The Molecular Mechanisms of Interaction Between C-reactive Protein and M-ficolin: Implications on Innate Immune Defense.

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ABSTRACT:

Innate and adaptive immunity are two antimicrobial defense systems. Innate immunity is the front-line defense, which protects the host prior to adaptive immune response. The innate immune system consists of phagocytic cells, and antimicrobial and inflammatory proteins. Complement activation is an important and powerful arm of innate immunity. The C-reactive protein (CRP) and M-ficolin are initiators of the complement pathways. From trace levels, the CRP becomes an abundant acute phase protein during inflammation. M-ficolin is a cell-associated pathogen-recognition receptor (PRR), recently suggested to be on the monocyte surface. This project seeks to understand the molecular mechanisms underlying the interaction between CRP and M-ficolin. Here, we confirm the localization of M-ficolin on the monocyte surface. We found that the interaction between CRP and M-ficolin regulates inflammation and antimicrobial effects. We provide evidence for anti- and pro-inflammatory functions of CRP in a pH-dependent manner. M-ficolin shows its essential role in triggering the NF-κB pathway leading to cytokine production. Furthermore, CRP and M-ficolin also probably interplay with other PRRs to potentiate the NF-κB activity.

I. INTRODUCTION

Our innate immune system possesses PRRs that specifically recognize PAMPs which are uniquely displayed on the pathogen surface. The plasma CRP level increases dramatically during infection, which is a well-known PRR. The M-ficolin is coincidentally an acute phase protein like the CRP, expressed in and on the monocytes and neutrophils, able to bind PAMPs and trigger immunity response. Thus, we hypothesize that plasma CRP interacts with monocyte membrane-associated M-ficolin, which activates the NF-κB pathway, leading to interleukin production in an inflammation response.

The objectives of this project are to:

- Characterize the interaction between M-ficolin and CRP under normal and infection conditions.
- Prove the molecular basis of interaction between M-ficolin and CRP and the biological significance through the trigger of the NF-κB pathway

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II. MATERIALS AND METHODS

Human pro-monocytic U937 and African green monkey kidney fibroblast (Cos-1) cell lines were used. To analyze the effect of CRP and M-ficolin toward U937 upon infection, we used IL8, a cytokine that produced by NF-κB pathway as the indicator. IL8 concentration of the cell supernatant was measure by hIL8 ELISA [BD Bioscience]. Besides, we used M-ficolin shRNA [Origene] to knockdown M-ficolin protein in the U937 cell for further characterization the role of M-ficolin in signaling inflammation signal. Using NF-κB luciferase assay [Promega], we checked for the role of M-ficolin in activation of NF-kB pathway.

III. THE MAIN FINDINGS OF THE PROJECT AND DISCUSSION

1. CRP was an anti-inflammation factor at pH of 7.4 but a pro-inflammation factor at pH of 6.5

U937 cell was incubated with different concentrations of CRP, in pH of 7.4 and 6.5. The supernatants were collected 24h later. IL8 concentrations were quantified by ELISA. At pH of 7.4, increasing CRP concentrations lead to decreasing in IL8 concentration. The opposite effect was observed with pH of 6.5. Thus, the effect of CRP in inflammation response was pH dependent.

2. M-ficolin was a membrane-associate protein of monocyctic cell.

Despite controversy about location of M-ficolin in the organism and the fact that M-ficolin apparently lacks the membrane-bound domain, by flow cytometry, we were able to detect M-ficolin on the surface of U937 cell. This finding is consistent with previous finding of Teh et al. (2002).

3. M-ficolin exhibits significant role in inducing NF-κB pathway upon LPS, ReLPS and Lipid A challenge. The effects were not apparent for GlcNAc, teichoic acid and peptidoglycan.

Knockdown M-ficolin cell line and the wild type cell were challenge with different PAMPs for 24h and measured IL8 concentrations in the supernatants. When challenge with lipid A, ReLPS and LPS, M-ficolin knockdown cell type showed reducing of IL8 concentrations in compare to wild type U937 36.64%, 39.19% and 53.52% respectively. No significantly different in IL8 concentrations when the cells challenged with GlcNAc, teichoic acid and peptidoglycan. We conclude that together with Toll like receptor, M-ficolin is a novel receptor for NF-κB pathway that locates on monocyctic surface.

4. M-ficolin probably interplays with others protein to elicit NF-κB pathways:

To separate the effect of M-ficolin toward NF-κB pathway from toll like receptor, we tranfected M-ficolin and NF-κB luciferase plasmid into Cos-1 cell which proved to not carry Toll like receptor. In the infection condition, if NF-κB in the Cos-1 cell is activated, NF-κB luc plasmid would be transcribed to firefly luciferase. Upon addition of subtract, firefly luciferase would give luminescence which would be detectable by
specific device. However, we observed no significant in NF-κB activation level between M-ficolin transfected and non-tranfected Cos-1 cell. We propose that there some protein that were absent in Cos-1 cell lead to disruption of the signaling pathway. Thus, M-ficolin probably interplay with other proteins in activating NF-κB signaling pathway.

IV. CONCLUSION

We have proven that CRP plays dual roles in pro- and anti-inflammatory activities in innate immune response, in a pH-dependent manner. M-ficolin, which is on the surface of monocytes, is triggered by selected PAMPs, to boosts inflammation response by increasing IL-8 production. However, the interaction between M-ficolin and CRP in vivo remains to be confirmed. Other cytoplasmic proteins that interplay with CRP-M-ficolin to potentiate the NF-κB pathway remain to be investigated.

V. FUTURE PERSPECTIVES

It will be imperative to confirm the binding of CRP to M-ficolin in vivo using co-immunoprecipitation. The proteins that cooperate with M-ficolin-CRP complex in inducing the inflammatory response may be identified using yeast-two hybrid analysis. The M-ficolin knockdown U937 cells can enable us to verify other potential factors in the orchestra of CRP-M-ficolin NF-κB pathway.

VI. REFERENCES


Paul Ehrlich the theory of the immune system (1890). Nobel price in Medicine 1908.


** These are selected references. For full reference, please refer to my thesis.