The significance of NLRP3 and inclusion body myositis.

NLRP3 is a crucial component of the innate immune system, forming an inflammasome that drives inflammation through the activation of proinflammatory cytokines and cell death pathways. In inclusion body myositis (IBM) NLRP3 is markedly upregulated in muscle tissue, linking chronic inflammation and mitochondrial dysfunction to muscle weakness. The strong association between NLRP3 activation, altered mitophagy, and IBM severity highlights NLRP3's significance as both a biomarker and a potential therapeutic target.

NLRP3 activation drives muscle degeneration in inclusion body myositis through a cascade of chronic inflammation and impaired cellular maintenance. Overactivation of the NLRP3 inflammasome in IBM muscle leads to increased production of proinflammatory cytokines, which stimulate muscle cell injury and promote protein aggregation within muscle fibers. Additionally, altered mitophagy disrupts the removal of damaged mitochondria, fueling oxidative stress and further NLRP3 activation in a vicious cycle that accelerates muscle weakness and atrophy, especially in type 2 fibres.

Selected references.

Bahat, A., Milenkovic, D., Cors, E., Barnett, M., Niftullayev, S., Katsalifis, A., Schwill, M., Kirschner, P., MacVicar, T., Giavalisco, P., Jenninger, L., Clausen, A. R., Paupe, V., Prudent, J., Larsson, N.-G., Rogg, M., Schell, C., Muylaert, I., Lekholm, E., … Langer, T. (2025). Ribonucleotide incorporation into mitochondrial DNA drives inflammation. *Nature*. https://doi.org/10.1038/s41586-025-09541-7

mtDNA is sensitive to the ratios of different types of nucleotides – deoxyribonucleotides (dNTPs) and ribonucleotides (rNTPs). When rNTPs are present in excess relative to dNTPs, RNA building blocks are misincorporated into the mtDNA. This imbalance compromises the stability and fidelity of mtDNA replication. This excess causes the mitochondria to expel these unbalanced mtDNAs.

Billingham, L. K., Stoolman, J. S., Vasan, K., Rodriguez, A. E., Poor, T. A., Szibor, M., Jacobs, H. T., Reczek, C. R., Rashidi, A., Zhang, P., Miska, J., & Chandel, N. S. (2022). Mitochondrial electron transport chain is necessary for NLRP3 inflammasome activation.

Nature Immunology, *23*(5), 692–704. https://doi.org/10.1038/s41590-022-01185-3

Demonstrated that **intact electron transport chain is required for NLRP3 activation**. Ties mitochondrial metabolism and ROS to inflammasome signaling.

Blevins, H. M., et al. (2022). The NLRP3 inflammasome pathway: A review of mechanisms and therapies in aging and age-related disease. *Frontiers in Aging Neuroscience, 14*, 879021. https://doi.org/10.3389/fnagi.2022.879021

This review discusses the recent advances in understanding the NLRP3 mechanism, its role in disease pathology, and provides a broad review of therapeutics discovered to target the NLRP3 pathway and their challenges.

