Scintillant BIOSCIENCE

Targeting the NLRP3 Inflammasome to Inhibit Inflammatory Disease: A Case Study

The Challenge

Only recently have we begun to understand the role of inflammation in the etiology of a broad spectrum of diseases, ranging from cancer, heart disease, neurodegeneration and beyond. This newfound appreciation for the pivotal role of inflammation coincides with the relatively recent discovery of inflammasomes and the elucidation of their functions within the innate immune system. These complex molecular structures assemble in response to infections and endogenous danger signals, resulting in the release of inflammatory cytokines from various cell types, including monocytes, macrophages, and microglia. The most extensively studied inflammasome is NLRP3, which is expressed widely throughout cell types of the innate immune system. Activation of the NLRP3 inflammasome leads to the secretion of inflammatory cytokines, IL-1β and IL-18. The targeted inhibition of the NLRP3 inflammasome offers the potential to mitigate, delay, or even reverse the progression of diseases associated with chronic inflammation.

The Solution

We conducted contract research in collaboration with a biotechnology company to support their efforts in discovering and developing drugs that target the NLRP3 inflammasome. Our involvement began in the early discovery phase and extended to their selection of a compound for human clinical trials. Our multifaceted approach included the following:

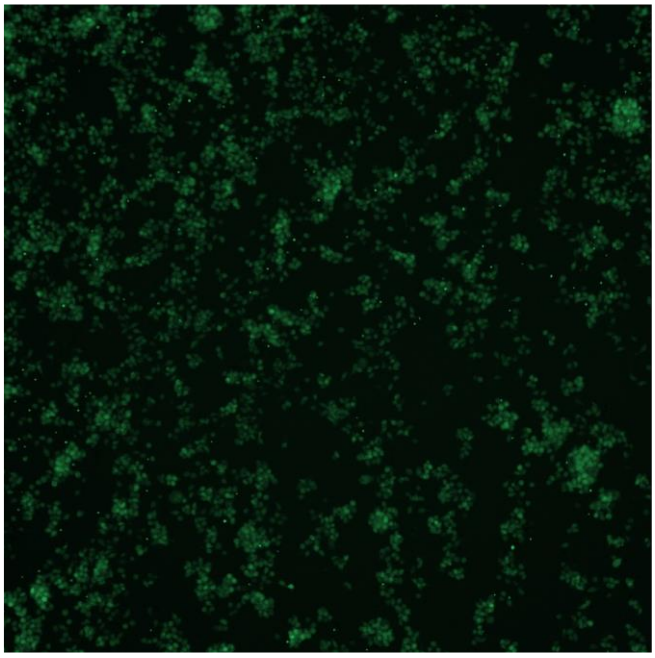
- We tested and rank-ordered our client's compounds based on their potency for inhibiting Caspase-1, a downstream effector of NLRP3 activation responsible for converting pro-IL-1β and IL-18 into their active secreted forms.
- We developed and implemented endpoint and time-lapse imaging assays to evaluate the priming of the NLRP3 inflammasome in response to lipopolysaccharide (LPS) and the activation of the NLRP3 inflammasome in response to nigericin. These assays utilized THP1-ASC-GFP cells, a reporter monocyte cell line expressing GFP fused to ASC, an essential inflammasome component. In some experiments, we pharmacologically induced differentiation of the monocytes to become macrophages. Various iterations of these assays, including time-course endpoints and time-lapse video analysis, were employed to assess the efficacy of our client's compounds for preventing or reversing inflammasome priming and activation.
- Most of our imaging assays with THP1-ASC-GFP cells were conducted in tandem with multiplex (10-plex) ELISA experiments to measure secretion of a panel of inflammatory cytokines.
- We extended these assays to other disease-relevant cell types, including human induced pluripotent stem cell (iPSC)-derived microglia. For example, we treated microglia with LPS and nigericin or ATP in the presence and absence of test compounds. At the assay endpoint, we removed cell-culture media for a multiplex ELISA, and then fixed the cells for immunocytochemistry of ASC to identify cells in which inflammasomes had assembled and activated, as measured by ASC speck formation and secretion of IL-1β and other inflammatory cytokines, including TNF-α.

