

# Potential Influences of Complement Factor H in autoimmune inflammatory and thrombotic disorders

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## List of abbreviations:

aHUS : atypical haemolytic uremic syndrome; AMD: age-related macular degeneration; aPL: anti-phospholipid antibodies; C3-NeF: C3- nephritic factor; CCP: Complement control protein; CEP: Carboxyethylpyrrole; CL: Cardiolipin; CNV: Copy number variation; CR: Complement receptor; DAF: Decay accelerating factor, CD55; DDD : dense deposit disease; ECM: Extracellular matrix; FH: Factor H; FHR: Factor H related; GAG: Glycosaminoglycan; GSL: glycosphingolipids; IAP: integrin associated protein; LA: Lupus anticoagulant; LDL: Low density lipoprotein; LXR $\beta$ : Liver nuclear X receptor  $\beta$ ; MAC: Complement Membrane attack complex; MBL: Mannose binding lectin; MCP: Membrane cofactor protein, CD46; MDA: Malondialdehyde; MPGN2: membranoproliferative glomerulonephritis type2; MSC: mesenchymal stem cell; OSE: Oxidation specific neo-epitopes; PMP: Platelet  $\alpha$ -granules and their micro particles; PNH: Paroxysmal nocturnal haemoglobinuria; PS: phosphatidylserine; RA: rheumatoid arthritis; RCA: Regulation of complement activation; Siglecs: Sialic-acid-binding immunoglobulin-like lectins; SLE: systemic lupus erythematosus; SNP: Single nucleotide polymorphism; TED: Thioester containing domain; TF: Tissue factor; TLR: Toll like receptors; TMA: Thrombotic microangiopathy; Tregs: regulatory T cells; TSP-1: thrombospondin-1; TTP: Thrombotic Thrombocytopenic Purpura; vWF: von Willebrand factor

## ABSTRACT

Complement system homeostasis is important for host self-protection and anti-microbial immune surveillance, and recent research indicates roles in tissue development and remodelling. Complement also appears to have several points of interaction with the blood coagulation system. Deficiency and altered function due to gene mutations and polymorphisms in complement effectors and regulators, including Factor H, has been associated with familial and sporadic autoimmune inflammatory - thrombotic disorders, in which autoantibodies play a part. These include systemic lupus erythematosus, rheumatoid arthritis, atypical haemolytic uremic syndrome, anti-phospholipid syndrome and age-related macular degeneration. Such diseases are generally complex – multigenic and heterogeneous in their symptoms and predisposition/susceptibility. They usually need to be triggered by vascular trauma, drugs or infection and non-complement genetic factors also play a part. Underlying events seem to include decline in peripheral regulatory T cells, dendritic cell, and B cell tolerance, associated with alterations in lymphoid organ microenvironment. Factor H is an abundant protein, synthesised in many cell types, and its reported binding to many different ligands, even if not of high affinity, may influence a large number of molecular interactions, outwith the accepted role of Factor H within the complement system. . Factor H is involved in mesenchymal stem cell mediated tolerance and also contributes to self-tolerance by augmenting iC3b production and opsonisation of apoptotic cells for their silent dendritic cell engulfment via complement receptor CR3, which mediates anti-inflammatory-tolerogenic effects in the apoptotic cell context. There may be co-operation with other phagocytic receptors, such as complement C1q receptors, and the Tim glycoprotein family, which specifically bind phosphatidylserine expressed on the apoptotic cell surface.. Factor H is able to discriminate between self and nonself surfaces for self-protection and anti-microbe defence. Factor H, particularly as an abundant platelet protein, may also modulate blood coagulation, having an anti-thrombotic role. Here we review a number of interaction pathways in coagulation and in immunity, together with associated diseases, and indicate where Factor H may be expected to exert an influence, based on reports of the diversity of ligands for Factor H.

## INTRODUCTION

The complement system has a dual innate immune role. It contributes to the maintenance of homeostasis by disposing of cell debris. On the other hand, complement, through its surveillance and recognition of microbes, mounts a defensive action. Recognition of modified self-tissue by complement may lead to injury and disease when the tissue is unprotected by factor H (FH). Both classical and alternative pathways may take part in this discrimination between self and non-self, substantially by negative regulator FH and homologues (Kajander et al. 2011; Ricklin et al. 2010; Zipfel and Lauer 2013). Besides these complement system related effects, FH can manifest non-canonical properties. Defects and malfunction in complement regulation have been associated with autoimmune, infectious and thrombotic disease susceptibility (Botto et al. 2009; Chen et al. 2010; Zipfel and Skerka 2009).

Complement and blood coagulation systems are evolutionarily and functionally related. Complement, like the coagulation cascade, is activated by successive limited proteolysis of serine protease zymogens and which are usually associated with non-catalytic cofactor proteins (Reid and Porter 1981; Tsiftoglou and Sim 2004; Sim and Tsiftoglou, 2004). There is cross-reactivity between complement and coagulation such as the action of complement C1 inhibitor, a serpin, disabling the production of vasoreactive bradykinin by kallikrein, and inhibiting coagulation factors XIa and XIIa (Davis et al. 2010). The MASP proteases of the complement lectin pathway also activate components of the coagulation pathway (Kozarcanin et al. 2016). A strong common link between diseases such as atypical haemolytic uremic syndrome (aHUS) and systemic lupus erythematosus (SLE) is inflammatory and thrombotic response of vascular endothelial cells, platelets and immune cells. Upon their injury by trauma and subsequent abnormal complement activation on their surface, these cells release pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6, and TNF- $\alpha$ , as well as pro-coagulant tissue factor (TF). TF is induced by complement anaphylatoxin C5a through its G-protein-coupled receptor on neutrophils, which are also chemo-attracted by C5a to an injury site (Ritis et al. 2006). TNF- $\alpha$  can also induce TF and reduce anti-thrombotic activated protein C and thrombomodulin receptor, whose expression inhibits blood coagulation on endothelial cells and platelets (Esmon 2004; Markiewski et al. 2007; Oikonomopoulou et al. 2012; Ricklin et al. 2010).

The complement system encompasses more than 40 secreted and membrane bound proteins, some of which recognise microbial and altered self-molecular patterns. Recognition proteins

include C1q of the classical pathway, and mannose binding lectin (MBL), ficolins and other collectins of the lectin pathway (Carroll and Sim 2011; Kemper et al. 2014; Walport 2001b). These recognition events trigger activation of C1r, C1s proteases and MBL associated serine proteases (MASPs), which initiate the catalytic cascade of C2, C4 effector proteins forming the C3 convertase C4bC2a. This enzyme complex cleaves C3 into active C3b moiety and C3a peptide, and C3b initiates formation of the alternative pathway C3 convertase, C3bBb. The alternative pathway is constantly maintained at a low homeostatic level through C3 autocatalysis *via* a hydrolysed intermediate C3(H<sub>2</sub>O) molecule which forms a C3 convertase C3(H<sub>2</sub>O)Bb, which itself cleaves C3 into reactive C3b and C3a molecules. This process can take place in plasma and on cell and microbe surfaces. The C3 convertases C4bC2a and C3bBb further form C5 convertases C4bC2aC3b and C3bBbC3b, which proteolytically cleave C5 protein into reactive C5b and C5a peptide. C5b propagates the terminal C5b-9 pathway culminating in cytotoxic membrane attack complex (MAC) (Law and Reid 1995; Walport 2001a; Walport 2001b). Cleavage products C3a and C5a are pro-inflammatory and chemo-attractant anaphylatoxins. Thrombin can also directly cleave C5 to produce C5a (Huber-Lang et al. 2006; Meri 2013). In these events, C3b is a cofactor to the serine protease zymogen factor B. The C3bB complex is activated by serine protease factor D; activated factor D cleaves factor B into the active C3bBb serine protease-complex. The classical pathway is also considered to undergo a constant low activation turnover, required in its dual role in the recognition, surveillance of bacteria and self-tolerance (Ricklin et al. 2010). In addition to cell and bacterial lysis by the MAC, the other immediate complement innate immune defence mechanism is microbe opsonisation through complement fragment deposition on the microbe, enhancing phagocytic cell uptake and microbe destruction, such as by macrophages and dendritic cells (DCs). This mechanism also serves for host apoptotic and necrotic cell clearance (Walport 2001b).

The complement proteolytic cascade is negatively regulated by complement control protein (CCP) domain containing glycoproteins, such as soluble FH and C4b binding protein (C4bp), and cell membrane bound complement receptor 1 (CR1/CD35), decay accelerating factor (DAF/CD55), and membrane cofactor protein (MCP/CD46). FH competes with factor B for C3b attachment, thereby limiting formation of the C3 convertase C3bBb. FH can also dissociate a formed C3bBb convertase complex, a process known as C3 decay acceleration (Figure 1) (Hourcade et al. 1989; Weiler et al. 1976). C4bp modulates C4b2a formation similarly to the action of FH on C3bBb. Properdin is a positive regulator of the alternative

pathway, which stabilises complement convertases against their decay by regulators (Fearon and Austen 1975).

C3 is composed of  $\alpha$  and  $\beta$  chains, cross-linked by two disulphide bridges. The  $\alpha$  chain harbours the N-terminal C3a peptide, various ligand binding sites, and a thioester group. C3 protein is activated by convertases, cleaving C3 into C3a peptide and C3b which undergoes conformational activation changes (Bokisch et al. 1975). C3b and C4b molecules are highly reactive, exposing their internal thioester through which they make ester and amide bonds at random, becoming covalently linked with close-by bacterial and host surfaces. C3b and C4b are short-lived, reacting rapidly with water if no surface is encountered (Dodds et al. 1996; Law and Dodds 1996; Sim et al. 1981a). The serine protease factor I cleaves C3b and C4b, only when they are in complex with FH or other regulators including C4bp, CR1 or MCP. C3b  $\alpha$ -chain is cleaved at two sites into 68, 43 and 3 kDa components. The 68 and 43kDa components are still attached to the C3b  $\beta$  chain, by disulphide links, forming a molecule designated as inactivated C3b (iC3b). The iC3b can be further cleaved by FI or by trypsin-like proteases such as plasmin or thrombin into C3dg or C3d and C3c, which dissociate from each other. If C3b is surface-bound, the iC3b and C3dg or C3d formed from it also remain surface-bound. The surface-bound C3 fragments thus covalently tag or opsonise bacterial and unprotected host cell surfaces for complement receptor binding and engulfment by phagocytic cells. C4b undergoes similar degradation by FI, but since C4 is less abundant than C3, surface-bound C4b or C4d are of much lesser importance than the corresponding C3 fragments (Carroll and Sim 2011; Davis and Harrison 1982; Rodriguez de Cordoba et al. 2004; Sim et al. 1981b).

Phagocytic cell complement receptors differ in structure and function. Human CR1 (CD35, C3b/C4b receptor) is composed of 23-37 (length polymorphism) complement control protein (CCP) modules, also called SCRs or Sushi domains, and is expressed on a variety of non-immune and immune cells, but largely on red blood cells capturing immune complexes bearing C3b/C4b for clearance by liver macrophages. By having both C3b and C4b binding domains, CR1 is a potent inhibitor of classical as well as alternative pathway through decay-acceleration of their convertases (Figure 1) (Krych-Goldberg and Atkinson 2001) CR2 (CD21), made up of 15 or 16 CCPs, is a B cell co-receptor for ligating C3dg-C3d/Ag complexes, for the enhancement of specific B cell receptor signalling (Heyman et al. 1990; Ricklin et al. 2010; van Lookeren Campagne et al. 2007; Walport 2001b). Macrophage CR1g,

a member of the Ig superfamily, ligates a  $\beta$ -chain region of C3b as well as of iC3b and C3c fragments, is expressed on resident liver Kupffer cells. Here, it is required for a fast and silent capturing and internalisation of a continuous bulk of pathogens from venous blood flow, in helping to maintain an anti-inflammatory-homeostasis. CR1g is a recycling phagosome traffic receptor, which fuses with, and separates from lysosomes (Bilzer et al. 2006; van Lookeren Campagne et al. 2007).

The integrins CR3 (CD11b/CD18) and CR4 (CD11c/CD18) are phagocytic cell signal transducing receptors for iC3b and its fragments. Through their  $\alpha$ I domains, CR3 and CR4 bind the C3dg region of iC3b, or the C3c region of iC3b, respectively (Bajic et al. 2013; Chen et al. 2012). Macrophage CR3-iC3b/apoptotic cell binding mediates their silent-tolerogenic phagocytosis, and is also engaged in B cell receptor mediated antigen presentation (Bajic et al. 2013). In bacterial infection, this pathway can turn pro-inflammatory, perhaps because bacteria lack surface PS. PS may be a leading co-stimulatory tolerogenic phagocyte ligand (Henson et al. 2001). Other CR3 ligands include  $\beta$ -glycan, ICAM and tolerogenic siglec CD22- BCR co-receptor (Diamond et al. 1991; Ding et al. 2013). FH has also an allotted binding site in iC3b thioester domain (Bajic et al. 2013) and may modulate CR3/iC3b/antigen phagocyte responses. FH also binds to CR3 (DiScipio et al. 1998) and to PS (Tan et al 2010), and so may have complex modulatory effects in this system.

Thus, complement receptors on phagocytic cells are involved in capturing and destroying pathogens, as well as in regulating complement activation together with FH. Expressed on antigen presenting cells (APC) such as monocytes-macrophages and DCs, they may be essential in antigen cross-presentation to adaptive immune cells. This issue is presented in more detail below.

### **Complement FH in self- and non-self-discrimination**

Complement FH (155 kDa) is an abundant and versatile plasma glycoprotein of variable concentration (range 128–654  $\mu$ g/ml) (Ansari et al. 2013) in human plasma. FH is composed of 20 complement control protein (CCP) modules, and is encoded within the chromosome 1q32 Regulators of Complement Activation (RCA) gene cluster, which contains FH related genes (FHR1-5) and its homologues such as C4bp, MCP (CD46), DAF, CR1 and CR2 (Ripoche et al. 1988; Rodriguez de Cordoba et al. 2004; Skerka et al. 2013) as well as plasma transglutaminase (Factor XIII) B chain.

FH N-terminal domains (CCP1-4) have mainly C3bBb decay accelerant and FI-cofactor regulatory function (Figure 1), whereas its CCP19-20 C-terminal domains bind to cell surface C3d thioester containing domain (TED) of the iC3b or C3d molecule, as well as to cellular anionic charge clusters, made up of, for example, of charged glycosaminoglycans (GAGs) or sialic acid in glycans (Aebi and Hennet 2001; Morgan et al. 2011). This direct dual C3-fragment-charge cluster FH cell interaction such as on vascular endothelial cells is required for self-protection from complement.

FH can bind surface-attached iC3b/C3d by two sites within CCP19 and 20, or GAGs with its CCP20 domain, and thus, bind to C3 fragment-bearing surfaces: this interaction increases the apparent avidity for the binding of C3b by the CCP1-5 region to hinder complement activation (Kajander et al. 2011). In contrast, bacterial surface molecules/antigens mainly lack such anionic charge clusters to bind CCP20, and so the overall avidity of FH CCP1-5 binding to the surface C3b is not augmented, allowing complement activation and opsonisation for their phagocytosis, or direct killing by terminal components MAC. Thus, FH aids in discrimination between normal self, altered-self and non-self-molecules (Kajander et al. 2011; Perkins et al. 2012). However, some bacteria have evolved proteins which selectively bind FH, and the bound FH appears to inhibit complement attack on the bacteria. Thus some microorganisms can disguise themselves as “self” by binding FH and/or its homologue C4bp, as discussed below. FHCCP19-20/GAG/C3b-TED complexes have been confirmed by X-ray and NMR structural analysis (Kajander et al. 2011; Morgan et al. 2011). Mutations of FH in its CCP-19 and CCP-20 domains which diminish C3d/GAG attachment are associated with risk for aHUS, and for membrano-proliferative glomerulonephritis type-2 (MPGN2-dense deposit disease DDD) (Figure 3) (Ferreira et al. 2009; Pickering et al. 2007).

Sialic acids, found on the terminal ends of glycans and linked to membrane glycoproteins, are widely expressed in humans and have a variety of biological functions, such as in DC development, antigen presentation and in regulation of B cell signalling (Crespo et al. 2013; Paulson et al. 2012). They engage a Siglec-like-binding site on FH CCP-20 domain, of which the key binding residues are conserved across mammalian species. A large number of FH mutations found in this sialic acid-binding site contribute to HUS susceptibility (Blaum et al. 2015). There is evidence that FH circulates as a compact omega-shaped molecule extending C-terminal CCP 19-20 domains for vascular GAG binding. It is suggested that only upon

such surface adhesion that the molecule opens to fully expose other binding sites and epitopes (Oppermann et al. 2006).

A FH polymorphism in its CCP7 domain increases substantially the risk for age-related macular degeneration (AMD). This polymorphism is replacement of Tyr402 by His; Y402H, sometimes also written as Y384H, depending on whether residue numbering includes the signal sequence (Day et al 1988; Clark et al 2006). CC6-8 is another important functional region of FH, associated with the binding of GAGs (Clark et al 2006; 2013) and surface proteins of many bacteria (Parente et al 2016).

FHR1-5 proteins had been relatively little studied up to about 8 years ago. Many potential roles have now been identified for them (Medjeral-Thomas and Pickering, 2016). In general they appear to oppose the function of FH, by promoting rather than downregulating C3 turnover. They are composed of different numbers of CCP domains, and they contain CCP domains which are very similar in sequence to FH domains 6, 7, 19 and 20. All of them appear to form homodimers or heterodimers, which will increase their binding avidity for surface ligands. They can therefore be expected to compete with FH for binding to some of its CCP6-8 and CCP19-20 ligands. An imbalance between FH and FHR proteins and their polymorphism, can contribute to susceptibility of several diseases, including aHUS and AMD (Skerka et al. 2013; Fritsche et al. 2010) (Figure 2).

### **Non-canonical roles of FH**

FH acts as an immune cell adhesion-migration molecule, recognising a range of cell ligands, including apoptotic cell phosphatidylserine (PS), ribonuclear antigens, extracellular matrix (ECM) proteins such as fibromodulin, osteoadherin and transmembrane signalling integrins (Kopp et al. 2012; Kouser et al. 2013). FH can mediate immune cell activation as well as their suppression. Complement receptor type 3 (CR3, an integrin also called CD11b-CD18,  $\alpha_M\beta_2$ , macrophage receptor-1; MAC-1) has been recognised as a FH receptor, which is expressed on monocytes, macrophages, dendritic cells (DCs), B cells, and neutrophils (Springer et al. 1979). Such FH adherence to human neutrophils *via* CR3 is required for their activation by C5a for oxidative burst and bactericidal effect (DiScipio et al. 1998). In addition, FH and FHR-1 mediate neutrophil adherence of *Candida albicans* for its engulfment and destruction (Losse et al. 2010). CR3 may ligate Fc $\gamma$ RIII for the generation of oxidative burst in neutrophils, as activated by agonist monoclonal antibodies against both

receptors (Zhou and Brown 1994). FH may modulate this Fc receptor function: in lupus prone mice, FH had a protective role in the development of severe lupus nephritis in processing immune complexes in the mesangium and capillary walls, as compared with FH-deficient mice (Bao et al. 2011). Recently, macrophage FH-CR3 interaction has been found to have a protective outcome in chronic immune complex-mediated glomerulonephritis in a mouse model of serum sickness and renal insufficiency; the protection depended on the presence of FH (Alexander et al. 2015). Other reported roles of CR3, presented in the next paragraph, may potentially be modified by FH binding, and since FH is an abundant protein, even low affinity binding of FH may be relevant.

CR3 can suppress TLR-mediated inflammatory signalling in monocytes and macrophages by activating tyrosine kinases, Src and Syk, enhancing MyD88 and TRIF adaptor destruction by ubiquitin ligase Cbl-b (Han et al. 2010; Mocsai et al. 2006). As another example, CR3 is also highly expressed on DCs, apparently mediating through antigen cross-presentation, an antigen specific Th cell type tolerance of a local lymph node draining region. For example, mice deficient in CR3 lacked oral tolerance to an immunising antigen, and developed Th17 inflammatory effector subset in intestinal region (Ehreichiou et al. 2007). CR3 is a marker of myeloid cell lineage, and is highly expressed on mature DCs (Springer et al. 1979). As studied in mice, CR3 is also found abundantly on regulatory DCs (DCreg), which arise in lymphoid organs such as spleen, lung and liver from differentiation of resident haematopoietic stem cell progenitors induced by organ stromal cell microenvironment (Li et al. 2008; Tang et al. 2006; Xia et al. 2008; Zhang et al. 2004). Splenic or lung endothelial-like stromal cells are also able to drive differentiation of mature or immature DCs, respectively, into DCreg cells (Li et al. 2008; Zhang et al. 2004). These DC effects occur on stromal cell contact or their secretion of TGF- $\beta$ , IL-10, or M-CSF. DCreg, in turn, secrete IL-10, nitric oxide (NO), and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) to ameliorate innate and adaptive immune cell-mediated inflammation, and to contribute to maintenance of homeostatic local tolerance (Li et al. 2008; Tang et al. 2006; Xia et al. 2008). DCregs inhibit naïve T cell proliferation induced upon antigen presentation by DCs. These inhibitory effects do not seem to involve anergic DCs, T cells, or Tregs. However, pulmonary DCregs could induce Tregs (Tang et al. 2006). Liver fibroblast-like stroma has been known to promote self-tolerance, and that of liver allografts (Xia et al. 2008). DCregs would seem to arise mainly from innate sources and possess a broad tolerogenic capacity on immune cells. CR3 is regarded a DCreg co-stimulatory molecule, together with CD40, CD80 and CD106. However, its ligand for this

purpose has apparently not been specified (Zhang et al. 2004). Among others, it might be FH or iC3b.

A SNP of CR3 alpha chain (rs1143679, at position 77 replacing Arg by His; R77H) has been associated with a significant risk of developing SLE in people of European and African descent (Nath et al. 2008). Studies with the mutated gene transfected into human and mouse cells, as well as with CR3 alpha knock-out mice, indicated that CR3 mediates B cell tolerance in controlling proliferation of self-reactive B cells (Ding et al. 2013). An allosteric defect in the R77H mutant variant in its propeller domain, impairing its ligand adhesion, has been revealed (Rosetti et al. 2015). CR3 adhesion to B cell-siglec CD22 relies on its sialylation (Ding et al. 2013). FH, as sialylated protein and a ligand of CR3, may potentially influence this receptor adhesion.

Human FH has been shown to be a natural ligand of human leukocyte adhesion molecule L-selectin, which is constitutively expressed on myeloid cells. FH in presence of calcium ions induced leukocyte TNF- $\alpha$  secretion. This FH function depended on its glycosylation (Malhotra et al. 1999). Two other selectin family members are P- and E- selectins expressed on activated vascular endothelial cells. Selectins are, *via* their cytoplasmic tail, cell signalling receptors (Bunting et al. 2002; Malhotra et al. 1999). Selectins together with their ligands strongly contribute together with  $\beta$ 2 integrins to adhesion and migration of circulatory leukocytes such as polymorphs and monocytes into extra-vascular regions for surveillance and an inflammatory response to infection. These events entail circulatory leukocyte tethering, rolling, activation and adhesion on post-capillary venule endothelia for their extravasation (Bunting et al. 2002). Various cytokines and chemokines are involved (Bunting et al. 2002; Zhu et al. 2008). The importance of selectins and of  $\beta$ 2 integrins (including CR3) in leukocyte trafficking became evident in rare hereditary leukocyte adhesion deficiency syndromes (LADs). LADI type is due to polymorphism in CD11/CD18 (leukocyte integrins, CR3, CR4 and LFA-1) receptor expression and structure defects (Guan et al. 2015) LADII results from a defective glycosylation by glycosylases of selectin ligands and other glycoconjugates (Becker and Lowe 1999; Bunting et al. 2002). A patient impaired in GDP-fucose transport into Golgi lumen also exhibited LADII-like symptoms (Lubke et al. 1999) LADI and LADII phenotypes are similar in showing growth/mental retardation, a severe recurrent/persistent infection and sepsis (Bunting et al. 2002; Sperandio et al. 2009). FH may

potentially contribute to myeloid leukocyte response modulation in infection surveillance, involving L-selectins and  $\beta 2$  integrins.

Human bone marrow-derived stromal progenitors, termed mesenchymal stem cells (hMSCs), possess pluripotent property to differentiate into various mesenchymal tissues. These include stromal cells in lymphatic tissues which support differentiation of haematopoietic cells (Majumdar et al. 1998). Besides, hMSCs have a direct broad anti-inflammatory and tolerogenic property in innate and adaptive immune cells by secreting corresponding cytokines and chemokines (Caplan 2009; Ma et al. 2014; Tu et al. 2010). Adult hMSCs constitutively secrete in a paracrine way a high gradient of FH which mediates the anti-inflammatory effects by these and adjacent cells in the microenvironment. Systemic FH produced by liver is ineffective in this aspect. Such FH production by hMSCs may be boosted by  $\text{INF-}\gamma$  and  $\text{TNF-}\alpha$ , and may thereby suppress complement activation (Tu et al. 2010). hMSC are found in most tissues including lymphoid organs and stroma (Ma et al. 2014; Tu et al. 2010). With human B cells, hMSCs inhibited their proliferation and differentiation as estimated by down-regulation of IgG and IgM and chemokine expression (Corcione et al. 2006). In a murine model of myelin oligodendrocyte glycoprotein induced T cell-mediated experimental autoimmune encephalomyelitis (EAE), MSCs substantially subdued nerve damage (Zappia et al. 2005). Human MSCs inhibited monocyte/DC differentiation as well as cofactor expression and IL-12 secretion of mature DCs, which is needed in their cross-presentation of antigens to T cells (Jiang et al. 2005). These events are reminiscent of gC1q receptor suppression of activation of DCs and of T cells (Waggoner et al. 2007). hMSC immunosuppressive and self-tolerogenic effects appear to be pleiotropic, and requiring inflammatory stimuli, such as  $\text{TNF-}\alpha$  (Ma et al. 2014; Tu et al. 2010). Suppression by an augmented FH of complement activation may be a component to regulate an exaggerated inflammation. FH may potentially engage its integrin and L-selectin receptors in conjunction with hMSC effects, in addition to its decay acceleration of C3 convertase.

### **Maintenance of self-tolerance: potential FH modulation of the actions of apoptotic cells, integrins, PS-binding ligands**

It has been suggested that a steady state homeostatic apoptotic cell turnover and clearance by phagocytes may maintain innate and adaptive immune cell peripheral tolerance to self-antigens (Savill et al. 2002; Steinman et al. 2000; Steinman et al. 2003). FH interactions with apoptotic cells, PS, iC3b, integrins and thrombospondin-1 may modulate some of the

relationships described below. The wide inter-individual variation in plasma FH will influence the magnitude of the effects of FH. Apoptotic cells induce, in monocytes and DCs, production and secretion of anti-inflammatory cytokines such as IL-10 and/or TGF- $\beta$ , which are also main tolerogenic factors of Tregs, and IL-10 in B regulatory cells (Tedder 2015). These effects depend on phagocyte cell type, ligand-receptor combination engaged, and cell microenvironment (Savill et al. 2002; Steinman et al. 2000; Steinman et al. 2003). Such apoptotic cell suppression of inflammation is an active process, since it can overcome nuclear factor- $\kappa$ B (NF- $\kappa$ B) dependent pro-inflammatory signalling. This has been demonstrated with human DCs and macrophages, engulfing apoptotic cells opsonised with iC3b, which is a major CR3 and CR4 ligand. Such stimulated macrophages produced IL-10 and suppressed pro-inflammatory cytokines including IL-1 $\beta$  and IL-6 (Amarilyo et al. 2010). A tolerogenic FH effect has been indicated in experiments with human monocytes engulfing apoptotic cells expressing PS. C1q-mediated apoptotic cell clearance via its monocytic C1qR is potentially a pro-inflammatory event. However, in the presence of FH, which ligated its monocyte receptor CR3, the C1q effects were substantially reduced (Kang et al. 2012). These potentially tolerogenic effects of FH on monocytes may be separate and opposing to those of C1q pro-inflammatory collagen domain receptor. The C1q globular domain receptor (gC1qR) may transduce tolerogenic effects *via* Th1 cells (Chen et al. 1994). This regulatory pathway appears to be separate from the DC and monocyte-macrophage tolerogenic responses to their apoptotic cell clearance. Studies with apoptotic cells, and also with *Plasmodium falciparum*-infected red blood cells cleared by human DCs showed such cells to assume an anergic state, failing to stimulate naïve Th cells for antigen presentation. However, in DCs, like in macrophages and neutrophils, this event was mediated by DC vitronectin- $\alpha$ v $\beta$ 3 integrin receptor, in conjunction with scavenger thrombospondin-1 (TSP1) CD36 receptor expressed on DCs (Urban et al. 2001; Savill et al. 1992). Later, it was shown, that TSP-1 protein, expressed on apoptotic macrophages, binds to DCs through its heparin binding domain, to several receptors including CD47 (integrin associated protein-IAP) and  $\alpha$ v $\beta$ 3. This receptor ligation induced in DCs the apoptotic cell phagocytosis, with a tolerogenic state, independently from apoptotic cell phosphatidylserine (PS) (Krispin et al., 2006). On the other hand, PS expressed on red cells binds to TSP-1 heparin-binding domain (Gayen and Setty, 2008). IAP was identified when it was co-purified with  $\alpha$ v $\beta$ 3 from placenta as a complex (Brown and Frazier, 2001). Further studies showed that as a cell ligand of IAP, TSP-1 together with its CD36 receptor signalling, can modulate through IAP, IAP-integrin complex

