Molecular Immunology 72 (2016) 37–48

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Complement factor H modulates the activation of human neutrophil granulocytes and the generation of neutrophil extracellular traps☆

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ARTICLE INFO

Article history:
Received 22 January 2016
Received in revised form 18 February 2016
Accepted 20 February 2016
Available online 1 March 2016

Keywords:
Complement
CR3
Factor H
Extracellular DNA
Neutrophil extracellular trap
Reactive oxygen species

ABSTRACT

Factor H (FH) is a major inhibitor of the alternative pathway of complement activation in plasma and on certain host surfaces. In addition to being a complement regulator, FH can bind to various cells via specific receptors, including binding to neutrophil granulocytes through complement receptor type 3 (CR3; CD11b/CD18), and modulate their function. The cellular roles of FH are, however, poorly understood. Because neutrophils are important innate immune cells in inflammatory processes and the host defense against pathogens, we aimed at studying the effects of FH on various neutrophil functions, including the generation of extracellular traps. FH co-localized with CD11b on the surface of neutrophils isolated from peripheral blood of healthy individuals, and cell-bound FH retained its cofactor activity and enhanced C3b degradation. Soluble FH supported neutrophil migration and immobilized FH induced cell spreading. In addition, immobilized but not soluble FH enhanced IL-8 release from neutrophils. FH alone did not trigger the cells to produce neutrophil extracellular traps (NETs), but NET formation induced by PMA and by fibronectin plus fungal β-glucan were inhibited by immobilized, but not by soluble, FH. Moreover, in parallel with NET formation, immobilized FH also inhibited the production of reactive oxygen species induced by PMA and by fibronectin plus β-glucan. Altogether, these data indicate that FH has multiple regulatory roles on neutrophil functions. While it can support the recruitment of neutrophils, FH may also exert anti-inflammatory effects and influence local inflammatory and antimicrobial reactions, and reduce tissue damage by modulating NET formation.

1. Introduction

The complement system is a major humoral component of innate immunity and plays important roles in antimicrobial defense and in maintaining host homeostasis (Ricklin et al., 2010). Complement components and their activation fragments generated upon triggering of the complement cascade also influence the activation and function of various cells through several receptors (Ricklin et al., 2010).

Factor H (FH) is a major inhibitor of the alternative pathway of complement in plasma and on host cellular and non-cellular surfaces (Ferreira et al., 2010; Kopp et al., 2012; Rodriguez de Cordoba et al., 2004). FH is a 155 kDa glycoprotein that is mainly produced in the liver and reaches a median plasma concentration of ~250 μg/ml (Kopp et al., 2012). FH is also produced locally by several types of cells, such as endothelial cells, monocytes and dendritic cells (Brooimans et al., 1990; Li et al., 2011; Whaley, 1980). FH regulates complement activation at the level of the central C3b component by acting as a cofactor in the cleavage of C3b by factor I and by inhibiting formation and accelerating the decay of the alternative pathway C3 convertase enzyme (Kopp et al., 2012; Rodriguez de Cordoba et al., 2004).

In addition to being a complement inhibitor, there is growing evidence for direct regulatory roles of FH on several cell types. FH has been shown to bind to neutrophil granulocytes via complement receptor type 3 (CR3; CD11b/CD18), and mediate adhesion and cell polarization (Avery and Gordon, 1993; DiScipio et al.,

Abbreviations: CR3, complement receptor type 3 (CD11b/CD18); DHR, dihydrorhodamine; DIC, differential interference contrast; FH, factor H; Fn, fibronectin; HSA, human serum albumin; NET, neutrophil extracellular trap; PMA, phorbol 12-myristate 13-acetate; ROS, reactive oxygen species.

☆ This work was presented in part at the 15th European Meeting on Complement in Human Disease, 27–30 June, 2015, Uppsala, Sweden (Mol. Immunol. 2015, 67: 179.).

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http://dx.doi.org/10.1016/j.molimm.2016.02.011
0161-5890/

1998; Losse et al., 2010). *Candida albicans*-bound FH facilitated fungal recognition and antifungal responses by neutrophil granulocytes (Losse et al., 2010). FH bound to *Streptococcus pneumoniae* was shown to mediate interaction of pneumococci with human neutrophils and epithelial cells, and facilitate the entry into host cells (Agarwal et al., 2010b). Moreover, FH was shown to facilitate adherence of *Neisseria gonorrhoeae* to CR3-expressing CHO-cells (Agarwal et al., 2010a). FH also binds to monocytes, macrophages, B cells and platelets (Hartung et al., 1984; Iferroudjene et al., 1991; Lambris et al., 1980; Vaziri-Sani et al., 2005). FH promotes the uptake of apoptotic cells by macrophages in a non-inflammatory manner (Mihlan et al., 2009) and has a chemotactic function on monocytes (Nabil et al., 1997). It was also shown that CR3 is involved in FH binding to monocytes and FH can inhibit the C1q-mediated uptake of apoptotic cells (Kang et al., 2012). On B cells, FH was reported to inhibit immunoglobulin secretion and cell differentiation (Tsokos et al., 1985), but the B cell FH receptor could not be identified at the molecular level (Erdei and Sim, 1987). However, these non-canonical, cellular roles of FH are poorly understood.