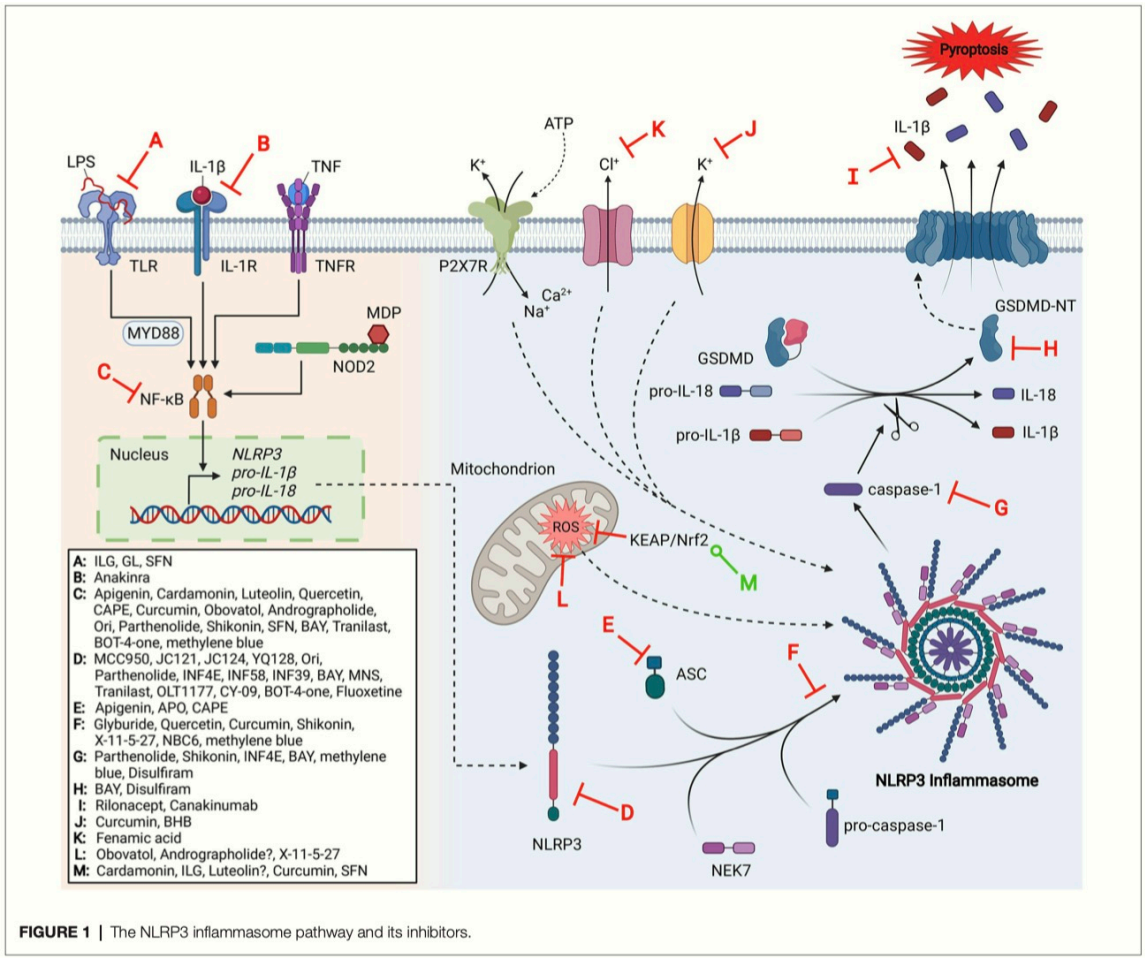

FIGURE 1 | The NLRP3 inflammasome pathway and its inhibitors.

Cordero, M. D., Williams, M. R., & Ryffel, B. (2018). AMP-Activated Protein Kinase regulation

of the NLRP3 inflammasome during aging. *Trends in Endocrinology & Metabolism, 29*(1), 8-17. https://doi.org/10.1016/j.tem.2017.10.009

Ding, W., et al. (2024). Mitochondrial DNA leakage triggers inflammation in age-related cardiovascular diseases. *Frontiers in Cell and Developmental Biology, 12*, 1287447. https://doi.org/10.3389/fcell.2024.1287447

Mitochondrial quality controls such as mitophagy could prevent mitochondria from triggering harmful inflammatory responses, but when this homeostasis is out of balance, mtDNA-induced inflammation could become pathogenic and contribute to age-related cardiovascular diseases. Here, we summarize recent studies on mechanisms by which mtDNA promotes inflammation and aging-related cardiovascular diseases, and discuss the potential value of mtDNA in early screening and as therapeutic targets.

Guglielmi, V., et al. (2024). Sporadic inclusion body myositis at the crossroads of inflammation and degeneration. *International Journal of Molecular Sciences, 25*(5), 2742. https://doi.org/10.3390/ijms25052742

Review integrating **degenerative (protein aggregation, mitochondrial dysfunction)** and **inflammatory (innate/adaptive immune activation)** components of IBM. Highlights NLRP3 as a unifying pathway.

Guo, H., Callaway, J. B., & Ting, J. P.-Y. (2015). Inflammasomes: Mechanism of action, role in disease, and therapeutics. *Nature Medicine*, *21*(7), 677–687. https://doi.org/10.1038/nm.3893

Inflammasomes have been linked to a variety of autoinflammatory and autoimmune diseases, including neurodegenerative diseases (multiple sclerosis, Alzheimer's disease and Parkinson's disease) and metabolic disorders (atherosclerosis, type 2 diabetes and obesity). In the initiation of inflammatory disease, inflammasomes play either causative or contributing roles, and also exaggerate the pathology in response to host-derived factors.

The mechanisms of NLRP3 activation supported by the most studies include potassium efflux out of the cell, the generation of mitochondrial reactive oxygen species (ROS), the

translocation of NLRP3 to the mitochondria, the release of mitochondrial DNA or cardiolipin, and the release of cathepsins into the cytosol after lysosomal destabilization. . . . the precise mechanism of NLRP3 activation is still debated.

Huang, Y., Xu, W., & Zhou, R. (2021). NLRP3 inflammasome activation and cell death. *Cellular & Molecular Immunology, 18*(9), 2114-2127. https://doi.org/10.1038/s41423-021-00740-6

Kelley, N., Jeltema, D., Duan, Y., & He, Y. (2019). The NLRP3 inflammasome: An overview of mechanisms of activation and regulation. *International Journal of Molecular Sciences, 20*(13), 3328. https://doi.org/10.3390/ijms20133328

Kummer, K., Bertram, I., Zechel, S., Hoffmann, D. B., & Schmidt, J. (2023). Inflammasome in Skeletal Muscle: NLRP3 Is an Inflammatory Cell Stress Component in Inclusion Body Myositis. *International Journal of Molecular Sciences, 24*(13), 10675. https://doi.org/10.3390/ijms241310675

Here, we identify a strong overexpression of NLRP3 inflammasome as a cell stress marker in the skeletal muscle of IBM patients and a well-established muscle cell culture model of the disease. Many overlaps between the previously known activators of this inflammasome and components of IBM protein aggregations suggest that the NLRP3 inflammasome is a central component of the interplay between inflammation and degeneration in IBM muscle and its model systems.

Naddaf, E., Nguyen, T. K. O., Watzlawik, J. O., et al. (2025). NLRP3 inflammasome activation and altered mitophagy are key pathways in inclusion body myositis. *Journal of Cachexia, Sarcopenia and Muscle, 16*(1), e13672. https://doi.org/10.1002/jcsm.13672

Showed **NLRP3 activation and impaired mitophagy** in IBM muscle tissue. NLRP3/ASC proteins were elevated in fibers and infiltrating immune cells; altered p-S65-ubiquitin correlated with weakness, suggesting mitochondrial distress–inflammasome coupling.

NLRP3. (2024, June 4). In *Wikipedia*. https://en.wikipedia.org/wiki/NLRP3

Rosa, C. P., et al. (2023). Reactive oxygen species trigger inflammasome activation: Mechanisms

and implications. *Life Sciences, 333*, 121719. https://doi.org/10.1016/j.lfs.2023.121719
Reactive oxygen species, derived from the cytosol or from mitochondria, can trigger inflammasome complexes, featuring as an important signal in their activation.
In this mini-review, it was demonstrated either the ROS production during pathogen infection activates inflammasomes or pathogen evasion mechanisms to overlap the immune system and survive in an intracellular harmful environment avoiding this important innate immune response. Therefore, ROS plays an important role in the host-pathogen relationship, directly impacting inflammatory activity, in which the understanding of how these intracellular microorganisms activate or inhibit the inflammasome sheds light on the development of therapeutic strategies.

Shimada, K., et al. (2012). Oxidized mitochondrial DNA activates the NLRP3 inflammasome during apoptosis. *Immunity, 36*(3), 401–414. https://doi.org/10.1016/j.immuni.2012.01.009
Seminal paper showing **oxidized mtDNA directly activates NLRP3**. Macrophages lacking mtDNA failed to secrete IL-1β. → Mechanistic foundation for mitochondrial DAMP → inflammasome link.