formation, such as with  $\alpha\beta3$  on macrophages and DCs (Gao et al., 1996a; 1996b). However, for an assembly of these proteins into a functional cell signalling supramolecular complex, IAP needs to recruit heterotrimeric Gi proteins (Frazier, et al 1999). IAP binding to cholesterol is also required, found enriched together with glyco-sphingolipids in cell plasma membrane domains, where assembly of these complexes and cell signalling mainly takes place (Green et al. 1999). TSP-1 can also ligate IAP with platelet specific  $\alpha\text{IIb}\beta3$  integrin, inducing activation (Chung et al. 1997).

As an integrin counter-inhibitory receptor, there exists the immunoinhibitory-signal regulatory protein- $\alpha$  (SIRP $\alpha$ ), which is a receptor for IAP, exhibiting on its cytoplasmic tail four immunoreceptor tyrosine- based inhibitory motifs (ITIMs). Its activation recruits cytosol tyrosine phosphatase src-homology (SHP-1) or SHP-2 domain. Together as a complex, these receptors transmit bi-directionally cell stimulatory and inhibitory signalling. SIRP $\alpha$  may prevail, and substantially contribute to a silent apoptotic cell clearance, by opposing TSP1/CD36 induced cell activation-inflammation (Barclay and van der Berg, 2014). CD47 is an ubiquitous, multiply membrane spanning Ig superfamily protein. It is a marker of normal cell- self. Its phagocytosis is prevented by SIRP $\alpha$  expressed on myeloid cells, through myosin type II deactivation, serving in phagocytosis as part of the cytoskeleton (Tsai and Discher 2008). FH is a high affinity ligand for TSP-1 and is also a ligand of the platelet integrin  $\alpha\text{IIb}\beta3$  (Vaziri-Sani et al., 2005; Carron et al, 1996; Parente et al., 2016).

FH is a major contributor to apoptotic cell iC3b opsonisation for the CR3-mediated tolerogenic-silent phagocyte uptake. As a cofactor to the serine protease FI, it augments conversion of C3b into iC3b on apoptotic cell surfaces (Amarilyo et al. 2010; Bajic et al. 2013). In these processes, complement regulators and receptors may engage other regulators such as the innate cell immunosuppressive TAM-tyrosine kinases Tyro3, Ax1 and MerTK. MerTK has been studied in a silent clearance of apoptotic cells by macrophages. In this task, MerTK relies on its co-receptor-ligands Protein S and growth arrest specific gene 6 (GAS6). TAM receptors function as an inhibitory feed-back to pro-inflammatory TLRs by inducing in phagocytes type-1 IFN- $\alpha$  production and the transcription factor STAT1. TAM receptors are vital in adult homeostasis, but not in embryos (Lemke and Rothlin 2008; Rothlin et al. 2007; van der Meer et al. 2014). Further systems involved are the PS specific T cell immunoglobulin variable (IgV) and mucin domain containing (TIM) family receptors. These are human Tim-1, Tim-3 and Tim-4 (mice also have Tim-2) (Freeman et al. 2010). The key

tolerogenic ligand is apparently PS expressed on early phase apoptotic cell outer membrane leaflet, which is specifically recognised by the Tim family, and is also a ligand of FH. Tim glycoproteins are type-1 transmembrane receptors, with extracellular IgV and mucin domains and cytoplasmic tails, which bear protein tyrosine phosphorylation signalling motifs. Tim-4 lacks this motif, but it is a ligand of Tim-1 (Freeman et al. 2010; Kobayashi et al. 2007; Miyanishi et al. 2007). Tim receptors are differentially expressed on innate and adaptive immune cells, involved in allergies, autoimmunity, self-tolerance and in transplantation allograft sensitivity (Freeman et al. 2010; Kuchroo et al. 2003; Kuchroo et al. 2008; Meyers et al. 2005b).

Tim-1 regulates Th2 cell responses to extracellular parasites by producing cytokines such as IL-4 and IL-13. Tim-1 is a well-known susceptibility gene for asthma, where it appears as a negative regulator (Meyers et al. 2005b). For example, a gain of normal function mutation of Tim-1 protected people from asthma (Sinha et al. 2015) whereas a loss of function polymorphism of Tim-1 was associated with allergic rhinitis (Mou et al. 2010). Allergy and asthma associated with Tim-1 may in some patients be mitigated by a concomitant hepatitis A virus (HAV) infection, which is a ligand for this receptor (Freeman et al. 2010). Tim-1 has also been associated with regulation of autoimmune disease such as multiple sclerosis (MS) and rheumatoid arthritis (RA). In RA patients, a correlation with Tim-1 gene polymorphism has been found (Freeman et al. 2010; Meyers et al. 2005a). Tim-1/Kim-1 (kidney injury molecule 1) is involved in proximal tubular epithelial cell uptake of apoptotic cells, and in their homeostasis (Ichimura et al. 1998). In B cells, Tim-1 is essential for regulatory B cells (Bregs) induction and maintenance in the context of apoptotic cells, which can then promote their tolerogenic effects also in Th2 cells (Ding et al. 2011; Xiao et al. 2015). However, in a B cell subset, Tim-1, expressed together with Tim-4, induces an inflammatory response against allografts and tumours (Mohib et al., 2014).

It has been shown that Tim-1 receptor on Th1 cells, interacting with Tim-4, can invoke a proliferative inflammatory response, producing a large amount of IFN- $\gamma$  and much less of IL-4 and IL-10. In Th2 cells, these cytokine amounts were inverted (Meyers et al., 2005a). Tim-4 is expressed on macrophages and DC (Meyers et al. 2005a). Such a cognate contact may occur at immunological synapse upon specific antigen cross-presentation by DCs to naïve T cells (Echbarthi et al., 2015). It has been demonstrated that Tim-4 can differentially ligate PS on apoptotic cell outer lipid bilayer, such as on functional activated Th1 cells, thus regulating

their number by elimination (Tietjen et al. 2014). This Th1 cell phenotype suppressed self-antigen pulmonary tolerance (Umetsu et al. 2005). As a counter-balanced loop response to spare the host, Tim-3 becomes activated on Th1 cells. Tim-3 is a major negative regulator of inflammation, promoting peripheral self-antigen tolerance, together with its soluble isoform ligand. This protein consists of only the Ig variant region of the full Tim-3 molecule (Sabatos et al. 2003; Sanchez-Fueyo et al. 2003). Recently, it has been indicated that such Th1/Tim-3 tolerance is itself regulated through heterodimerization with the carcinoembryonic antigen cell adhesion molecule-1 (CEACAM-1) (Huang et al. 2015). Tim-3 is also expressed on myeloid cells, such as circulating monocytes, peritoneal exudate macrophages, and CD8<sup>+</sup>DCs, co-expressed with CD11c/CD18 (CR4). It is involved in their apoptotic cell phagocytosis and in the self-antigen cross-presentation to T cells, resulting in their death as a way of self-reactive T cell negative selection (Nakayama et al. 2009).

Tim-4 can be co-expressed on peritoneal resident macrophages (pRMs), together with the FH receptor CR3 and with MerTK (Meyers et al. 2005a; Nishi et al. 2014) suggesting a cooperation in a silent apoptotic cell clearance. MerTK may be the tolerogenic component in these receptor combinations. In DC-mediated apoptotic cell clearance, MerTK inhibited pro-inflammatory NF- $\kappa$ B signalling for their tolerogenic phenotype (Sen et al. 2007). Engulfment by murine peritoneal resident macrophages of apoptotic cells proceeded in two steps. Firstly, Tim-4 binds apoptotic cell PS for adherence to the cells, followed by their MerTK mediated phagocytosis (Nishi et al. 2014). Most likely, MerTK also inhibited the macrophage pro-inflammatory cytokine production. It was reported previously that apoptotic cells induce in macrophages such effects (Cvetanovic and Ucker 2004). Human blood macrophage apoptotic cell clearance through CR3 inhibited NF- $\kappa$ B signalling induced by zymosan (Amarilyo et al. 2010). Apoptotic cell TSP-1 heparin binding domain induced in DCs a tolerogenic state (Krispin et al. 2006). Potentially, a MSC paracrine FH may mediate its tolerogenic effects through the receptor, CR3 or by ligating TSP-1 of macrophages and DCs.

There are further apoptotic cell/PS binding receptors in macrophages and DCs such as MFG-E8 (milk fat globule EGF factor VIII), which is able to synergistically work with Tim-4, to induce immunosuppressive effects (Miyanishi et al. 2012). In mice, blockade of Tim-4 as well as of Tim-3 by monoclonal antibodies resulted in delayed apoptotic cell phagocyte uptake and in appearance of anti-cardiolipin and anti-dsDNA autoantibodies (Kobayashi et al. 2007; Miyanishi et al. 2007; Nakayama et al. 2009; Nishi et al. 2014).

