the mitochondrial membrane. In response to cellular stress, its externalization occurs, which is necessary for autophagy and cell death. It was found that mitochondrial dysfunction allows direct interaction between cardiolipin and NLRP3 through the LRR domain, activating the inflammasome. In another study, the effect of LPS on the binding of cardiolipin with caspase-1 was identified, leading to inflammasome activation.^{84,97} Adenosine triphosphate is a central product of the mitochondria and

a known activator of NLRP3 through the involvement of the P2X7R receptor. Adenosine triphosphate can also enhance the inflammasome-induced production of IL-1 β and IL-18 in innate immune cells.⁹⁸ It should be noted that NLRP3 has a motif that binds and hydrolyzes ATP in the NACHT domain, called Walker B, which is required for inflammasome activity. Recent studies have identified that MCC950 – a common inhibitor – binds to this motif, preventing NLRP3 from attaining an active conformation.⁹³

NLRP3 activation in response to lysosome destabilization correlates with lysosomal membrane permeabilization (LMP) and the release of lysosomal hydrolases, such as cathepsins, into the cytosol. Lysosomal membrane permeabilization is induced by a wide range of pro-inflammatory stimuli, e.g., monosodium urate (MSU) and crystalline cholesterol. This leads to a potassium efflux, consequently increasing mtROS and cardiolipin externalization.⁸⁴

As mentioned at the beginning of the chapter, post-translational processes of NLRP3 are likely to play an important role in its assembly. Ubiquitination contributes to inflammasome activation through deubiquitinating enzymes (DUBs). Phosphorylation events, in turn, control the assembly and activation of inflammasomes.⁹⁹

During activation, as a result of the previously described structural changes in NLRP3, activated caspase-1 transforms pro-IL-1 β and pro-IL-18 into their mature forms. Another critical and direct substrate of caspase-1 is gasdermin D (GSDMD) – the principal executor of pyroptosis. Caspase-1 processes cytosolic GSDMD to release its N-terminal domain with a high affinity for the cell membrane, where it inserts and forms pore-like structures on the membrane. This particular structure allows the release of IL-1 β and IL-18 into the extracellular space (Fig. 3). Besides releasing pro-inflammatory cytokines and other contents, the pores in the membrane lead to pathological ion flows, ultimately resulting in cell death, known as pyroptosis.¹⁰⁰ Pyroptosis is characterized by nuclear shrinkage and nuclear DNA fragmentation. The genetic material damage during pyroptosis is limited, and the overall nuclear structure remains intact. Inflammatory mediators that are released during the process recruit and activate immune cells, enhancing the body's immune response. Unbalanced activation of this form of cell death can lead to an inflammatory response that disrupts host homeostasis.¹⁰¹

The apoptotic effectors BAX and BAK induce NLRP3 inflammasome activation and IL-1 β secretion. It is worth noting that a large number of cell death effectors, such as caspase-8, MLKL and GPX4, directly or indirectly regulate NLRP3 inflammasome activation in response to various PAMPs and DAMPs.¹⁰² NLRP3 activation may affect the induction of autophagy, and, on the other hand, autophagy may modulate the inflammasome, reducing its activity. According to researchers, this bidirectional regulation ensures a balance between the required host defenses and the prevention of excessive inflammation.¹⁰²

