Review

Tamas Gyula Fülöp*, Mihály Józsi, Josbert Metselaar, Gert Storm, Laszló Rosivall and Janos Szebeni

The possible role of factor H in complement activation-related pseudoallergy (CARPA): a failed attempt to correlate blood levels of FH with liposome-induced hypersensitivity reactions in patients with autoimmune disease

Abstract: Factor H (FH) is a natural inhibitor of the alternative pathway (AP) of complement (C) activation, an abundant protein in blood whose reduced level has been associated with proneness for increased C activation. There are also 5 FH-related proteins (FHR), which have different impacts on C function. After brief outlines of the C system and its activation via the AP, this review focuses on FH and FHR, collecting data from the literature that suggest that reduced levels or function of FH is associated with C activation-related hypersensitivity reactions (HSRs), called C activation related pseudoallergy (CARPA). Based on such observations we initiated the measurement of FH in the blood of patients with inflammatory bowel disease (IBD) and rheumatoid arthritis (RA), and examined the correlation between FH levels and HSRs following i.v. administration of PEGylated liposomal prednisolone phosphate (PLPP). ELISA assay of FH was conducted on plasma samples before treatment, immediately after treatment and at follow-up visits up to 7 weeks, and an attempt was made to correlate the FH levels obtained with the presence or absence of HSR that occurred in five of twenty patients. However, the initial data presented here on three reactive and three non-reactive patients showed FH levels >600 μg/mL, while the normal range of FH is 2–300 μg/mL. This unexpected outcome of the test led us to realize that the ELISA we used was based on antibodies raised against the short consensus repeats (SCR) in FH, which are also present in FHR. Thus the kit cannot distinguish these proteins and we most likely measured the combined levels of FH and FHR. These initial data highlighted an unforeseen technical problem in assessing FH function when using a FH ELISA that cross reacts with FHR, information that helps in further studies exploring the role of FH in CARPA.

Keywords: chronic inflammatory disease; complement; FHR; hypersensitivity reactions; liposomes.

DOI 10.1515/ejnm-2015-0004
Received January 8, 2015; accepted February 11, 2015

***Corresponding author: Tamas Gyula Fülöp,** PhD Student, Nanomedicine Research and Education Center, Semmelweis University, Budapest 1089 Nagyvárad tér 4, Budapest, Hungary, Phone: +36/305677602, E-mail: fulopgyulatamas@gmail.com; and MIRA Institute, Department of Targeted Therapeutics, University of Twente, Enschede, The Netherlands
Mihály Józsi: MTA-ELTE "Lendület" Complement Research Group, Eötvös Loránd University, Budapest, Hungary
Josbert Metselaar: MIRA Institute, Department of Targeted Therapeutics, University of Twente, Enschede, The Netherlands; and Department of Experimental Molecular Imaging, University Clinic and Helmholtz Institute for Biomedical Engineering, RWTH-Aachen University, Aachen, Germany
Gert Storm: MIRA Institute, Department of Targeted Therapeutics, University of Twente, Enschede, The Netherlands; and Utrecht Institute for Pharmaceutical Sciences, Department of Pharmaceutics, Utrecht University, Utrecht, The Netherlands
Laszló Rosivall: Institute of Pathophysiology, Semmelweis University, Budapest, Hungary
Janos Szebeni: Nanomedicine Research and Education Center, Semmelweis University, Budapest, Hungary

Introduction: A primer in complement and factor H

The complement (C) system

One of the major tasks of the C system is to mark and dispose of potentially dangerous particles such as

Brought to you by | University Library Utrecht
Authenticated
Download Date | 2/18/16 3:28 PM

pathogenic microbes and altered host cells (1). This is achieved by the recognition of and targeted activation on foreign surfaces and modified host targets, such as apoptotic cells. The classical and lectin C pathways are activated upon recognition of certain molecular patterns associated with microbes or altered self, whereas the alternative pathway is activated constantly at a low rate and in an indiscriminative manner. Importantly, complement regulators protect the host from bystander damage.

Artificial surfaces such as stents, cannulae, nanoparticles, liposomes etc. represent foreign surfaces that may cause activation of the complement system. Complement activation can occur via the three above-mentioned pathways and may proceed, if not attenuated by regulators, to the initiation of the terminal pathway. The terminal pathway is activated upon the cleavage of C5, which generates the inflammatory mediator C5a, and C5b, which by binding additional components can result in the formation of terminal complement complexes (C5b-9 or TCC). C5b-9 when inserted in the target cell membrane forms pores (termed membrane attack complex; MAC) that result in lysis.