The Results

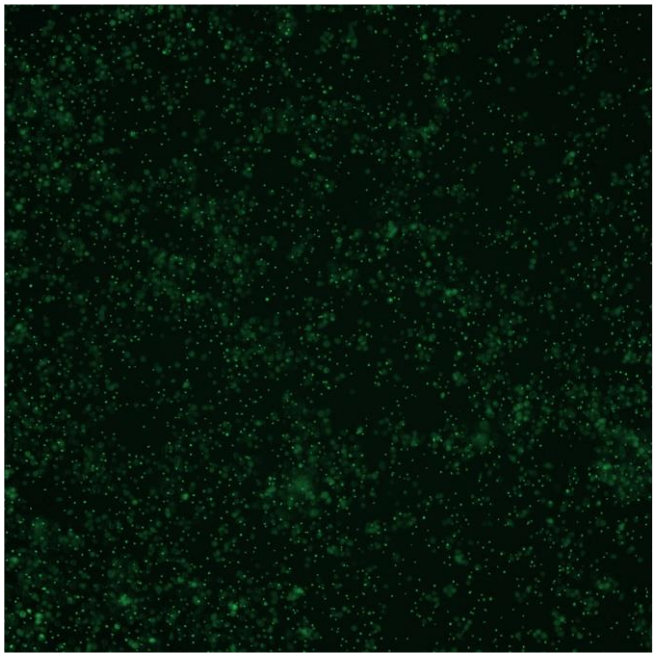
Our efforts contributed significantly to our client's success, helping them secure over $20 million in venture capital. Our time-lapse imaging videos played a pivotal role in demonstrating the inhibitory effects of their lead compound on inflammasome priming and activation. We also provided essential datasets that aided our client in ranking their compounds and selecting a candidate for their Investigational New Drug (IND) application.

Inhibition of NLRP3 Inflammasome Activation in THP1-ASC-GFP Cells

THP1-ASC-GFP cells after LPS priming and Nigericin triggering in the presence of a test compound that prevented ASC speck formation.



THP1-ASC-GFP cells after LPS priming and Nigericin triggering that induced ASC speck formation, as indicated by ASC-GFP specks.



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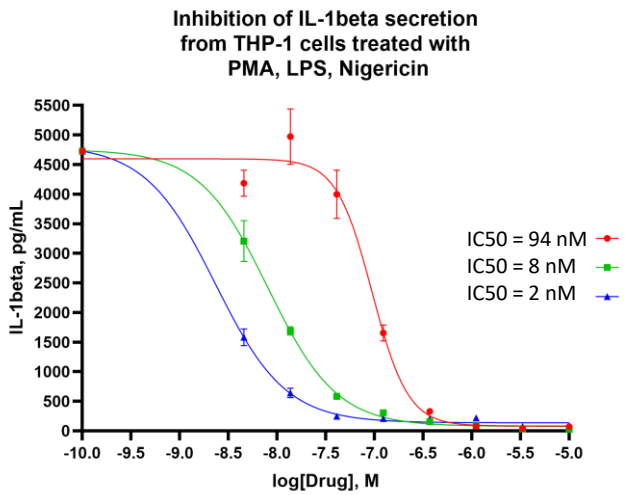
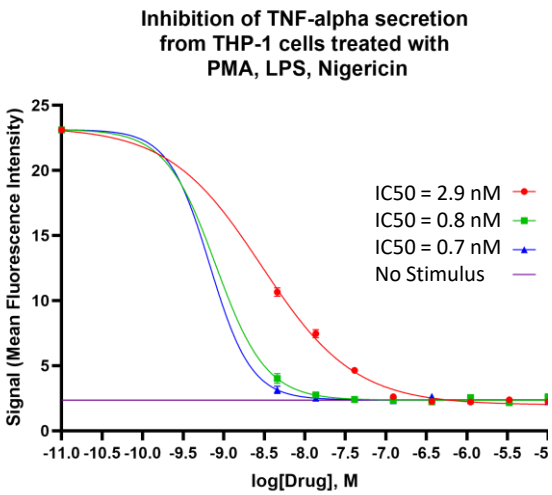
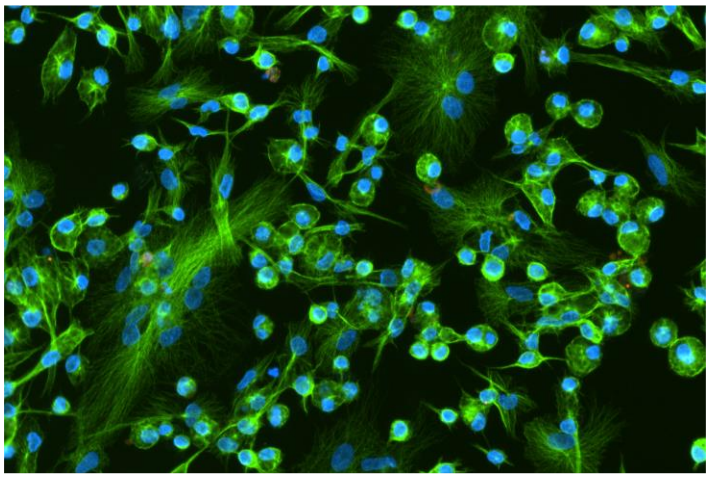




Inhibition of NLRP3 Inflammasome Activation in Human iPSC-Derived Microglia

Human iPSC-derived microglia after LPS priming and Nigericin triggering in the presence of a test compound that prevented ASC speck formation.



Human iPSC-derived microglia after LPS priming and Nigericin triggering that induced ASC speck formation, as indicated by ICC with an ASC antibody (orange dots).