Ph.D. Thesis

Investigation of the complement inhibitory molecule factor H and the pathological role of the FHR molecules

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2019
Introduction:

The immune system is an important defense system of our body. Its main function is to recognize self-, altered self- and foreign structures and to protect against infections. It responds with tolerance to structures recognized as self, while eliminating those (altered) self materials that are recognized as dangerous. Two types of immunity developed during evolution, mediated by the ancient innate immune system and the adaptive immune system. In the case of infection, first the soluble and cellular elements of the innate immune system try to eliminate the pathogen that has entered into the body. A major effector component of the innate immune system is the complement system, which plays an important role in the elimination of pathogenic microbes and altered host cells, and forms a bridge between innate and adaptive immunity. Depending on the activating agent, the complement cascade can be activated via one of three pathways: classical, lectin or alternative pathways. Factor H is the main soluble regulatory protein of the alternative pathway. In addition to factor H, the factor H protein family includes factor H-like protein 1 (FHL-1), an alternative splice variant of factor H and five factor H-related proteins (FHR-1 to FHR-5).

Gene variations in the CFH/CFHR gene cluster may be associated with various diseases. In general, mutations and autoantibodies affecting the N-terminal regulatory domains of factor H result in dysregulation of the alternative pathway in the plasma and on certain surfaces, which primarily causes the deposition of C3 fragments in the glomeruli and associate with C3 glomerulopathy (C3G). Mutations and autoantibodies affecting the C-terminal domains of factor H which are responsible for recognizing different surfaces primarily cause disturbance in the control of alternative pathway activation on surfaces, and are associated with atypical hemolytic uremic syndrome (aHUS). Research on human FHR proteins is of interest due to their link with various diseases identified by genetic testing. However, FHR proteins can also be found in non-human species, such as mice. Another inhibitor of the alternative pathway is the CRIg molecule, which selectively inhibits the alternative pathway C3 and C5 convertases (both in vitro and in vivo).

Factor H mutations, the unknown functions of human and murine FHR proteins, and their relationship with various diseases, as well as the need for the development of effective complement inhibiting molecules justify research that can lead to a better understanding of the function of these proteins.
**Aims:**

During my doctoral studies, we aimed at the functional characterization of the N-terminal, disease-associated factor H W198R mutation, the understanding of its role in physiological and pathological processes; functional examination of the mouse FHR-B protein; and the characterization a factor H-based complement inhibitor molecule. The factor H mutation and the mouse FHR-B molecule were studied because of their relationship to the diseases mentioned above, and the CRIg-FH recombinant protein was designed as a potential complement inhibitory molecule to investigate its potential therapeutic use.

We planned to examine the following:

- The heterozygous W198R factor H mutation associated with two different kidney diseases (C3G and aHUS) was planned to be created as a recombinant mutant protein consisting of the factor H regulatory domains (CCP 1-4) and was to be compared with the wild-type factor H fragment to determine how the mutation affects the factor H regulatory functions.

- The under- or overactivity of the complement system can be caused by various mutations (e.g., in factor H) and autoantibodies, which can result in various diseases (e.g., aHUS, C3G). Of the diseases associated with the complement system, aHUS can be treated by eculizumab, which is the only approved biological agent. Our aim was to investigate the function of the new hybrid protein (CRIg-IgV_FH18-20), as a potential complement inhibitory molecule, in various complement activation assays.

- More and more data are available on human factor H-related proteins, but in order to aid future investigation of the physiological and pathological functions of FHRs in vivo using different animal models, we aimed at the functional characterization of the mouse FHR-B molecule (interaction with C3b and pentraxin molecules, ECM and necrotic cells).

Main methods used in the experiments:

ELISA; SDS-PAGE; Western-blot; PCR; recombinant protein expression and purification; flow-cytometry; surface plasmon resonance; C3 convertase-, complement activation-, cofactor- and hemolytic assays.
Results and discussion 1:

The W198R N-terminal factor H mutation sequentially manifested as glomerulonephritis (probably C3G) and then as aHUS in the same patient. In the patient, the W198R mutation is present in heterozygous form; the mutation did not result in a lack of factor H because the serum level of factor H in the patient was in the normal range.

The FH_{W198R} mutation results in a strong decrease in complement regulatory activity.