The alternative pathway of C activation

The internal thioester bond in C3 can undergo spontaneous hydrolysis, resulting in C3(H_2O), which is able to form the C3 converting enzyme C3(H_2O)Bb. This enzyme cleaves C3 molecules into C3a and C3b. C3a is an anaphylatoxin and C3b can bind covalently via its thioester group to nearby hydroxyl or amino groups thus C3b can deposit to target surfaces. In addition, C3b can form fluid phase or surface alternative pathway C3 convertase enzyme (C3bBb). Because each of the three complement pathways leads to the cleavage of C3 into C3b, the alternative pathway can amplify the cascade reaction started by any pathway (called the "amplification loop"). Thus, even if initial activation is due to the classical or lectin pathway, alternative pathway activity may be responsible for the majority of observed total complement activation (1, 2).

Because of this amplification function of the alternative pathway, its proper regulation in the host is particularly important in order to maintain cell and tissue integrity (2). Indeed, a number of complement regulatory proteins, both fluid-phase inhibitors and cell membrane bound proteins, act at the C3 level(3).

This also implies that therapeutic alternative pathway inhibitors, particularly those acting at C3/C3b, such as FH, can effectively down-regulate C3 fragment deposition and terminal pathway activation.

FH structure and function

FH is the main inhibitor of the alternative complement pathway [reviewed in (4, 5)] (Figure 1A). It is a 155-kDa plasma glycoprotein with a serum concentration of ~250 μg/mL. It is a cofactor for factor I in the enzymatic degradation of C3b and also inhibits the C3bBb convertase by preventing its assembly and, when C3bBb already formed, accelerating its decay. Factor H is composed of 20 short consensus repeat (SCR) domains (also termed complement control protein domains, CCPs) (Figure 1B).

The cofactor and decay accelerating activities reside in the N-terminal SCRs 1-4, which also represent one of the two main C3b binding sites within the molecule. The C-terminal SCRs 19-20 contain the second main C3b binding site and this site interacts with the TED domain (residing in the C3d fragment) of the C3b molecule. These domains also harbor a major binding site for cell surface polyanionic host markers, such as sialic acid and glycosaminoglycans. This allows factor H to bind to C3b/C3d deposited on host cell surfaces and thus to discriminate between self and

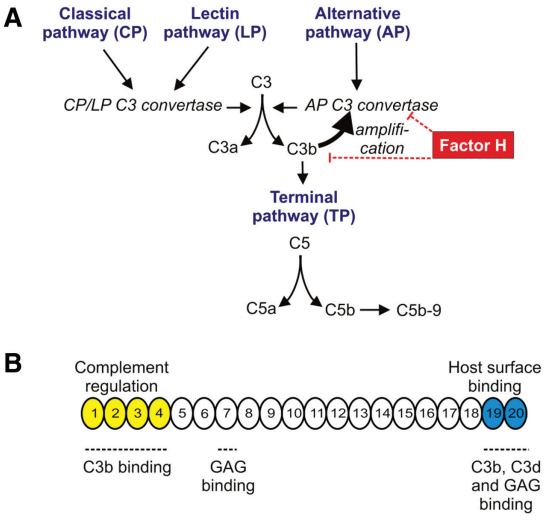

Figure 1: Complement activation and the role of factor H.
(A) Complement is activated via three major pathways, the classical, the lectin and the alternative pathway. Activation generates C3 converting enzymes that cleave C3 into C3a and C3b. C3b feeds into the alternative pathway, allowing an amplification loop of complement activation. C3b also binds to the C3 convertases which then are able to cleave C5 into inflammatory C5a and C5b, which by binding additional components can form lytic C5b-9. Factor H inhibits the AP by facilitating the cleavage of C3b by factor I and the inactivation of the AP C3 convertase. (B) Schematic structure of FH. FH is composed of 20 SCR domains. SCRs 1-4 mediate the complement regulatory functions of FH and SCRs 19-20 allow binding of FH to host cell and tissue surfaces. C3b binding sites are located in the same domains; in addition, glycosaminoglycan (GAG) binding sites are found in SCR7 and SCRs 19-20.

Brought to you by | University Library Utrecht
Authenticated
Download Date | 2/18/16 3:28 PM

non-self (6). Thus, Factor H acts in the fluid phase (i.e., in plasma and other body fluids) and also inhibits complement activation on host surfaces, such as cells and basement membranes, where it can bind via its glyosaminoglycan/sialic acid binding site. In contrast, microbes normally do not express host-like polyanionic molecules and factor H cannot bind to them, allowing C3b deposition unchecked and progression of the activation cascade (4).

FH is the prototypical member of a family of related proteins, which include FH-like protein 1 (FHL-1), a 42-kDa serum glycoprotein derived via alternative splicing from the FH gene, and five FH-related proteins (FHR-1 to FHR-5) that derive from separate genes adjacent to the FH gene (Figure 2) [reviewed in (7)], FHL-1 includes the SCRs 1-7 of FH plus a four amino acid-long unique C-terminal end and possesses FH-like cofactor and convertase decay accelerating activities. In contrast to this, the five FHR proteins lack domains related to the SCRs 1-4 of FH and lack such activities. The FHRs have 4-9 SCR domains that exhibit various degrees of amino acid sequence identity to certain FH domains, ranging from distant homology (e.g., 36–42% of SCRs 1-2 of FHR-1, FHR2 and FHR-5 to SCRs 6-7 of FH) to high similarity (e.g., 95–100% in the three C-terminal SCRs of FHR-1 to FH domains SCRs 18-20). Recent data

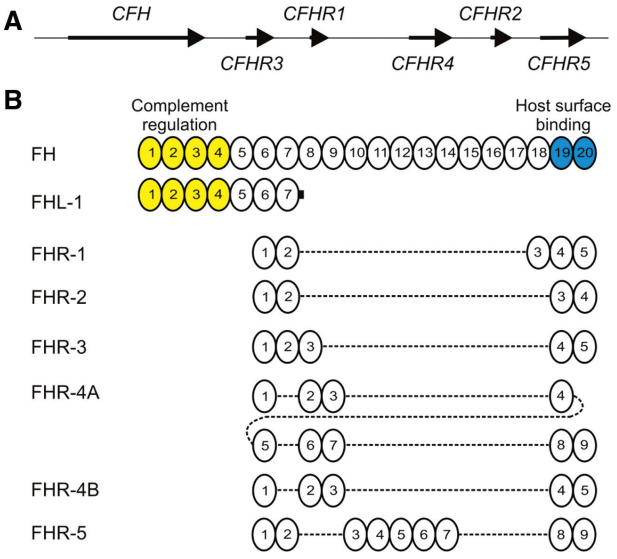

Figure 2: FH-like proteins.
(A) In humans, six genes on the long arm of chromosome 1 encode the members of the FH protein family. The CFH gene gives rise to FH and FHL-1 and five CFHR genes encode the FHR proteins. (B) The domain composition of the FH, FHL-1 and FHR proteins. FH is composed of 20 SCR (or CCP) domains. FHL-1 is identical to the N-terminal SCRs 1-7 of FH plus includes 4 aminoacids at its C terminus. The individual FHR proteins include four to nine SCRs, which are shown in vertical alignment to the homologous domains of FH. The similarity of homologous domains varies from 32 to 100% (not shown in the figure).

suggest that the FHR proteins can antagonize FH function by competing with FH for ligands such as C3b, thus these proteins seem to enhance complement activation through this mechanism termed de-regulation (8–10). Thus, the relative amounts of FH and FHRs as well as their affinities to common ligands and surfaces will determine the degree of AP C inhibition by FH locally. A further important consequence of the existence of these closely resembling proteins is that most antibodies, both monoclonal and polyclonal, raised against FH cross-react with one or more of the FHR proteins and FHL-1. Thus, simple immunostaining or ELISA detecting "FH" may be misleading if there is no precise information available on the cross-reactivity of the applied Abs.

FH deficiency in diseases

There are a number of pathological cases arising from FH dysfunction due to its central role in complement regulation (4, 11). Overactive FH leads to reduced complement activity against pathogenic agents, thus increasing the susceptibility to microbial infections. However, underactive FH can increase unwanted C activity against healthy host cells leading to complement-mediated disease. These pathologies usually are the results of mutations in the protein or polymorphism. As an example, patients with homozygous FH deficiency leading to underactive FH can suffer from recurrent bacterial infections like *Neisseria sp*., furthermore vasculitis and glomerulonephritis. In studies of patients with hypocomplementemic glomerulonephritis heterozygous deficiency with mutations of conserved cysteine residues in SCR 9 and 16 were described. These mutations disrupt intrachain disulfide bridges, thereby perturbing the higher order structure of FH. This leads in turn to a profound selective block in secretion of the FH (12). On the other hand, recent studies showed hyperactivation of the alternative C pathway in neuropsychiatric disorders like schizophrenia: single nucleotide polymorphisms (SNPs) of gene encoding FH was found, CFH rs424535 (2783-526T>A) SNP was positively associated with schizophrenia. Moreover, SNPs rs800292(184G>A) was positively associated with stroke in ischemic stroke studies (13).

FH deficiency correlating with radiocontrast reactions

A thorough survey of the literature on the relationship between FH levels and HSRs in patients led to the group of

Brought to you by | University Library Utrecht
Authenticated
Download Date | 2/18/16 3:28 PM

Phillip Lieberman et al., who published two papers focusing on HSRs to radiocontrast agents (14, 15). Radiocontrast agents are a type of medical contrast medium used to improve the visibility of internal bodily structures in X-ray based imaging techniques such as computed tomography (CT) and radiography. They analyzed the adverse reactions to metrizamide, iohexol, ioversol, iopamadol and concluded that next to penicillin these contrast materials are the most significant causes of anaphylactoid reactions worldwide. According to the quoted statistics, the above iodinated radiocontrast materials caused HSRs in approximately 5–9% of patients treated.

Importantly, among the risk factors that showed significant association with radiocontrast reactions in the studies of Lieberman et al., reduced FH levels or FH dysfunction were listed. Radiocontrast reactions, on the other hand, are known to be caused, at least in part, by C activation (16, 17). The two facts taken together suggest that reduced FH levels or FH dysfunction might sensitize people for not only radiocontrast agent-induced, but all drug-induced CARPA because of reduced natural suppression of C activation via the AP. Consequently, reduced FH levels can be used as a laboratory predictor of i.v. drug-induced CARPA.

FH – as predictor of liposome-induced HSRs

Based on the above theory on the potential use of FH as a biomarker for drug-induced CARPA, we recently carried out a study wherein the protein profile in the plasma of a normal human subject was analyzed, who showed proneness for C activation by liposomal doxorubicin (Caelyx) in vitro (referred to as Caelyx-sensitive plasma, CSP) (18). The aim was to find one or more specific changes that could be considered as a biomarker for increased susceptibility for C activation. The proteome profiling was done with a library of human plasma proteome specific mAbs on chips (PlasmaScan-380TM) that have been printed on a microscope slide-sized glass plate (six/plates), each containing 380 different mAbs raised against non-redundant (with respect to epitopes). The analysis revealed 8 proteins that were differentially represented in CSP in comparison with Caelyx-insensitive control plasma (Table 1).

Among the significant changes we found that the level of FH decreased 1.9-fold in CSP, while the level of FHR was increased 3.4-fold (Table 1) (18). The decrease in FH was consistent with proneness for increased C activation, while the increase of FHR was difficult to interpret.

In another recent study Kuznetsova et al. showed the presence of FH, along with C3b, among the proteins bound in plasma to liposomes loaded with diglycerade conjugates of melphalan and methotrexate (Mlph-DOG and MTX-DOG). Interestingly, neither fragment C3 nor FH was detected in the protein "corona" in the absence or decreased MTX-DOG content, suggesting that the drug has significant impact on C protein deposition on liposomes. It was concluded that the liposome composition defines the surface properties, which in turn determines the set of plasma proteins bound and thus causes inertness or reactivity of liposomes in circulation (19).

The above information on an inverse correlation between FH levels and risk of HSR to radiocontrast reactions, the in vitro preliminary data on FH levels in a

Table 1: Protein representation changes in Caelyx sensitive relative to Caelyx insensitive plasmas.

Protein	Direction of change	Fold change: Caelyx vs. Caelyx insensitive plasmas		
FH	Down	**−1.89±0.03**	**−2.25±0.68**	−1.08±0.05
FH and fHRP	Up	**3.42±0.41**	1.88±1.35	1.03±0.71
Serum amyloid P	Up	**4.40±1.33**	1.78±1.59	**7.53±5.97**
Fibronectin	Up	1.37±0.48	**3.23±0.75**	1.35±0.61
Apolipoprotein B100	Up	1.33±0.12	1.35±0.15	**1.66±0.38**
Alpha-2-HS-glycoprotein	Up	1.66±0.45	2.21±1.98	**2.54±1.62**
C4A	Up	**1.90±0.45**	1.47±1.05	0.86±0.44
Protrombin	Up	1.49±1.09	**2.37±1.10**	1.01±0.89

Values in the table show the averaged global normalized intensity ratio of the different plasma samples (mean±SD). Bolded entries are ratios when the corresponding residuals were higher than 2 × of SD of the calculated residual mean. The table shows the monoclonal antibody array-bound labeled proteins, whose raw and globally normalized pixel intensities were significantly lower (down) or higher ("up") than those in (3 different) Caelyx insensitive plasma samples. These proteins repeatedly showed statistically significant deviation from the microarray's inherent variations in at least two out of 3 similar hybridizations. Their identities were determined via immunoprecipitation using dynabeads, followed by MS and Western blot analyses, as described. Table reproduced from (18) with permission.

Brought to you by | University Library Utrecht
Authenticated
Download Date | 2/18/16 3:28 PM

hypersensitive serum and the information on liposome content influencing FH binding to liposomes led us to analyze samples from the clinical study described below to further our understanding of the role of FH in HSRs to i.v. drugs, including liposomes.

Preliminary results of a clinical study testing the correlation between FH levels in the blood of PEGylated liposomal prednisolone phosphate-treated patients and rise of HSRs

Patients and Samples

Blood samples were collected from patients suffering from autoimmune diseases (inflammatory bowel disease and rheumatoid arthritis), who participated in two clinical trials wherein they were treated with PEGylated-liposomal prednisolone phosphate (PLPP) administered in infusion. All participants provided written informed consent. The clinical trials were approved by the local institutional review board and conducted according to the principles of the International Conference on Harmonisation–Good Clinical Practice guidelines (Clinicaltrials.gov registration NCT01039103, NCT01647685). In total 22 patients were treated with two injections of PLPP (150 mg) with a two-week interval. Plasma samples were taken before treatment, immediately after treatment and at times of their follow-up visits, initially weekly, later two-weekly. Samples were stored at –80C until shipping to Hungary on dry ice for the FH assays, in the framework of collaboration. Further details and ultimate evaluation of the clinical study will be described elsewhere.

FH ELISA

The human FH ELISA is a standard immune assay using primary monoclonal and secondary (peroxidase bound) antibodies which recognize the SCR on the FH molecule (Figure 1). The HK342 Human Complement FH ELISA kits were obtained from TECO*medical* (HK342 Human Complement FH ELISA kit, Hycult Biotech, Uden, The Netherlands). The assay was performed by following the manufacturer's instructions.

Clinical observations

Hypersensitivity reactions were seen in 5 out of 22 patients in these studies. Additionally, two other subjects experienced mild symptoms consistent with an infusion reaction which were not reported as such. Symptoms by which an infusion reaction could be identified were erythema, shortness of breath, itching, flushing and shivers. The infusion reactions were mostly reported as mild and resolved without sequelae after temporarily halting the infusion. In one case a fever was reported (39°C).

Plasma FH levels

After appropriate dilutions, all OD values in the test samples were within the specified effective dynamic range of the assay (5–120 ng/mL) (Figure 3), thus, the ELISA proved to work as expected.

Figure 4A and B show the FH levels in the blood of patients displaying or not displaying HSRs, respectively.

The values did not show any trend in initial levels or subsequent changes that would reflect a role in the rise of HSRs. However, it is important to point out that FH levels were in the 600–3000 μg/mL range. According to literature, while previous studies reported plasma FH concentrations ranging 265–684 μg/mL (20), recent studies using monoclonal antibodies reported mean FH concentrations of 233 μg/mL and 269 μg/mL (21). On the contrary, the FH ELISA kit used in the current study measured 400–800 μg/mL normal range according to its manual. So even if we accept the higher normal range, in the group of patients with HSR, patient 23 had higher baseline (pre-infusion sample) value than the other two shown, and also the samples taken from this patient were abnormally

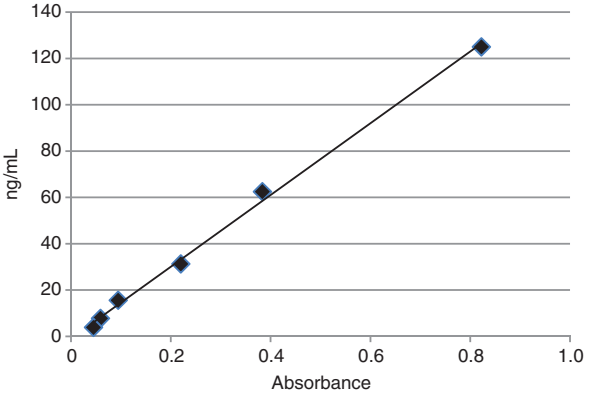

Figure 3: Standard curve of the Hycult FH ELISA kit.

Brought to you by | University Library Utrecht
Authenticated
Download Date | 2/18/16 3:28 PM

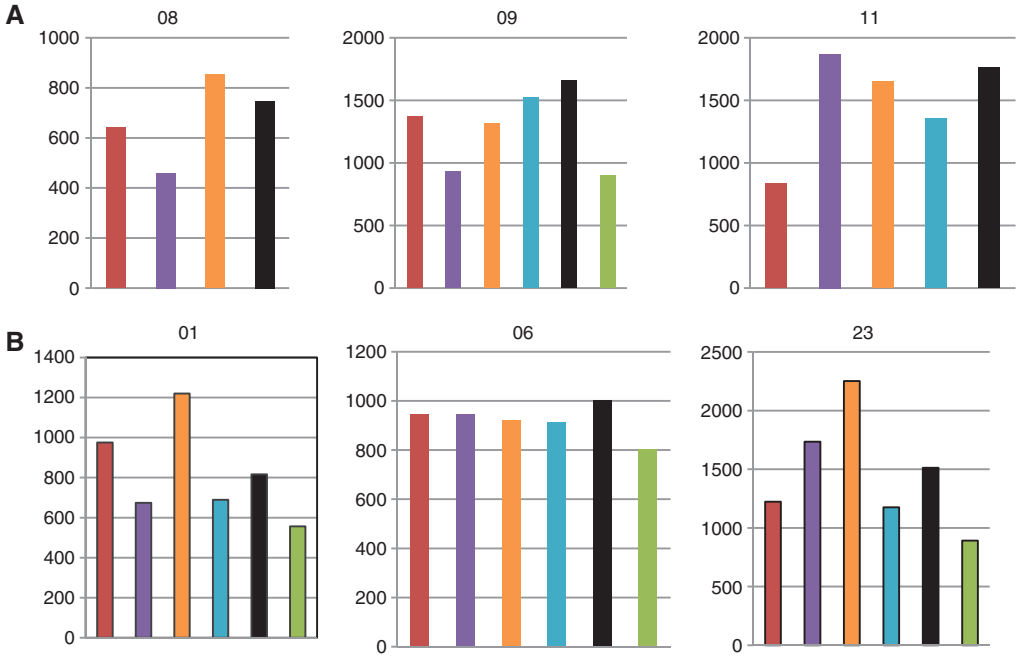

Figure 4: FH levels in patients who did not display HSR during treatment with PLPP (A) and in those who got HSR (B). Samples were taken pre and post treatment, then at 4, 5, 6 and 7 weeks later. Factor H levels were measured in the samples as described in the methods. Color code: Red: Pre Inj., Purple: Post inj., Orange: week 4, blue: week 5, Black: week 6, Green: Week 7. Y values, representing FH concentrations, are given in μg/mL. The numbers above the charts are patient numbers.

higher than the other patients'. At the patient group with no HSR, patient 09 had a high baseline (pre-infusion), and patient 9 and 11 had abnormally high FH values (See Figure 4A and B). These observations can be most easily rationalized by the fact that the ELISA was not measuring only FH but also FHR, which also carry the SCR antigen for which the ELISA was developed. Alternatively, the high FH levels could be attributed to the autoimmune disease of the patients, although the major rise we observed would be unexpected on the basis that autoimmune disases are often associated with FH deficiency (see earlier comments on FH diseases).

Discussion and outlook

Complement activation induced by CARPA-genic materials, such as liposomes and radiocontrast media, may be inhibited by engineered or natural C inhibitors. Therefore, it is plausible that levels of such natural inhibitors like FH in plasma of patients with HSRs can influence proneness to and severity of HSRs. Indeed, previous studies suggested that FH levels may be correlated with severity of HSRs (14, 15). However, determination of FH levels is complicated by the presence of at least six other related proteins (FHL-1 and FHRs) in plasma/serum samples (4). Increased FHR levels could also result in less regulation by FH due to their antagonistic effect on FH function via competition with FH for certain ligands and surfaces. This may explain the association of increased FHR protein level in Caelyx sensitive plasma compared with Caelyx non-sensitive plasma (18).

The FH measurements presented here did not show any possible correlation between FH level changes and HSR occurrence, most likely because the FH ELISA kit measures not only FH but also FHR, which also carry the SCR antigen for which the ELISA was developed. For overcoming of this technical barrier, a truly FH specific ELISA would be needed that excludes any FH-related proteins in the measurements.

Acknowledgments: The work of M.J. is supported by the Hungarian Academy of Sciences (grant LP2012-43). This work was undertaken in the framework of a collaboration agreement between Semmelweis University in Budapest and Twente University in the Netherlands. Support by the Phospholipid Research Center in Heidelberg (grant no. 309.26319) is gratefully acknowledged.

Brought to you by | University Library Utrecht
Authenticated
Download Date | 2/18/16 3:28 PM

References

1. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. Nat Immunol 2010;11:785–97.
2. Thurman JM, Holers VM. The central role of the alternative complement pathway in human disease. J Immunol 2006;176:1305–10.
3. Zipfel PF, Skerka C. Complement regulators and inhibitory proteins. Nat Rev Immunol 2009:9:729–40.
4. Kopp A, Hebecker M, Svobodová E, Józsi M. Factor H: a complement regulator in health and disease, and a mediator of cellular interactions. Biomolecules 2012:2:46–75.
5. Makou E, Herbert AP, Barlow PN. Functional anatomy of complement factor H. Biochemistry 2013;52:3949–62.
6. Blaum BS, Hannan JP, Herbert AP, Kavanagh D, Uhrín D, Stehle T. Structural basis for sialic acid-mediated self-recognition by complement factor H. Nat Chem Biol 2015:11:77–82.
7. Józsi M, Meri S. Factor H-related proteins. Methods Mol Biol 2014:1100:225–36.
8. Goicoechea de Jorge E, Caesar J, Malik TH, Patel M, Colledge M, Johnson S, et al. Dimerization of complement factor H-related proteins modulates complement activation in vivo. Proc Natl Acad Sci USA 2013:110:4685–90.
9. Tortajada A, Yébenes H, Abarrategui-Garrido C, Anter J, García-Fernández JM, Martínez-Barricarte R, et al. C3 glomerulopathy-associated CFHR1 mutation alters FHR oligomerization and complement regulation. J Clin Invest 2013:123:2434–46.
10. Chen Q, Wiesener M, Eberhardt HU, Hartmann A, Uzonyi B, Kirschfink M, et al. Complement factor H-related hybrid protein deregulates complement in dense deposit disease. J Clin Invest 2014:124:45–55.
11. Ferreira VP, Pangburn M, Cortés C. Complement control protein factor H: the good, the bad, and the inadequate. Mol Immunol 2010:47:2187–97.
12. Sculier JP, Coune A, Brassinne C, Laduron C, Atassi G, Ruysschaert JM, et al. Intravenous infusion of high doses of liposomes containing NSC 251635, a water-insoluble cytostatic agent. A pilot study with pharmacokinetic data. J Clin Oncol 1986;4:789–97.
13. Boyajyan A, Ghazaryan H, Stepanyan A, Zakharyan R. Genetic polymorphisms of complement factor H in schizophrenia and ischemic stroke. Mol Immunol 2013;56:294.
14. Lieberman P, Siegle R, Treadwell G. Radiocontrast reactions. Clin Rev Allergy 1986:4:229–45.
15. Lieberman P. Anaphylactoid reactions to radiocontrast materia. Clin Rev Allergy 1991;9:319–38.
16. Szebeni J. Complement activation-related pseudoallergy caused by liposomes, micellar carriers of intravenous drugs and radiocontrast agents. Crit Rev Ther Drug Carr Syst 2001;18:567–606.
17. Szebeni J. Hypersensitivity reactions to radiocontrast media: the role of complement activation. Curr Allergy Asthma Rep 2004;4:25–30.
18. Szebeni J, Kádas J, Lázár J, Takács L, Kurucz I. Plasma proteome profiling with monoclonal antibody libraries: a pilot biomarker analysis for nanomedicine-induced complement activation. Adv Nanopart 2013;2:133–44.
19. Kuznetsova NR, Vodovozova EL. Differential binding of plasma proteins by liposomes loaded with lipophilic prodrugs of methotrexate and melphalan in the bilayer. Russian in Biokhimiya 2014;79:797–804.
20. De Paula PF, Barbosa JE, Junior PR, Ferriani, VP, Latorre MR, Nudelman V, et al. Ontogeny of complement regulatory proteins – concentrations of factor H, factor I, C4b-binding protein, properdin and vitronectin in healthy children of different ages and in adults. Scand J Immunol 2003;58:572–7.
21. Hakobyan S, Harris CL, Tortajada A, Goicochea de Jorge E, García-Layana A. Measurement of factor H variants in plasma using variant-specific monoclonal antibodies: Application to assessing risk of age-related macular degeneration. Invest Ophthalmol Vis Sci 2008;49:1983–90.

Bionotes

Tamas Gyula Fülöp
PhD Student, Nanomedicine Research and Education Center, Semmelweis University, Budapest 1089 Nagyvárad tér 4, Budapest, Hungary
fulopgyulatamas@gmail.com
and MIRA Institute, Department of Targeted Therapeutics, University of Twente, Enschede, The Netherlands

Gyula Tamás Fülöp is a PhD student at the Nanomedicine research and education center at Semmelweis Medical University of Budapest, Hungary. He got his bachelor and also master degree in Medical and Pharmaceutical Biotechnology on the University of applied sciences Krems, Austria.

Mihály Józsi
MTA-ELTE "Lendület" Complement Research Group, Eötvös Loránd University, Budapest, Hungary

Mihály Józsi is working in the field of complement research. He graduated in 1997 and obtained his Ph.D. in immunology at Eötvös Loránd University (ELTE), Budapest, Hungary. He worked as a post-doctoral fellow at the Lebniz Institute for Natural Product Research and Infection Biology, Jena, Germany, where he led a research group between 2006 and 2012. Since 2012, he is a senior research fellow and head of the MTA-ELTE „Lendület" Complement Research Group of the Hungarian Academy of Sciences. His research area is the role of complement, particularly factor H and related proteins, in health and disease. Recently, he is interested in mechanisms of liposomal- and other nanodrug-induced complement activation and possibilities to prevent this by natural and engineered complement inhibitors.

Brought to you by | University Library Utrecht
Authenticated
Download Date | 2/18/16 3:28 PM

Josbert Metselaar
MIRA Institute, Department of Targeted Therapeutics, University of Twente, Enschede, The Netherlands

Josbert Metselaar (Rotterdam, July 6th 1971) obtained a MSc degree in Pharmaceutical Sciences in 1995 and a PharmD (Doctor of Pharmacy) degree in 1998, both at Utrecht University. During his study he completed a research internship in pharmacology and PK/PD modeling at the Dept of Pharmaceutics, University of Florida, US. In 1999 he started his PhD at the Dept of Pharmaceutics and the Dept of Immunology Veterinary Medicine in Utrecht within the scope of a large academic research collaboration funded by the Japanese pharmaceutical company Yamanouchi (nowadays Astellas). He studied novel targeted formulations of anti-inflammatory compounds in autoimmune diseases. After completing his PhD in 2003 and a Post Doc fellowship in the same field of research in 2005, he decided to on translating his academic accomplishments into novel clinical and industrial investigational products. To this end he founded his own University spin off company Enceladus Pharmaceuticals, with which he raised significant funding over the years.

Gert Storm
MIRA Institute, Department of Targeted Therapeutics, University of Twente, Enschede, The Netherlands; and Utrecht Institute for Pharmaceutical Sciences, Department of Pharmaceutics, Utrecht University, Utrecht, The Netherlands

Gert Storm is a (bio)pharmaceutical scientist at Utrecht University. He studied biology and obtained his PhD at the Dept. of Pharmaceutics of the same university. His research interests are in the fields of biopharmaceutics and drug targeting. In his early career, he worked at Liposome Technology Inc. (USA), at the San Francisco (UCSF) School of Pharmacy and at Pharma Bio-Research Consultancy B.V. (Netherlands). In September 1991 he took up his position at Utrecht University. In 1999, he was appointed adjunct professor at the Royal School of Pharmacy, Copenhagen, and since 2009 he has been Honorary Professor in Biomacromolecular Drug Delivery at the University of Copenhagen. In 2000 he was appointed as a professor (Targeted Drug Delivery) at Utrecht University. Since 2012 he is also professor (Targeted Therapeutics) at the MIRA institute of the University of Twente (Netherlands). Besides, he keeps a position at the University Medical Center Utrecht (UMCU) within the Centre for Image-Guided Oncological Interventions.

Laszló Rosivall
Institute of Pathophysiology, Semmelweis University, Budapest, Hungary

Laszlo Rosivall is the head of the department Pathophisiology at Semmelweis Medical University, Budapest, Hungary. He is also the leader of the PhD School of Medical Sciences at Semmelweis, Head of Joint Nephrology Research Group of Hungarian Academy of Sciences and Semmelweis University, and owner of several scientific awards. He got his medical degree in 1973, then his PhD in 1980 at Semmelweis, in 2005 became a member of European Academy of Science and Arts. He led several researches in physiology, pathophysiology, nephrology, hypertension.

Janos Szebeni
Nanomedicine Research and Education Center, Semmelweis University, Budapest, Hungary

Janos Szebeni is the head of the Nanomedicine Research and Education Center at Semmelweis Medical University, Budapest, Hungary. He is an internationally recognized expert in the field of complement research. He has published many research papers in the field and has given numerous presentations at various scientific forums. He also introduced the term Complement Activation Related Pseudoallergy (CARPA), and is editor of the book Complement: Novel Roles in Health and Disease. His work in the past has encompassed a range of topics, including liposome based blood substitutes, HIV pharmacotherapy, and bone marrow transplantation. He has spent most of his professional career in the United States at the National Institutes of Health and other research institutes.

Brought to you by | University Library Utrecht
Authenticated
Download Date | 2/18/16 3:28 PM