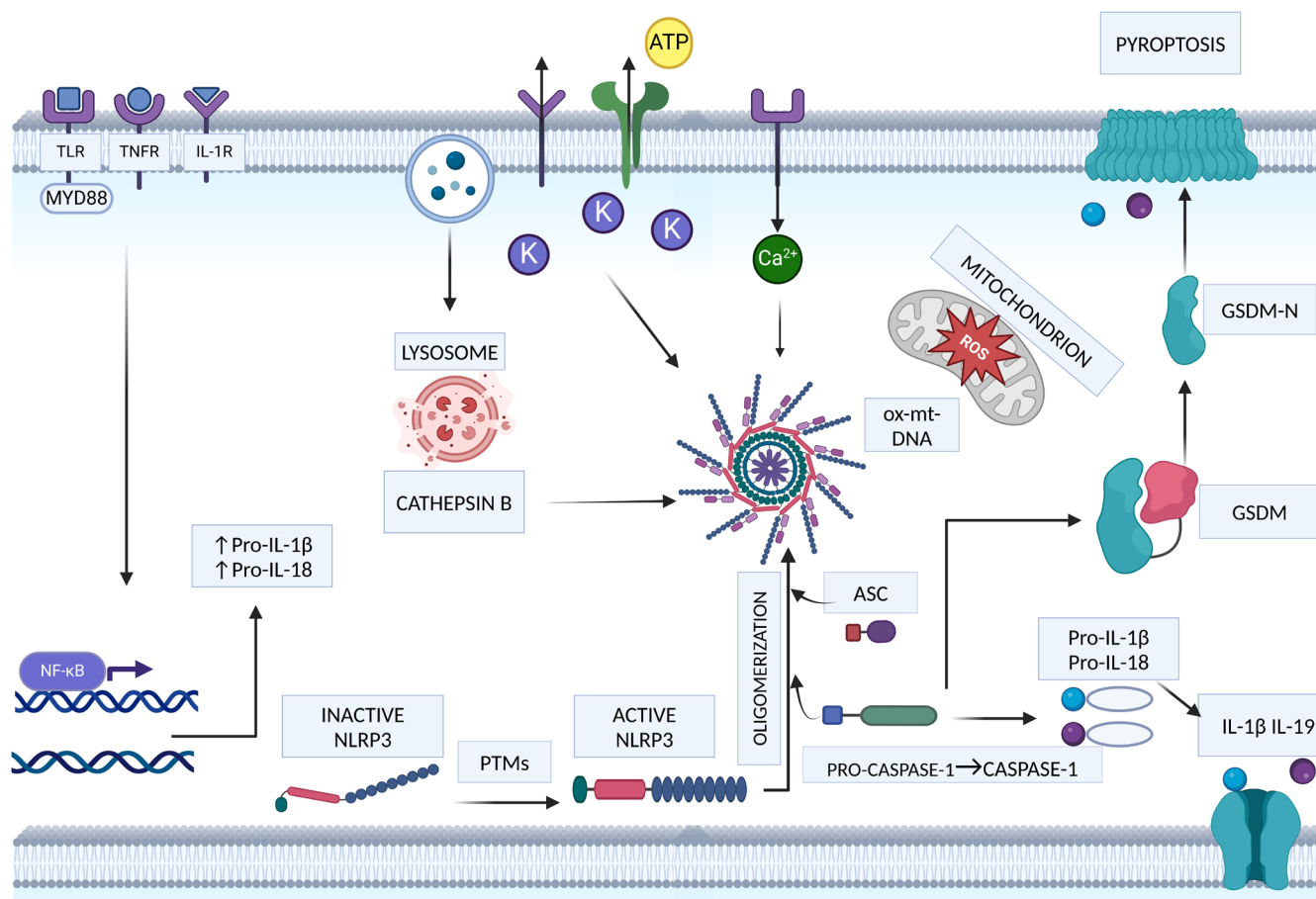


Fig. 3. Simplified scheme of NLRP3 inflammasome activation

NF-κB – nuclear factor κB; TLR – toll-like receptor; TNFR – tumor necrosis factor receptor; IL-1R – interleukin 1 receptor; GSDM – gasdermin D; GSDM-N – N-terminal fragment of gasdermin D; PTMs – posttranslational modifications; ROS – reactive oxygen species; ox-mtDNA – oxidized mitochondrial DNA.⁷⁶

There are also inhibitory factors related to these protein complexes. An example is the cytokine IFN-γ, which affects the immunopathologic response by regulating nitric oxide production and inhibiting NLRP3 inflammasome formation through its nitrosylation. Moreover, carbon monoxide (CO) acts as an inhibitor of caspase-1 activation and the secretion of IL-1β and IL-18.¹⁰³

The cytosolic response pathway to LPS, involving caspase-4/5 in humans and caspase-11 in mice, was termed non-canonical due to the signal received by TLR4-TRIF. Lipopolysaccharides from Gram-negative bacteria, which is delivered into the cytosol via outer membrane vesicles (OMVs), are taken up by cells, with the simultaneous initiation of interferon type I, which affects caspase-11 expression and generates specific guanylate-binding proteins (GBPs) and immunity-related GTPase family member b10 proteins (IRGB10) that directly target the cell membrane of the engulfed bacteria in a GBP-dependent manner, disrupting the microorganism's structural integrity and releasing LPS and lipid A into the cytoplasm. Part of the lipid A molecule directly interacts with the proper caspase-4/5/11 recruitment domain, leading to their

oligomerization and activation. The subsequent steps of the process resemble the canonical pathway.⁹⁰

Alternative NLRP3 inflammasome activation requires only 1 signal. Toll-like receptors ligands are sufficient to initiate NLRP3 inflammasome activation in human monocytes. The main differences between alternative and classical inflammasome activation (canonical and non-canonical) involve a lack of dependence on the flow of potassium and pyroptosis.⁸⁰

Functions of the inflammasome in the body

The inflammasome plays a crucial role in regulating the inflammatory state and protecting the body. The NLRP3 inflammasome can be activated by bacterial and viral components, as well as intracellular signals such as mitochondrial damage, protein aggregates or abnormal ion levels. Cell death following inflammasome activation, known as pyroptosis, also serves as an important protective mechanism against bacterial infections. It inhibits

pathogen replication within cells, promotes the phagocytosis of remaining bacteria, and induces the release of pro-inflammatory proteins involved in the pathogenesis of various chronic inflammatory diseases.¹⁰⁴ The NLRP3 inflammasome is activated in the presence of bacterial infections, including *Staphylococcus aureus*, group B *Streptococcus*, *Listeria monocytogenes*, and *Neisseria gonorrhoeae*. Meanwhile, the AIM2 inflammasome plays a critical role in alerting the innate immune system during invasion by certain extracellular and intracellular bacteria, as exemplified by *Streptococcus pneumoniae*.^{83,105} Viral nucleic acids are typically detected by AIM2 (ALR) inflammasomes; however, several viruses and their components, including the influenza virus, poliovirus and enterovirus, are sufficient to stimulate the NLRP3 inflammasome. Consequently, NLRP3 inflammasome activation inhibits virus replication, reducing mortality in murine models.¹⁰⁶

Dysregulation of NLRP3 inflammasome complex formation may promote chronic inflammation by increasing IL-1 β release. However, improper activation of the complex may also lead to severe pathological damage.¹⁰⁴ The response to DAMPs in the absence of a microbial factor is termed sterile inflammation, occurring in conditions such as atherosclerosis, T2D, neurodegenerative disorders, and cancer.⁷⁸

The inflammasome coordinates the body's immune response to engage protective mechanisms during disturbances in physiological processes. There is evidence linking the NLRP3 inflammasome to organ injury, neurologic diseases and CV diseases.^{104–106} Increased NLRP3 expression was documented in atherosclerotic plaques, correlating with the severity of coronary artery disease (CAD). Inflammasome activation in endothelial cells is associated with impaired coronary flow, while IL-1 β and IL-18 may lead to improper vasodilation. Activation of the NLRP3 inflammasome was identified as a potent mediator of the inflammatory response, releasing pro-inflammatory mediators IL-1 β and IL-18 that enhance lipid deposition and foam cell accumulation, ultimately contributing to the progression of atherosclerosis.^{107,108} Furthermore, there was NLRP3 inflammasome activation in cardiomyocytes during acute myocardial infarction (AMI). This results in inflammation and cell death in the form of pyroptosis. In neutrophils, interleukins promote the release of ROS and enzymes, causing damage to cardiomyocytes.¹⁰⁹

Chronic inflammation is a significant aspect of the pathogenesis of T2D. Activation of the NLRP3 inflammasome occurs in response to glucose, free fatty acids and mtROS.¹¹⁰ The production of IL-1 β also plays a crucial role, leading to the induction of insulin resistance and impaired pancreatic β -cell function.¹¹¹

In many studies investigating inflammasome activation during cerebral I/R events in rodents, there was an increased expression of inflammasome components such as NLRP3, caspase-1, IL-1 β , and IL-18 already in the initial hours, which is consistent with the hyperacute and

acute phases of strokes in humans.^{112–114} Therefore, there were harmful inflammatory effects of complex activation. The use of an inflammasome inhibitor reduces the expression of inflammatory mediators. Franke et al. induced ischemic strokes in mice through a 60-min middle cerebral artery occlusion followed by 3-h, 7-h and 23-h reperfusion periods. Besides confirming the effectiveness of the inhibitor, they observed reduced infiltration of immune system cells in the ischemic hemisphere, along with preserved blood–brain barrier (BBB) integrity.¹¹³ Furthermore, in a more recent study, it was proved that the addition of the MCC950 inhibitor resulted in a reduction in brain injury caused by oxidative stress in rats.¹¹⁴

Inflammasome in the kidney transplant process

Improving the effectiveness of RRT in the form of transplantation involves, among other things, reducing IRI, which is also the underlying pathophysiology of dysfunction in many organs. Increased production of ROS, cell damage and cell death can mobilize DAMPs that are necessary for initiating the inflammasome activation pathway, thus leading to an intensified cascade of the inflammatory process.¹¹²

Ischemia/reperfusion is a potential cause of early kidney rejection due to increased susceptibility of the kidneys to ischemia. A complex sequence of biochemical events resulting in renal cell death due to reduced oxygen levels and subsequent reperfusion determines the severity of the damage and initiation of the inflammatory response, leading to renal dysfunction.^{31–45} Currently, there are few scientific studies related to NLRP3 inflammasome activation during the kidney transplant process. In 1 publication, there was a decline in transplant efficacy due to the intensified effects of WIT, which was associated with increased NLRP3 expression resulting from prolonged organ storage in hypothermia.¹¹⁵

Tissue ischemia, which is a main cause of AKI, serves as a potential source of ligands for NLRP3 activation. Inflammasome-related studies were conducted on both murine and human renal tissues subjected to I/R, where effectors in the form of caspase-1, IL-1 β and IL-18 were also measured. High levels of NLRP3 and its effectors in human and murine renal tubular epithelial (RTE) cells indicate a crucial role in initiating inflammatory responses during tissue damage. Lack of NLRP3 provides protection against renal cell death during IRI and loss of kidney function; however, silencing the ASC domain alone or effectors (caspase-1, IL-1 β and IL-18) did not yield such positive effects.^{116,117} Confirmation of these observations was obtained in another study, where various renal tubular cell lines, primary mouse renal tubular cells lacking NLRP3 (knockout, KO) and a unilateral ureteral obstruction (UUO) model were used in vitro. Cellular hypoxia induced a significant increase in NLRP3 regardless

of ASC, caspase-1 and IL-1 β . During oxygen deprivation, NLRP3 in renal tubular cells was relocated from the cytosol to the mitochondria and was bound to mitochondrial antiviral signaling protein (MAVS). Deletion of NLRP3 or MAVS weakened mtROS production and depolarization of mitochondrial membrane potentials under hypoxic conditions. In response to unilateral ureteral obstruction, mice with silenced NLRP3 exhibited less fibrosis and apoptosis compared to wild-type mice.¹¹⁸ Findings from a more recent study on the acute and chronic phases of ischemic AKI indicated persistent overexpression of NLRP3, which is associated with abnormal renal tubular repair and correlated with infiltrating macrophages and fibrosis. The hypothesis was raised that elevated NLRP3 levels after AKI could serve as a new biomarker for chronic kidney lesions, predicting the transition from AKI to CKD, and a marker for monitoring long-term effects, similar to KIM-1 and NGAL. However, this requires confirmation in further clinical studies.¹¹⁹ One promising therapeutic strategy for renal IRI is to prevent NLRP3 activation through the use of inhibitors. An example is a study using H₂S, which acts to inhibit cell pyroptosis via the NLRP3/caspase-1 pathway and reduce I/R-induced AKI. The exact mechanism of action remains subject to further research.¹²⁰ An alternative approach involves using endothelial progenitor cells (EPCs), which play an important role in maintaining vascular integrity and endothelial repair. The EPCs were cultured from human peripheral blood and administered to mice 5 min before reperfusion. This resulted in a reduction in NLRP3 expression levels and cleaved caspase-1 compared to the control group. Inflammasome-induced inflammation is a potential target of EPCs to treat I/R-induced AKI and prevent progression to CKD.^{121,122}

Conclusions

Kidney transplants are one of the greatest achievements of modern medicine, since the number of patients suffering from severe kidney diseases is continuously increasing. However, improvements in long-term outcomes remain a challenge in the field of transplantology. The transplanted organ is predisposed to multiple injuries related to the body's immune response, toxicity resulting from pharmacotherapy, and IRI. This review proved that the activation of NLRP3 inflammasomes is a crucial element of kidney injury during transplant procedures. We have described the pathways of inflammasome activation indicating the NLRP3 inflammasome as a potential target for future preventive/therapeutic strategies.

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