- Wild-type (FH1-4_{WT}) and mutant (FH1-4_{W198R}) forms of the factor H complement regulatory domains were produced as a recombinant protein.
- We have shown by ELISA and SPR measurements that the FH_{W198R} mutation leads to a significantly reduced C3b binding, resulting in a decrease in fluid phase cofactor activity, and a decreased activity of the mutant Factor H fragment to accelerate the decay of the surface-bound C3 convertase.
- The mutant fragment was less effective in inhibiting complement activation, i.e. protecting sheep and rabbit red blood cells from complement-mediated lysis than the wild-type factor H fragment.

Our results suggest that the FH_{W198R} mutation leads to an impairment of complement alternative pathway regulation. The heterozygous nature of this mutation may explain the sequential manifestation of two different complement mediated kidney diseases several years apart.
Results and discussion 2:

The CRIg-Factor H hybrid protein inhibits complement activation

- The C3b binding capacity of the CRIg-Factor H hybrid protein was significantly increased compared to factor H.
- CRIg-IgV_FH18-20 is able to inhibit the amount of soluble C5b-9 complex formed by zymosan- and nanoparticles (AmBisome, CrEL, Abelcet) induced complement activation.
- CRIg-IgV_FH18-20 protects sheep and rabbit red blood cells from human complement mediated lysis.

Our results suggest that with this novel complement inhibitor molecule it is possible to inhibit the alternative pathway at the C3 level. Since the three activation pathways merge upon the cleavage of C3 molecule, C3 may be a therapeutic target for modulating the complement cascade.

Results and discussion 3:

Mouse FHR-B promotes complement activation

- We have shown that FHR-B binds to human C3b and competes with human factor H for C3b binding.
- Like human FHR proteins, mouse FHR-B is able to activate the alternative complement pathway and supports the assembly of functionally active C3bBb alternative pathway C3 convertase via its interaction with C3b.
- According to our results, FHR-B is capable of binding to mouse pentraxins (C-reactive protein and pentraxin 3) and extracellular matrix (ECM), and enhances complement activation when bound to these ligands.
- FHR-B, similarly to some human FHR proteins, is able to bind to necrotic cells and to increase the deposition of C3 fragments on cell surfaces.

Our results support a role for the FHR proteins as deregulators of complement activation and antagonists of factor H. We have described new ligands for FHR-B: mouse PTX3, mouse CRP, ECM, and necrotic cells. In addition, our results may help to further develop animal models of various complement-related diseases to study the in vivo functions of FHR proteins.
Summary:

In my doctoral dissertation we examined the role of the complement system from several directions. We searched for the functional consequences of a C3G and aHUS associated mutation in the factor H regulatory domains. We also investigated the function of the FHR-B protein, which has not been studied so far, and whether its role in complement activation is similar to that of the human FHRs. Finally, we investigated the potentially inhibitory effect of a CR1g-Factor H hybrid protein on complement activation. For this we characterized the interactions of recombinantly produced proteins (FH1-4W198R, FHR-B, CR1g-IgV_FH18-20) with C3b, pentraxins, and ECM in ligand binding and various complement activation assays in vitro.

We have shown that the FH\textsubscript{W198R} mutation resulted in decreased C3b binding in ELISA and SPR assays, and the mutant protein had a reduced cofactor activity and a lower rate of decay accelerating activity compared to the wild type protein. The mutant fragment was less effective in protecting sheep and rabbit red blood cells from complement mediated lysis.

Mouse CRP, mouse PTX3 and ECM were identified as ligands for FHR-B. In addition, C3b when bound to FHR-B is capable of generating a functionally active C3bBb convertase on FHR-B. FHR-B was shown to be capable of activating complement and by binding to necrotic cells, increases the deposition of C3 fragments on these cells, similarly to human FHR proteins.

The C3b binding capacity of the CR1g-Factor H complement inhibitor protein was significantly increased compared to that of factor H. We found that the hybrid protein is capable of inhibiting complement activation induced by zymosan or liposomal nanodrugs and it is able to protect the host-like cells from complement-mediated lysis.

Our results contribute to a better understanding of the physiological role of factor H and FHRs, their functions and the pathomechanism of certain diseases. In addition, our results may help to further develop animal models of various complement-related diseases to study the in vivo functions of FHR proteins, to examine them as potential therapeutic targets, and to interpret the results.
Publications related to the Ph.D. thesis:


Other publications:


Conference abstracs and publications:


