PhD Thesis

The role of factor H-related proteins FHR-1, FHR-3 and FHR-5 in the regulation of the complement system

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Introduction

The complement system as a part of innate immunity is essential to remove pathogens, apoptotic cells and immune complexes from the body. At the same time, it has a crucial role in immune homeostasis by interacting with other physiological mechanisms as well as with the adaptive immune system. The regulation of this enzyme cascade system is therefore indispensable, and is performed by numerous molecules acting at different levels of complement activation. Perhaps the most effective complement pathway, the so-called alternative complement pathway is primarily regulated by factor H (FH). This soluble glycoprotein has the capacity to interact with different, mostly polyanionic, ligands resulting in inhibition of complement activation on cell surfaces as well.

Between 1991 and 2001 a number of proteins that were found to be structurally very similar to FH were discovered and were given the name: factor H-related proteins (FHRs). Based on their homology and high degree of sequence similarity with FH they were neglectfully thought to function as „small FH” molecules. It must be noted, however, that the FHR proteins lack domains related to the complement regulatory domains of FH. Furthermore, in the nearly 20 years since their discovery, several genetic studies have demonstrated their association with different diseases.

It is now indisputable that FHR-1, FHR-3 and FHR-5 are related to various pathologic conditions, including age-related macular degeneration (AMD), atypical hemolytic uremic syndrome (aHUS), C3 glomerulopathies, IgA nephropathy or systemic lupus erythematosus. An example of the genetic findings is the concomitant deletion of the genes coding for FHR-1 and FHR-3. Interestingly, this double ΔCFHR3-CFHR1 gene deletion is a predisposing factor in aHUS, but at the same time it protects from IgA nephropathy or AMD.

The mostly unknown function of the FHR proteins and their association with severe diseases justify research aiming to explore and better understand their role and nature.
Objectives

We planned to examine the function and role of FHR proteins FHR-1, FHR-3 and FHR-5 in physiologic and pathologic conditions. These proteins were chosen due to their association with the diseases mentioned above. Specifically, we aimed to analyze the following:

- We aimed to recombinantly express and purify FHRs, and then examine the interactions of the FHR proteins with pentraxins (C-reactive protein [CRP], pentraxin 3 [PTX3]) and with extracellular matrix (ECM) proteins, that are disease-related and known FH ligands, as well.
- We chose to investigate whether these FHR proteins were able to interfere with (inhibit) the complement regulator function of FH.
- Based on previous results for FHR-4, we wished to analyze whether FHR-1, FHR-3 or FHR-5 could directly activate the alternative complement pathway by binding C3b.
- As controversial observations have been published, we planned to clarify whether these FHR proteins themselves were able to inhibit complement activation at the level of C3 or at the level of the terminal pathway.

The following methods were used: PCR, protein expression in insect cells, protein purification, ELISA, SDS-PAGE, Western blot, C3-convertase and cofactor assays.
Results and interpretation

1. The binding of FHR-1 to CRP, and the analysis of the interaction

- We have shown that FHR-1 binds to the known FH ligand monomeric CRP (mCRP) in a pH- and dose dependent manner. The binding site was localized in the C-terminal part of FHR-1.
- Based on our results, FHR-1 is unlikely to compete with CRP under normal conditions. However, complement deregulation by FHR-1 (by competing with FH for mCRP) might be relevant when local protein concentrations are changed, or when avidities change due to mutations or inflammation. This interaction could potentially take place in the eye of AMD patients, where the inflammatory pH shift might be favorable for the FHR-1 – CRP binding.

2. The binding of FHR-3 to CRP, PTX3 and malondyaldehyde, and characterization of these interactions

- We identified the native, pentameric CRP (pCRP) and PTX3 as new FHR-3 ligands. In our experiments, FHR-3 bound PTX3 retained its capacity to bind C1q. We demonstrated that FHR-3 increased complement activation in human serum.
- We have shown that FHR-3 also binds to the oxidative stress marker malondyaldehyde, and that FHR-3 is able to compete with FH for this ligand, as well.
- Competition of FHR-3 with FH for pentraxins and malondyaldehyde is a way of indirect deregulation of the complement system. This mechanism facilitates complement activation and may be an explanation of the protective role of the ΔCFHR3-CFHR1 double gene deletion in AMD.

3. The binding of FHR-5 to CRP, PTX3 and ECM, and characterization of the interactions

- We identified PTX3, mCRP and ECM as ligands of FHR-5.
• We concluded that FHR-5 could effectively compete in physiological concentrations with FH for these ligands, and that FHR-5 could deregulate complement by that competition, as confirmed by *in vitro* cofactor assays.
• We demonstrated the ability of FHR-5 to modulate complement activation in human serum.

4. The direct role of FHR-1, FHR-3 and FHR-5 in complement activation

• After immobilizing FHR-1, FHR-3 and FHR-5, and adding purified components we detected the formation of the alternative complement pathway C3 convertase.
• We confirmed the generation of the convertase enzymes by measuring enzyme components after incubating the FHR proteins with human serum. This indicates a direct mechanism of complement activation by FHR-1, FHR-3 and FHR-5.

5. FHR-1, FHR-3 and FHR-5 do not have FH-like, complement inhibiting properties

• Based on our *in vitro* assays performed at the level of C3 and at the level of the terminal pathway, we conclude that the analyzed FHR proteins lack FH-like, complement regulatory activity.
Summary

In our research presented in this dissertation, we aimed to assess the function of the factor H (FH)-related proteins FHR-1, FHR-3 and FHR-5 in the course of complement activation. The role of these members of the complement system has been hitherto poorly explored. We examined the interactions of the recombinant FHR proteins with pentraxins, extracellular matrix (ECM) and C3b utilizing in vitro binding assays and complement activation experiments.

We showed that FHR-1 bound the monomeric form of C-reactive protein (mCRP), a known FH ligand. The complement deregulation by FHR-1 (due to competition with FH for CRP) may be relevant when a change in avidity occurs, due to mutation, change in concentrations or during inflammation. The interaction between FHR-1 and CRP may be important in the eye of patients with age-related macular degeneration (AMD), where local inflammation may cause a shift in pH.

We identified the native, pentameric CRP (pCRP) and pentraxin 3 (PTX3) as ligands of FHR-3 and demonstrated the ability of FHR-3 to modulate complement activation when bound to these ligands. We also found that FHR-3 bound to the oxidative stress marker malondialdehyde, and that FHR-3 could readily compete with FH for this ligand. The deregulation, however, cannot take place when FHR-3 is absent due to gene deletion. The complement activating properties of FHR-1 and FHR-3 thus provide a possible explanation for the protective role of the ΔCFHR3-CFHR1 double gene deletion in AMD.

We found that PTX3, mCRP and ECM were new ligands of FHR-5. We demonstrated the competition of FHR-5 with FH for these ligands, as well as the consequent deregulation of complement. We also showed that FHR-5 enhanced complement activation in human serum when bound to pentraxins or to the ECM.

In contrast to previous results, we could not show the complement inhibitory capacity of FHR-1, FHR-3 and FHR-5. On the contrary, we found that they enable the assembly of the alternative complement pathway C3-convertase by binding C3b, and eventually they promote complement activation. Under certain circumstances they are also able to compete with FH ligands resulting in increased complement activation. These FHR proteins should thus be considered as positive complement regulators.
The dissertation is based on the following publications:


Other publications:


Abstracts:


Csincsi ÁI, Pouw RB, Tortajada A, de Córdoba SR, Wouters D, Józsi M. **Factor H-related protein 3 (FHR-3) inhibits factor H binding to pentraxins and malondialdehyde epitopes, and activates the alternative pathway via C3b binding.** *Immunobiology* 2016 10:1177.