Neutrophil granulocytes are major inflammatory cells and key players during infections, since they provide the first line of host cellular defense (Mocsai, 2013; Nathan, 2006). They are rapidly recruited to infected tissues and have several killing mechanisms to eliminate pathogens (Kolaczkowska and Kubes, 2013). In addition to phagocytosis and intracellular killing, and the release of antimicrobial factors, neutrophils can trap microorganisms by releasing neutrophil extracellular traps (NETs) (Brinkmann et al., 2004; Nathan, 2006). These web-like structures are formed by activated neutrophils and composed of nuclear chromatin associated with nuclear histones and granular antimicrobial proteins (Brinkmann et al., 2004). Thus, NETs probably do not only function as a trap, but they are also able to play a direct role in killing pathogens (Brinkmann et al., 2004; Kolaczkowska and Kubes, 2013). NETs are formed in response to a variety of pro-inflammatory stimuli, such as LPS, IL-8 and TNF-α, as well as several microorganisms (Brinkmann et al., 2004; Remijsen et al., 2011). In vitro, phorbol 12-myristate 13-acetate (PMA) is considered the most potent agent to induce NET formation (Brinkmann et al., 2004; Remijsen et al., 2011). In addition to particularly hyphal forms of fungi (Svobodova et al., 2012; Urban et al., 2006), immobilized, purified fungal β-glucan together with fibronectin as an extracellular-matrix component can also stimulate NET formation (Byrd et al., 2013). However, NETs represent not only an effective protection when phagocytosis is not possible due to the large size of microbes (Branzk et al., 2014), but could also be a potential immunostimulatory agent if NET elimination is not completely performed under non-infectious conditions (Farrera and Fadeel, 2013; Leffler et al., 2013; Mocsai, 2013). In addition to the prolonged inflammatory environment and continuous tissue damage, NETs can contribute to the production of autoantibodies (e.g., anti-dsDNA and anti-histones), which may play a role in autoimmune and inflammatory diseases (Leffler et al., 2013; Mantovani et al., 2011; Saffarzadeh and Preissner, 2013; Sur Chowdhury et al., 2014).

The FH receptor CR3 is also a main receptor for recognizing fungal ligands, including β-glucan, on human neutrophils (Losse et al., 2011; Ross et al., 1987; van Bruggen et al., 2009) and it also plays an essential role in immune-complex induced (Behnen et al., 2014) and β-glucan plus fibronectin-induced formation of NETs (Byrd et al., 2013). The present study was designed to investigate the role of FH in modulating the activation and cellular functions of human neutrophils, particularly the generation of NETs.

2. Materials and methods

2.1. Materials

Purified human FH, C3b, factor I, and polyclonal goat anti-human FH antibody were purchased from Merck Ltd. (Budapest, Hungary). Human iC3b was obtained from Complement Technology Inc. (Tyler, Texas). Bovine serum albumin (BSA) was from Applichem (Darmstadt, Germany) and human serum albumin (HSA) was from Sigma-Aldrich Inc. (St. Louis, MO). Horseradish peroxidase (HRP)-conjugated goat anti-human C3 antibody was obtained from MP Biomedicals (Solon, OH). HRP-conjugated rabbit anti-goat immunoglobulins and goat anti-mouse immunoglobulins were from Dako (Hamburg, Germany).

2.2. Cells

Human neutrophil granulocytes were isolated from peripheral blood of healthy individuals. All blood donors gave informed consent. In some cases, neutrophils were isolated from buffy coats obtained from healthy blood donors and provided by the Hungarian National Blood Transfusion Service. The studies were approved by the respective national authorithy (TUKEB ETT, permission number 838/PI/12). Mononuclear cells were removed by Ficoll-Hypaque (Sigma-Aldrich) density gradient centrifugation, then dextran sedimentation using Dextran T-500 (Pharmacia Fine Chemicals, Uppsala, Sweden) was performed. Red blood cells were lysed in hypotonic buffer. Purity of isolated neutrophils was analyzed by flow cytometry using anti-CD16 and anti-CD14 antibodies (BD Biosciences, Heidelberg, Germany) and was over 95%.

2.3. Colocalization assay

FH binding to neutrophils was analyzed by flow cytometry as previously described (Losse et al., 2010). To measure colocalization between FH and CD11b, 10^6 neutrophils were first incubated with 50 μg/ml FH for 30 min at 22 °C in modified Hank's buffer (142 mM NaCl, 1 mM Na_2SO_4, 5 mM KCl, 1 mM NaH_2PO_4, 1 mM $MgCl_2$, 2.5 mM $CaCl_2$, 5 mM glucose, 10 mM HEPES; pH 7.4). After washing with PBS, Fc receptor blocking reagent (Miltenyi Biotec, Bergisch Gladbach, Germany) was added to reduce nonspecific Ab binding, then a goat anti-human FH antibody (1:500 in PBS containing 1% FBS) was added for 30 min at 4 °C, followed by Alexa-488-conjugated rabbit anti-goat Ig (Molecular Probes-Invitrogen, Carlsbad, CA) for 30 min at 4 °C. For detection of CR3, CD11b was labeled with biotinylated anti-CD11b (clone M1/70.15; Molecular Probes-Invitrogen) and streptavidin-PE (Sigma-Aldrich). The colocalization was quantified by calculating Pearson's correlation coefficients from at least 100 cells in each sample (Adler and Parmryd, 2010).

2.4. Cellular cofactor assay

2×10^6 neutrophils were incubated with 10 μg/ml FH for 30 min at 22 °C in modified Hank's buffer. After washing twice with PBS to remove unbound FH, 3 μg/ml purified C3b and 5 μg/ml factor I were added to the cells in 200 μl final volume in PBS and incubated for 1 h at 37 °C. The supernatants were separated on 10% SDS-PAGE gel, transferred to nitrocellulose membrane and analyzed by Western blot using HRP-conjugated anti-C3 antibody to detect cleaved C3b fragments. As a positive control for cofactor activity, FH, C3b and factor I were mixed together in PBS, without cells. The blots were developed by enhanced chemiluminescence (Merck-Millipore).