Swanson, K. V., Deng, M., & Ting, J. P.-Y. (2019). The NLRP3 inflammasome: Molecular activation and regulation to therapeutics. *Nature Reviews Immunology, 19*(8), 477-489. https://doi.org/10.1038/s41577-019-0165-0

Xian, H., et al. (2022). Oxidized DNA fragments exit mitochondria via mPTP/VDAC to activate cytosolic innate immunity. *Immunity, 55*(8), 1370–1385.e8. https://doi.org/10.1016/j.immuni.2022.07.008
Mitochondrial DNA (mtDNA) escaping stressed mitochondria provokes inflammation via cGASSTING pathway activation and when oxidized (Ox-mtDNA), it binds cytosolic NLRP3, thereby triggering inflammasome activation. NLRP3 inflammasome activation follows a “two-step” route: priming and activation. Priming is initiated by Toll-like receptors (TLRs), which sense pathogen (PAMPs) or danger (DAMPs) associated molecular patterns and trigger nuclear factor kappa-lightchain-enhancer of activated B

cells (NF-κB)-induced NLRP3 and pro-IL-1β transcription. Activation entails NLRP3 inflammasome assembly, Casp1 activation and IL-1β maturation. In addition to their key role in cell survival and death, mitochondria have emerged as central regulators of inflammation. Mitochondria and NLRP3 inflammasome intersect at multiple facets and in different diseases. Our results further establish mitochondria as primary targets for diverse NLRP3 activators.

Yang, S., Huang, G., & Ting, J. P. Y. (2025). Mitochondria and NLRP3: To die or inflame. *Immunity*, *58*(1), 5–7. https://doi.org/10.1016/j.immuni.2024.12.007

The NLRP3 inflammasome is a cytosolic complex that recognizes pathogenic insults and cellular perturbations. Its activation typically requires two signals: The first priming signal, activated by pattern recognition receptors or cytokine receptors, transcriptionally upregulates inflammasome components and post-translationally modifies NLRP3 from an auto-inhibitory to a signal-competent state. The second signal involves detecting numerous damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs), leading to NLRP3's interaction with the adaptor molecule ASC and procaspase-1, nucleating the inflammasome. Subsequent signaling drives the cleavage of caspase-1, promoting the maturation of IL-1β, IL-18, and gasdermin D, which mediates the release of proinflammatory cytokines and pyroptotic cell death. Mitochondria, as hubs of the respiratory chain and oxidative phosphorylation complexes, influence the NLRP3 inflammasome in different aspects. Mitochondrial dysfunction results in the accumulation of reactive oxygen species (ROS) and the release of oxidized mitochondrial DNA (mtDNA), both potent initiators of NLRP3 nucleation. Additionally, as central regulators of intrinsic apoptotic cell death, mitochondria play critical roles in deciding between apoptosis and pyroptosis, which remains undefined.

Zanini, G., et al. (2023). Mitochondrial DNA as inflammatory DAMP: A warning of an upcoming age. *Biochemical Society Transactions, 51*(2), 735–749. https://doi.org/10.1042/BST20221010

Review on **mtDNA as a DAMP** in aging/inflammation ("inflammaging"). Supports

systemic dimension relevant to IBM as an age-associated disease.

Zheng, D., et al. (2020). Inflammasome activation and regulation: Toward a better understanding of complex mechanisms. *Cell Discovery, 6*, 36. https://doi.org/10.1038/s41421-020-0167-x

Appropriate inflammasome activation is vital for the host to cope with foreign pathogens or tissue damage, while aberrant inflammasome activation can cause uncontrolled tissue responses that may contribute to various diseases, including autoinflammatory disorders, cardiometabolic diseases, cancer and neurodegenerative diseases. Therefore, it is imperative to maintain a fine balance between inflammasome activation and inhibition, which requires a fine-tuned regulation of inflammasome assembly and effector function. Recently, a growing body of studies have been focusing on delineating the structural and molecular mechanisms underlying the regulation of inflammasome signaling. In the present review, we summarize the most recent advances and remaining challenges in understanding the ordered inflammasome assembly and activation upon sensing of diverse stimuli, as well as the tight regulations of these processes. Furthermore, we review recent progress and challenges in translating inflammasome research into therapeutic tools, aimed at modifying inflammasome-regulated human diseases.