APOL2 & APOL3 proteins

Introduction

The Inflammasome Pathway and the APOL family

When a cell is infected with LPS, the non-canonical inflammasome pathway is triggered. This in turn activates caspase 4/5(in mice) or caspase 11(in human). Caspases are enzymes that have the ability to cleave substrates such as GSDMD and IL-1B. The cleavage of GSDMD causes the formation of pores on the cell, resulting in pyroptosis, during which IL-1B, a proinflammatory cytokine, is released. It is well known that caspase has hundreds of other substrates that are yet to be characterized.

The APOL family constitutes a cluster of 6 genes, most of which are induced by IFN-gamma. APOL1 is the best characterized and is known to be a secreted protein that is part of human serum. Not much is known about the other proteins, but it is established that they are mostly intracellular and lack a secretion signal.

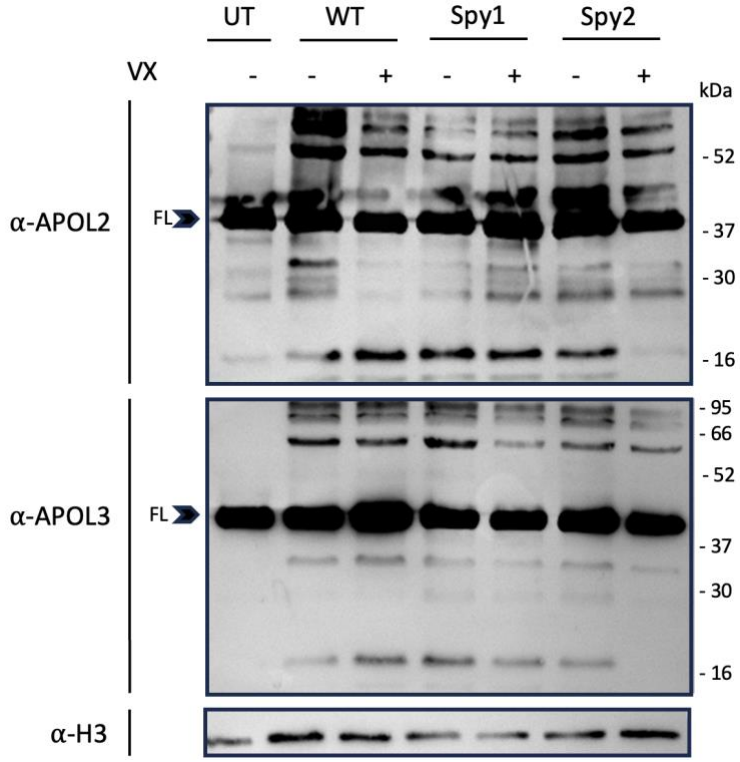
One paper focusing on APOL3, stated that it could restrict bacterial replication. They hypothesized that APOL3 targeted the inner membrane of bacteria and was aided by GBP1 in this process. GBP1, is a protein that can associate with a bacteria containing vacuole, causing the formation of a GBP complex, which can serve as a platform for inflammasome signaling and can facilitate lytic killing. The weakening of the outer membrane by GBP1 is thought to enable access for APOL3 to the inner membrane. However, APOL3 was stated to function independently of the non-canonical inflammasome pathway such that it does not impact it but can occur in parallel. However, my supervisor's proteomics did not show the APOL3 protein. Therefore, we decided to focus on 2 main research questions:

1. Are APOL2 and APOL3 proteins cleaved by caspase?
2. What is the relationship between APOL2 and Caspase4?

To do this, we used methods such as cell culture work, bacterial infection, Western Blots, LDH cell death assay, siRNA techniques and protein purification.

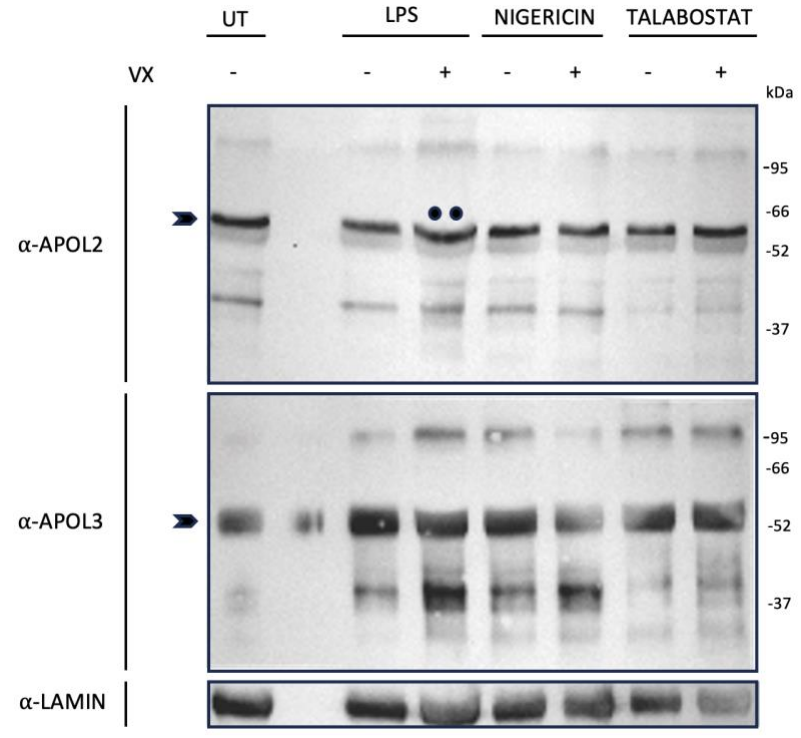
Results

HIEC cells treated with mutated Salmonella.



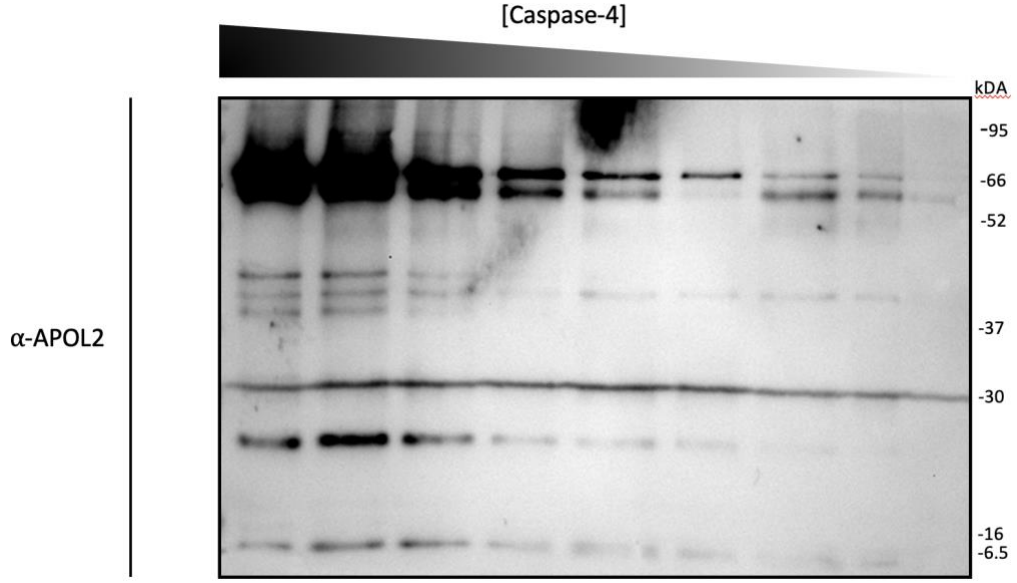
HIEC cells were treated with mutations of Salmonella, namely Spy1 and Spy2. The full length of APOL2(37kDA) and APOL3(44kDA) are clearly visible. There is lots of cleavage with APOL2, suggesting that it is cleaved by caspase.

Neutrophil cells



Neutrophils were transfected with LPS, Nigericin and Talabostat. The choice was made to use neutrophils because they are good for APOL2 protein expression. Nigericin should activate the NLRP3 inflammasome while Talabostat should activate the NLRP1 inflammasome pathway. Full length APOL proteins are not visible but there is cleavage with APOL2.

Purified protein



Western Blot was performed on purified proteins. Different concentrations of pure caspase 4 were added to consistent concentrations of pure APOL2. Full length APOL2 is not visible, however last 2 bands in the blot correspond to cleavage. Strangely, higher concentrations of caspase 4 corresponded to darker and more bands, as opposed to the other way around.

Discussion

It is undeniable that APOL2 is cleaved by caspases, but it seems to present differently in different cells. The above Western Blots suggest that APOL2 and caspase could aggregate together. Alternatively, APOL2 could aggregate with itself to form oligomers, supported by the smearing on the blot. APOL2 hence has an interesting relationship to caspase 4 that requires investigation. Despite having the knowledge of APOL2 being a substrate of caspase 4, downstream activities of the protein remain unclear. Further, it is also clear that APOL3 does not seemed to be cleaved by caspases.