Tim-3 appears to co-operate with the gC1qR, which is also expressed on Th1 cells. gC1qR inhibits Th1 proliferation, similarly to that by Tim-3 (Carroll 2004; Chen et al. 1994; Ghebrehiwet et al. 2014; Yao et al. 2008). gC1qR may contribute to self-tolerance as a co-ligand to Th1 cells, regulated by CEACAM1/Tim-3 complex adhesion, by inhibiting Th1 cell differentiation through suppressing IL-12 production by DCs (Huang et al. 2015; Waggoner et al. 2007). Viruses, including hepatitis C (HCV) and HIV, exploit this gC1qR negative pathway for their disease chronicity (Waggoner et al. 2007; Yao et al. 2008).

Early phase PS recognition is important for a prompt and silent clearance of apoptotic cells, before they release apoptotic blebs containing inflammatory-immunogenic molecules or undergo secondary necrosis. *In vivo* studies found that macrophages, through engulfing apoptotic cells in a PS-dependent manner, secrete TGF- $\beta$ 1 which promoted wound healing (Huynh et al. 2002). PS, an anionic phospholipid, is an integral part of cell membrane lipid structure, expressed in their inner leaflet. On an injury, infection, apoptosis or physiologically, this asymmetry is perturbed by scramblase, distributing PS also on the outer membrane of the lipid bilayer (Zwaal et al. 2005).

Tim-1 is a marker of regulatory B cells (Bregs) (Tim-1<sup>+</sup> B cells), but is also expressed temporarily on other B cell types. As studied in mice, activated through its ligation by a natural specific antibody, Tim-1 may promote peripheral tolerance (Ding et al. 2011). Tim-1 was found to be paramount in sustained production by Bregs of IL-10 in constraining inflammatory processes. It binds apoptotic cells to Bregs, which is needed for their IL-10 production (Xiao et al. 2015). Tim-1 mucin domain defective mutant mice exhibited a massive mononuclear cell infiltration in various organs. Their B cells produced inflammatory IL-1 $\beta$  and IL-6 cytokines, instead of normal IL-10. They induced Th1 and Th17 type expression, while inhibiting Foxp3<sup>+</sup> Tregs. Such B cells aggravated murine EAE, in contrast to normal Bregs, which ameliorate this brain pathology (Xiao et al. 2015). Such Tim-1- Breg-EAE suppressive effects might be similar to those of MSCs in anergic T-cells, expressing FH (Zappia et al. 2005). CR3 is also involved in B cell receptor (BCR) mediated tolerance maintenance (Ding et al. 2013).

Tim-3 has divergent functions expressed on Th1 cells, and possibly on B cells, it suppresses inflammation. On innate immune cells, where it can be constitutively expressed, including monocytes, microglial cells, DCs, it promotes inflammation, e.g. in microglial border region with experimental autoimmune encephalomyelitis (EAE) lesions (Anderson et al. 2007;

Monney et al. 2002; Sanchez-Fueyo et al. 2003). In these effects, its suppression or activation is aided by its cellular ligand galectin-9. For example, in antigen presenting DC-CD11b<sup>+</sup>, it cooperates with TLRs, inducing NF-κB transcription and pro-inflammatory cytokines (Anderson et al. 2007). Such inflammatory burst is needed for their maturation, but can be afterwards suppressed by engulfed apoptotic cells, which induce IL-10 production in DCs (Urban et al. 2001). Tim-3 was the first family member to be discovered on Th1 cells in which it down-regulates IL-2, IFN-γ, TNF-α and lymphotoxin production, associated with intracellular pathogens and with organ-specific autoimmune diseases. It promotes Tregs mediated self-immune tolerance (Meyers et al. 2005b; Monney et al. 2002).

*In vivo* studies demonstrated that Tim-3 as well as Tim-4 are vital in a steady state clearing of apoptotic cells by ligating their PS. Blockade by monoclonal antibodies against Tim-3, but also against Tim-4, induced in mice dsDNA autoantibodies (Nakayama et al. 2009). These studies also revealed that Tim-3 is important in antigen cross-presentation. Apoptotic cell antigens captured by splenic lymphoid CD8<sup>+</sup>CD11c<sup>+</sup> DCs are presented at immunological synapses to naïve Th cells together with MHC class I molecules for induction of specific cytotoxic CD8<sup>+</sup> T cells. However, such self-reactive cell production can be abortive, followed by their destruction in lymphatic tissues, which is a way of self-tolerance maintenance. Monoclonal anti-Tim-3 antibodies reduced such cross presentation (Nakayama et al. 2009). Similarly, a specific T-cell tolerance was induced upon DC-CD11c<sup>+</sup> presentation to an antigen, coupled to apoptotic cells (Ferguson et al. 2002). Interestingly, PS was transiently expressed on human CD8<sup>+</sup> T cells upon antigen stimulation, which may serve in their adhesion in immunological synapse (Fischer et al. 2006). Such cell-cell cognate immunological synapses are organised and regulated by tetraspanin CD81 (Rocha-Perugini et al. 2013).

Another PS receptor for an enhanced clearing of apoptotic cells is the brain-specific angiogenesis inhibitor-1 (BAI1) which is also expressed in spleen, bone marrow, and constitutively in monocytes. BAI1, an adhesion type G-protein-coupled 7 transmembrane receptor, ligates PS through its extracellular five thrombospondin type 1 repeats. Apart from PS recognition, BAI1 also mobilizes Rac protein for cytoskeleton actin-mediated internalization of apoptotic cells by human monocytes-macrophages (Park et al. 2007). There seems to be only indirect evidence for a connection of Tim receptors with FH, because FH may interact in an inhibitory fashion with CR3 which is co-expressed on macrophages with

Tim-4. Experiments on this possible connection- signalling complex formation appear to be lacking. Similarly, FH may potentially interact on B cells with CR3 and influence this integrin ligation with inhibitory CD22 receptor (Ding et al. 2013). A possible link with Tim-1-B cell regulation has apparently not been studied (Xiao et al. 2015).

FH-PS binding may act as an apoptotic cell bridging reaction, provided via the integrin CR3, an anti-inflammatory phagocytic component, in conjunction with iC3b-CR2 ligation. This potential FH binding may be enhanced by TSP-1 binding. FH additionally binds late phase apoptotic cell nuclear proteins and lipids mainly through their attached-adducted neo-epitopes such as malondialdehydes (MDAs), which are apoptotic bleb peroxidation products (Weismann et al. 2011).

### **Potential Factor H roles in innate-adaptive B cell immunity**

Immunological studies have been concerned with T cell and B cell self-antigen responses such as tolerance, anergy, and negative selection of auto-reactive B cells, *versus* positive B cell selection against pathogen antigens, in which DCs play a prominent role (Steinman et al. 2000). B cells are mainly regulated through their antigen specific membrane IgM-, IgG or IgD-B cell receptors (mIg-BCRs), and through BCR positive and negative co-receptors, these interactions taking place in lymphatic organs. These include CR1 (CD35), CR2 (CD21) and CR3 (CD11b/CD18) involved in antigen presentation to mIg-BCR by DCs, or directly by macrophages (Bajic et al. 2013). Further stimulatory co-receptors are CD19, and Bruton tyrosine kinase (Btk). These are opposed by negative Siglec-CD22 co-receptor of mIg-BCRs, associated with CR3 ligation (Ding et al. 2013). These co-receptors are, or involve mainly tyrosine kinases, and protein tyrosine phosphatases, respectively, and inhibitory tyrosine motif harbouring molecules (Carter and Fearon 1992; Dempsey et al. 1996; Sinclair 2000). B cell progenitors undergo several differentiation stages in bone marrow, and following on their migration to peripheral lymphatic organs, from immature to mature stages with plasma cell differentiation, or becoming antigen memory cells. Early human precursors abound with self-reactive B cells, most of them are being BCR edited, or negatively selected through their apoptosis (Tiegs et al. 1993; Wardemann et al. 2003). Even so, 30-40 % of such self-antigen reactive B-cells escape these mechanisms, and their activation signalling has to be constantly kept at bay by negative mIg-BCR co-receptors such as CD22 in conjunction with CR3, or by B-regulatory cells by inducing their anergy as part of peripheral tolerance (Ding et al. 2013). Induction of tolerogenic IL-10 is found in a B cell subset with tolerogenic property, and in

Bregs (Tedder 2015). An early broad antigen reactive B cell subset, designated B1, is an important immediate first line bacterial defence (Hutzler et al. 2014). Conventional B2 cells at their transition stages, changing antigen IgH<sup>-</sup> IgM<sup>hi</sup> -BCR to IgH<sup>+</sup> IgM/IgG- BCR type, is critical for their survival. Newly described membrane molecule Dickkopf-3 modulates B cell differentiation, antibody production and antigen specific recall of memory B cells. It can also inhibit CD8 T cells (Ludwig et al. 2015). Upon their stimulation through cross-linking mIg-BCR receptors by their specific antigens, immature B cells are prone to apoptosis, whereas mature type B cells respond with their activation of differentiation markers and proliferation. There is a plasticity in such intrinsic developmental dichotomy, influenced by outside factors such T cell dependence (King and Monroe 2000; Petro et al. 2002).

In antigen presentation to mIg-BCRs in draining lymph nodes, both follicular DCs (FDCs) and resident macrophages may be engaged. FDCs, upon their tissue migration antigen sampling and their processing, present antigen/C3dg covalent complexes. This process occurs on a close contact-interaction with B cell surface co-receptor CR2 (CD21), together with antigen specific mIg-BCRs. CD21 captures the C3dg moiety of the complex, thus facilitating antigen binding to its specific mIgM/IgG-BCR, which appears in up to hundred copies on a B cell membrane. For an effective B cell proliferation, a cross-linkage of several mIg-BCR copies is required. An avidity excess 3/1 C3dg/antigen ratio may be optimal. This is especially valid in response to a primary infection, where mIg-BCR activation threshold can be lowered up to 10,000 times by such cross-linking (Carroll 2004; Carter and Fearon 1992; Dempsey et al. 1996). In addition, lymph node sub-capsular sinus macrophages express CR3/iC3b-C3dg TED/antigen complexes which may be presented directly via CD21 ligation to specific mIg-BCRs. These complexes have been visualized by crystallography. The resident macrophages store a variety of antigens which can be presented by FDCs to B cells, utilising CR1 and CR2 (Bajic et al. 2013; Carroll 2004; Fang et al. 1998). This may be a way to present the CR3 $\alpha$ I-iC3b linkage of an antigen complex to B cells, which may be tolerogenic to self-antigens (Bajic et al. 2013).

Upon antigen binding and cross-linking, a mIgM/IgG-BCR complex becomes activated by phosphorylation of its two cytoplasmic co-receptors CD79 A and B via signalling molecules Src-family protein tyrosine kinases (PTKs), including Lyn, Vav, and Syk (p72<sup>Syk</sup>). This stimulation is reciprocal with that of co-receptor CD19 tyrosine molecules on its cytoplasmic domain, which ligates PTKs, and thus, augments mIg-BCR signalling for T cell-dependent

antigens (Fujimoto et al. 1999; O'Rourke et al. 1998). For these positive antigen/C3d effects, a cognate ligation of CD19 by CD21 and BCR is needed, but which may become signal suppressive with a higher concentration of C3d/specific antigen complexes (Lee et al. 2005). Antigen mIg-BCR activation of tyrosine kinases is also required in the stimulation of Burton's tyrosine kinase (Btk), as well as of negative co-receptors CD22, IgG-FcR2 and CD72. In addition, CD19/Vav complex can also activate phosphatidylinositol-3 kinase and phospholipase-C for intracellular  $Ca^{2+}$  release, as well as ERK2 and MARK pathways for antigen-specific B cell proliferation and differentiation (Li and Carter 2000; O'Rourke et al. 1998; Rickert et al. 1995). mIg-BCR inhibitory co-receptors possess on their cytoplasmic tails conserved tyrosine pattern, termed immune receptor tyrosine-based inhibitory motifs (ITIMs). mIg-BCR Lyn phosphorylated-ITIMs recruit protein tyrosine phosphatases SHP-1 and Grb2 which dephosphorylate tyrosine kinases from their substrates (Fujimoto and Sato 2007; Otipoby et al. 1996). CD22 glycoprotein belongs to the sialic-acid-binding immunoglobulin-type lectin (Siglecs) family, which together with Siglec-G in mice (human ortholog Siglec-10), control B-cell tolerance against self-reactive B cells producing anti-dsDNA and other autoantibodies (Muller and Nitschke 2014). CD22 is expressed on mature mIgD<sup>+</sup> mIgM<sup>+</sup> BCR cells. CD22 substrates are sialylated glycans and are thymus-independent (Otipoby et al. 1996). CD19 co-receptor is directly linked with Lyn-BCR-SHP-1 pathway by the SHP-1 negative loop, opposing BCR protein tyrosine kinases A and B activation, and that of CD19. In this way, these factors may maintain a B cell activation threshold (Fujimoto et al. 2000; Fujimoto and Sato 2007).

CR3 subunit CD11b has been recognized as a tolerogenic co-receptor, in conjunction with CD22, in subduing autoreactive B cells (Ding et al. 2013). CR3 adheres, owing to its sialylated glycans, to BCRs and to its negative co-receptors and signalling factors such as CD22, while stabilising their mutual ligation. This CR3-CD11b glycan function is lost due to the SNP exchange of arginine for histidine residue 77 (R77H). This CD11b mutation is found in European, African, Asian, and South American people, conferring a risk to develop SLE (Nath et al. 2008). On the other hand, Btk following activation by mIg-BCRs sets a threshold for B cell survival. *Btk* over-expressing transgenic mice develop autoreactive B cells and SLE-like pathology, exhibiting anti-nuclear antibodies and resistance to FAS apoptosis (Kil et al. 2012). *Btk* deficiency can result in X-linked agammaglobulinaemia (XLA) (Kil et al. 2012).

In aspects of antigen presentation and mIgBCR cross-linking, it has been proposed that such mIg-BCR signalling amplification mechanisms distinguish between harmful bacterial and innocuous self-antigens (Carter and Fearon 1992). FH unprotected microbial antigens are much more likely to be tagged by C3dg fragment in order to engage their specific B cells for their proliferation, antibody production and memory recall (Rickert 2005). In contrast, B cells may respond to self-antigens *via* their negative CD22-CD11b/CD18 co-receptors, inducing a tolerogenic B cell state (Ding et al. 2013). A distinction in tolerogenic PS expression on host cells, and not on bacteria, may also come into play (Henson and Bratton, 2001). FH also binds to sialylated glycans, PS, iC3b-C3dg-TED, and to CR3, and may thus modulate B cell responses to antigens (Bajic et al. 2013; Tan et al. 2010). B cell subsets, like other immune cells, can assume a tolerogenic phenotype for their balance in B cell responses (Tedder 2015). FH has been shown to inhibit poke weed mitogen or Epstein-Barr virus -activated human B cells in their differentiation and Ig secretion, but not for their proliferation (Tsokos et al. 1985). By an analysis of tonsil B cell FH ligation, a possible receptor for FH on these and Raji cells has been characterized (Erdei and Sim 1987). FH interactions with GAGs and ECM proteins in self- protection and in cell receptor ligation may be fortified by coagulation factor FXIII transglutaminase, making covalent cross-links between such molecules.

### **Complement FH crosslinking by coagulation factor XIII**

Blood coagulation factor XIII (FXIII), a circulating transglutaminase zymogen, was firstly found in stabilizing blood clots (Laki and Lorand 1948). FXIII and tissue transglutaminase (TG) family members 1-7 are generally involved in organ growth and homeostasis *via* modifying protein and other molecules containing primary amines such as serotonin. It is also involved in blood pressure regulation (Richardson et al. 2013). FXIII catalyses glutamine residue ( $\gamma$ -glutamyl)-lysine residue ( $\epsilon$ -lysyl) amide-bond formation within and between proteins (Richardson et al. 2013). FH has been found to be a good substrate of FXIII, and FH can be cross-linked to fibrinogen (Sim et al. 2008; Ferluga et al. 2014). It is thus likely that FXIII may bond FH with its ECM protein substrates such as fibrinogen, fibronectin, thrombospondin, collagen and integrins in various tissues and on platelets. FXIII cross-bonding may stabilise FH cell protective function afforded by its interaction with GAGs, proteoglycans with cell surface C3d and with its integrin receptors. Such putative fortified FH protection may be particularly relevant in vascular injury, exposing collagen and basement membrane (Richardson et al. 2013). Collagen is also an anchorage for pro-thrombotic vWF, a

FXIII substrate (Richardson et al 2013). vWF captures activated platelets which release their thrombin, initiating platelet rich thrombi formation (de Groot et al. 2012). Damaged vascular tissue may also release pro-thrombotic TF.

FXIII is a hetero-tetramer of 2 zymogen A protein subunits, which are protected by its 2-B sub-units, each of which consists of 10 CCP domains, which are homologues of FH (Souri et al. 2008). A subunit zymogen undergoes conformational-activation changes on separation from B subunits, brought about by thrombin and  $Ca^{2+}$  ions. FXIII is also found abundantly in its dimeric A-form in cytoplasm such as of platelets, which contain about half of circulating FXIII, monocytes, vascular endothelial cells and of placenta (Yee et al. 1994). A low stabilised level of platelet-thrombin-XIII activation on vascular endothelia is part of haemostasis (de Groot et al. 2012; Lorand and Graham 2003). FH has also a potential protective role in blood coagulation system and in thrombosis, by masking cell membrane anionic phospholipids such as PS, required in coagulation factor activation, a role which might be fortified by FXIII.

### **Complement FH as potential anti-coagulant glycoprotein**

A homologue of FH,  $\beta$ 2-glycoprotein-1 ( $\beta$ 2-GP-1) (Steinkasserer et al, 1991) has been studied in anti-phospholipid syndrome (APS), which is partly mediated by autoantibodies against this glycoprotein (Guerin et al. 1997).  $\beta$ 2-GP-1 and FH have been found to have common anti-coagulant properties (Ferluga et al. 2014). Both glycoproteins bind to membrane negatively charged pro-coagulant phospholipids such as PS, and thereby competitively inhibit Hageman factor (FXII) system contact activation by PS (Ferluga et al. 2014; Schousboe 1988). Generally, coagulation factors require anchorage on a PS or cardiolipin (CL) platform such as on activated platelet membrane for their complex assembly and activation (de Groot et al. 2012; Meri 2013). For example,  $\beta$ 2-GP-1 inhibits in this way activation of platelet pro-thrombinase (aFX-aFV- $Ca^{2+}$ ) complex for its prothrombin-thrombin conversion (Nimpf et al. 1986). FH may have a similar effect on this pro-enzyme. PS becomes expressed e.g. on activated platelet outer plasma membrane leaflet by inner leaf inversion upon their activation. FH, like its homologue  $\beta$ 2-GP1, likely binds to anionic phospholipids through its cationic amino acid motif on its 19-20 CCP domains. An extended anti-inflammatory and possible anti-coagulant property of FH is its binding to oxidized lipid neo-epitopes such as on apoptotic blebs for their clearance and protection.

## **Complement FH as an oxidative stress protector in cellular debris clearance**

Mitochondrial oxidative phosphorylation drives body processes such as development, cell signalling and energy metabolism in homeostasis via ATP production, and via its by-product reactive oxygen species (ROS) as in cardiogenesis (Chung et al. 2007). However, ROS is also needed in defence against microbes via its production by macrophages and neutrophils. It is modulated by cell and plasma redox systems, including reducing agent glutathione, and by enzymes superoxide dismutases, hydrogen peroxide catalase and glutathione peroxidase (Baud et al. 2004). It can be exaggerated as oxidative stress, which could be chronic in a tissue environment, e.g., in atherosclerotic plaques, and in AMD, which are strongly associated with oxidised cell debris (Handa 2012; Weismann et al. 2011). Apoptotic cell blebs and unsaturated lipids are constantly subjected to ROS peroxidation, which have to be subdued and removed in a non-inflammatory manner by phagocytes as part of homeostasis (Weismann et al. 2011).

FH can contribute substantially to clearing of such inflammatory cell debris. FH can ligate specifically a major oxidation marker, malondialdehyde (MDA) assisted by malonacetaldehyde (MAA), which covalently bond-tag cell debris proteins and lipids through their primary amines, i.e.  $\epsilon$ -lysyl residues in proteins (Weismann et al. 2011). MDA is one of such markers termed oxidation-specific epitopes (OSEs) (Weismann and Binder 2012). FH ligates MDAs *via* its CCP7 and CCP20 domains, by specific molecular pattern recognition, which is  $\text{Ca}^{2+}$  independent. Further OSEs include carboxyethylpyrrole (CEP), phosphocholine (PC), oxidised PS (OxPS), oxidised cardiolipin (OxCL) and oxidised low density lipoprotein (OxLDLs). OSEs are predominant neo-epitopes in apoptotic cell debris (Weismann and Binder 2012). Besides FH, MDA is ligated by macrophage scavenger receptor SR-A, while PC-OSEs are recognised by SR-CD36 and SR-B1 (Weismann and Binder 2012). Since CD36 is foremost a receptor for TSP-1, FH might ligate TSP-1 in this process.

MDA-tagged apoptotic and necrotic cell ligation by FH may protect such tissue sites from complement-induced inflammation and from endothelial cell oxidative stress. FH can augment iC3b production and deposition on their surface for their opsonisation in CR3-mediated silent engulfment by phagocytes, similarly as suggested for early phase PS tagged apoptotic cells. It may well be that both MDAs-SR-A receptor engagement, and that of FH-iC3b are required in macrophage phagocytosis for a firm cell adhesion and a silent removal

of cellular debris, respectively (Kang et al. 2012; Weismann et al. 2011). This is consistent with findings that iC3b opsonised apoptotic human thymocytes induced in macrophages upon their phagocytosis a blockade of pro-inflammatory NF- $\kappa$ B signal ligand via IL-10 secretion. Both CR3 and CR4 could be engaged (Amarilyo et al. 2010).

Phagocyte scavenger receptor inflammatory responses may be obligatory in microbe clearance and killing, but may be actively inhibited by engaging CR3 as well as in apoptotic cell removal (Urban et al. 2001). This observation apparently applies also to OSE-tagged cell debris. There may be subsets of phagocytes in this aspect (Amarilyo et al. 2010). As recently found, FH C-terminal MDA recognition site appears to be ionic, as is the one associated with aHUS related polymorphism, impairing FH binding to GAGs on cell surface for their protection (Hyvarinen et al. 2014).

OSEs are immunogenic and are targets of autoantibodies, such as of specific natural IgM autoantibodies produced by B1 cell subset, which are of relatively low affinity (Wardemann et al. 2003). OSEs are natural predominant targets of autoantibodies which are mainly of MDAs-LDLs and OxLDL specificities, as examined in human cord blood (Chou et al. 2009; Wardemann et al. 2003). FH may also ligate other apoptotic cell recognition molecules such as acute phase C-reactive protein (CRP). Monomeric CRP is a ligand of FH. Such mCRP/FH complexes bind to phosphocholine OSEs protein-lipid tags, protect injured or apoptotic vascular endothelial cells from complement attack, inhibit pro-inflammatory cytokines, and augment FI-C3b inactivation into iC3b production and surface deposition (Mihlan et al. 2009). FH protective property (against complement attack) has been studied in AMD where cell debris membrane deposits, termed drusen, have been found to contain oxidised lipid products including MDA and carboxy-ethylpyrrole (CEP) (Zipfel and Skerka 2009). Such OSEs are ligands for FH CCP7 domain. FH by binding to and clearing OSE adducts protects the tissue cell environment from oxidative stress and inflammation (Weismann et al. 2011). A SNP mutation in FH CCP7 domain, replacing tyrosine with histidine at position 402, confers a strong risk for AMD development. Mutated FH-His-form binds more weakly to cell debris affecting their clearance (Clark et al. 2013)

### **Complement Factor H in platelet regulation and protection**

Platelet precursors are major producers of FH, and FH released from platelets may contribute to vascular cell protection and their apoptotic cell clearance. Platelets represent a border area

between coagulation and innate immune systems. They have a major regulatory and effector role in haemostasis and thrombotic events, by interacting via their receptors with vascular endothelial cells and immune cell ligands, as evident in their inherited disorders (Nurden and Nurden 2015; Vieira-de-Abreu et al. 2012; Yip et al. 2005). They provide a platform for surface complement and coagulation reactions by expressing PS and ECM proteins, as common binding anchors and activators for coagulation factor assembly (Bergmeier and Hynes 2012; de Groot et al. 2012). Platelets produce via their precursors-megakaryocytes, or acquire and store in their granules most of complement and coagulation proteins, including  $\alpha$ -granule FH, vWF, TSP-1, and TGF- $\beta$ . These proteins are released upon platelet activation by collagen, adenosine diphosphate (ADP), and thrombin at the site of vascular injury (Markiewski et al. 2007). Platelet involvement in complement deficiency disorders is indicated by accompanying thrombocytopenia, such as in aHUS and TTP. Activated platelets release a concentrated amount of FH, as compared with plasma FH level, which may be more efficient in inhibiting platelet pro-thrombotic effects, by masking their PS (Devine and Rosse 1987). FH is a ligand of platelet specific pro-thrombotic  $\alpha$ IIb $\beta$ 3 integrin, as it is also of TSP-1. FH strongly binds to TSP-1, and both ligands together strengthen their binding to and stimulation of this integrin receptor (Vaziri-Sani et al. 2005). Additional ligands of  $\alpha$ IIb $\beta$ 3 include fibrinogen, vW factor A1 domain (vWF-A1), and CD40L. Their ligation to  $\alpha$ IIb $\beta$ 3 stabilises platelet aggregation by fibrinogen and platelet vascular endothelial-ECM ligation in thrombus formation. Fibrinogen also binds to endothelial cell  $\alpha$ v $\beta$ 3 (vitronectin receptor) (Andre et al. 2002; Bergmeier and Hynes 2012; Sanchez-Cortes and Mrksich 2009). FH/TSP ligation of  $\alpha$ IIb $\beta$ 3 may competitively inhibit binding of such pro-thrombotic proteins, in contribution to thrombosis regulation. It has been proposed that pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6 and TNF- $\alpha$  induced by complement or bacterial LPS on monocytes and vascular endothelial cells can stimulate TF expression on these cell membranes (Drake et al. 1989). This effect is augmented by activated platelet and endothelial cell P-selectin (Shebuski and Kilgore 2002). TF, as a cofactor, may in combination with FVII zymogen, initiate through FX activation of the extrinsic and common coagulation pathways, resulting in prothrombinase activation, thrombin production and fibrin clot formation. The complement C5b-9 complex induces, in platelet membrane, PS expression (Esmon 2004; Markiewski et al. 2007). Platelets appear to be resilient to MAC lysis (Devine and Rosse 1987). Platelet  $\alpha$ -granules and their microparticles contain P-selectin, FH, FD, TF and other coagulation components to trigger the coagulation cascade, as well as complement cascade propagation on the platelet surface (Del Conde et al. 2005; Noris et al. 2012; Peerschke et al. 2008). P-

selectin harbours a consensus binding motif able to interact with various complement components (Peerschke et al. 2008).

### **Autoimmune diseases**

Systemic inflammatory thrombotic autoimmune diseases (Fig. 2) appear to be mainly due to defects in the classical/lectin complement pathway components C1/MBL, C2 and C4, and in their regulator C1 inhibitor associated with hereditary angioedema (Botto et al. 2009). On the other hand, diseases such as aHUS and MPGN-2 have been linked mainly with mutations of the alternative pathway components and regulators. These defects arise from over-activation of complement with resulting inflammation and thrombosis on damaged vascular endothelia (Botto et al. 2009; Meri 2013; Noris and Remuzzi 2009). Such autoimmune diseases may, to various extents, be inherited or be acquired by development of autoantibodies, which simulate mutation defects on their targets. On the whole, these diseases arise on a polygenic and polymorphic background and are triggered by environmental factors.

The aHUS pathology includes glomerular arteriole and capillary thrombosis, termed thrombotic microangiopathy (TMA), which often leads to renal failure (Noris and Remuzzi 2009) aHUS, and tHUS (the latter associated with *E. coli* infection), thrombotic thrombocytopenic purpura (TTP), MPGN-type 2 and DDD share microvascular thrombotic lesions in renal and other organs such as brain and heart. Albeit arising from different causes, TMA lesions appear to spring from endothelial vWF-exaggerated platelet adhesion and activation (Benz and Amann 2009; Reininger et al. 2006; Skerka et al. 2009).

Defects of several known and unknown gene factors contribute to disease susceptibility. There is a strong redundancy, deduced from disease rarity (Kavanagh et al. 2013). Approximately, 50% of aHUS patients carry quantitative loss-of-function mutations such as of complement regulators FH, MCP (CD46), and of thrombomodulin as cofactors of FI, which itself may be mutated (Noris and Remuzzi 2009). Effectors C3 and FB may carry gain-of-function mutations, resulting in over-stabilization of C3bBb convertase, resisting its decay by regulators, which also leads to pathogenic over-activation of complement (Goicoechea de Jorge et al. 2007; Roumenina et al. 2009). Autoantibodies to FH contribute to the disease susceptibility by preventing its adhesion to anionic GAGs for self-protection (Kavanagh and Goodship 2011; Kavanagh et al. 2013).

Thrombomodulin expressed on endothelial cells protects these cells from inflammation and thrombosis. Its missense mutations may contribute to inflammation and thrombosis via its disabled facilitation of thrombin-catalysed activation of pro-carboxypeptidase, which inactivates C3a and C5a anaphylatoxins. Thrombomodulin also enhances activation of protein-C by thrombin, taking place on vascular endothelial cells, which is a major negative regulator of blood coagulation (Kavanagh et al. 2013). Thrombomodulin also enhances the degradation of C3b into iC3b by FI and FH (Tateishi et al. 2016).

It has been suggested that FH mutation-polymorphism especially in its C-terminal CCP19/20 domains (Kavanagh et al. 2013), may contribute differentially to the development of aHUS, DDD, and other disorders including AMD, albeit also depending on other genetic and environmental factors (Botto et al. 2009; Dragon-Durey et al. 2004; Pickering et al. 2007; Ruggenti et al. 2001; Skerka et al. 2009; Zipfel et al. 2011; Zipfel and Lauer 2013). For a thrombotic aHUS phenotype, presence of C5 is required, suggesting a C5a induced TF expression on vascular endothelia and platelets (Goicoechea de Jorge et al. 2011).

Primary anti-phospholipid syndrome (APS) is largely mediated by pathogenic IgG/IgM autoantibodies to  $\beta$ 2-GP1-cryptic epitopes. APS can be secondary to SLE, displaying lupus anticoagulants (LA), anti-phospholipid antibodies (aPL), and susceptibility to arterial and venous thrombosis. In both disorders, C4 and C3 fragment vascular deposition, and their consumption has been detected, suggesting complement activation (Oku et al. 2009). Renal disease of various forms, including TMA and immune complex deposit-associated glomerulonephritis, is a frequent complication in these syndromes, together with thrombocytopenia (Benz and Amann 2009; Meri 2013; Ricklin and Cines 2013). In addition to aHUS, anti-FH autoantibodies have been found in APS, SLE and RA (Zadura et al. 2012; Guerin et al. 1997; Kavanagh and Goodship 2011). Anti-neutrophil cytoplasmic antigen (ANCA) autoantibodies are found in patients suffering with systemic and renal vasculitides, including Wegener's granulomatosis, engaging inflammatory C5a product of complement activation (Chen et al. 2010). An impairment of inflammatory TNF- $\alpha$  signalling regulation has been correlated with thrombosis in several autoimmune disorders including SLE and APS, and in experimental mice model of sepsis (Namjou et al. 2012; Ochoa et al. 2013; Xu et al. 2014). Mutation defects in FH-FI-iC3b-CR3 anti-inflammatory and tolerogenic pathway, which normally can withstand inflammatory cytokines, may be part of susceptibility to these

diseases. Renal deposition of C3 and C4 fragments may be due to an insufficiency in CR1 as well as insufficiency in the protective role of FH.

### **Systemic lupus erythematosus (SLE)**

SLE is a complex multigenic autoimmune inflammatory disease-syndrome of various tissues and organs such as kidney, brain, joints and blood cells. SLE is clinically heterogeneous and variable, seemingly exhibiting various disease symptoms as a syndrome, at alternate phases and remissions, and a range of autoantibodies. Neuropsychiatric signs can also occur (Agmon-Levin et al. 2011; Morrison et al. 2014). Symptoms include acute flairs, skin rashes, photosensitivity, facial erythema, oral ulcers, synovitis, serositis, vasculitis, meningitis, brain damage, lupus nephritis, arthralgia, haemolytic anaemia, leukopenia, thrombocytopenia, features which may also persist in chronic phase (Walport et al. 1998). Major autoantibodies include anti-nuclear-antibodies (histones) (ANA), dsDNA, anti-cytoplasmic auto antigens, lupus anti-coagulant (LA), and anti-phospholipid antibodies (aPL) (Pons-Estel et al. 2014). Further, there are anti-C1q and anti-FH autoantibodies, contributing to the autoimmune disease.

SLE heterogeneity is reflected in the associated gene polymorph-variants, found in wide population studies, some targeting particular phenotypes, rather than the disease. These include those of complement receptor CR3 (CD11b/CD18) and of FH and the FHR1-3 glycoproteins. The extensive multi-population study on CR3 polymorphism in B cells found certain variants to be associated with SLE (Nath et al. 2008). A study with European-American and African- American people, confined to FH/FHR gene haplotype polymorphism in chromosome 1q32 region, revealed an increased risk for SLE with FHR1-3 deletion mutation in this haptotype. Symptoms included serositis and not anti-dsDNA autoantibodies (Zhao et al. 2011). FHR1 is a negative regulator of C5 convertase, including complement terminal pathway and inflammatory- chemo attractant C5a production (Heinen et al., 2009). C5a is a major prerequisite in inflammatory diseases (Zhao et al. 2011). A FH deficiency might be additive, i.e. contributing to renal glomerulonephritis, as suggested by a FH deficient mice model prone to SLE, in which the fatal disease was accelerated (Bao et al. 2011). On the other hand, lack of FHR1-3 competition with FH may augment FH efficiency, which benefits AMD resistance in people prone to this disease, the susceptibility of which is apparently inherited but without an autoimmune component (Zhao et al. 2011).

Anti-dsDNA antibody phenotype component in SLE risk is associated with polymorphism in STAT4, CR3, interferon regulatory factor 5 (IRF5) and in MHC proteins (Chung et al. 2011). A previous study with Caucasian and Chinese SLE patients revealed a link with CR2 (CD21) polymorphism in the disease (Wu et al. 2007). SNP in the TNF receptor associated factor 6 (TRAF6) encoded on chromosome 11p12 has been assessed for linkage with SLE in a large population study, corroborating a risk for thrombocytopenia and rheumatoid arthritis. TRAF6 encompasses also TLR-signalling via NF- $\kappa$ B and activator protein 1, including MAPKs for immune cell proliferation and survival. A strong association of SLE was found in a SNP of protein tyrosine phosphatase, non-receptor type 22 (PTPN22). These signalling protein variants are also associated with several systemic and organ specific diseases including type-1 diabetes and myasthenia gravis (Namjou et al. 2012; 2013). A further study was concerned with a tendency of familial clustering of autoimmune diseases on genetic overlap basis. SLE loci were compared with a range of other autoimmune diseases including RA, type 1 diabetes and Crohn's disease. There seems to be no general common susceptible genetic locus, but appearance of distinct pleiotropic disease loci. MHC genes were excluded from the study (Ramos et al. 2011). aPLs and C1q autoantibodies often occur in SLE patients, aPLs representing a link with secondary APS, associated with arterial thrombosis in SLE (Peerschke et al. 2009; Walport 2002).

Complement is prominently involved in SLE development (Walport 2002). Earlier studies focused on classical C1, C2 and C4 proteins, since their genetic deficiency phenotype in familial SLE patient is highly penetrant. For example, in people with homozygous C1q and C4 deficiency, the frequency risk for SLE is up to 90% and 80%, respectively, but these are very rarely found in the population. Perhaps most homozygous early embryos are lost prenatally. C1q is known to be required in developmental cell migration in lower vertebrates (Nayak *et al.*, 2010; 2012). However, in heterozygous-recessive and sporadic SLE cases, other complement quantitative and qualitative abnormalities came more to light, such as in C3, C5, their regulators and receptors, such as FH and its receptor CR3 (Amarilyo et al. 2010; Manderson et al. 2004; Walport et al. 1998; Walport 2002). These studies were concerned with apoptotic cell recognition, phagocytic clearance and complement regulation of inflammation and self-tolerance (Amarilyo et al. 2010).

Two mutually inclusive views have been expressed. SLE would develop due to lack of C1q-dependent apoptotic cell clearance, and subsequent accumulation of altered-self

immunogenic nuclear and other cellular debris (Manderson et al. 2007). In a recent study, C1q was shown to recognize exclusively the late phase apoptotic cells, termed secondary necrosis, when processed by serum-bound DNase I, for their efferocytosis by professional and non-professional phagocytes, such as epithelial and endothelial cells and fibroblasts (Liang et al. 2014). Alternatively, the apparent C1q self-tolerogenic property would be lost. Such deficiency would allow for natural self-reactive B cell anti-dsDNA autoantibody production and complement activation of phagocytes, producing pro-inflammatory cytokines. Nuclear proteins and DNA are highly conserved across animal species and bacteria, and there is cross-reactivity (Carroll 1998; Carroll 2004). This notion is compatible with C1q/gC1qR – Th1/Tim-3 and B cell anti-inflammatory and tolerogenic pathway (Chen et al. 1994; Ghebrehiwet et al. 2014; Yao et al. 2008). However, alternative C3 tolerogenic pathway employing FH-iC3b-CR3 expressed on monocytes, DCs and B cells, is also a good candidate for maintenance of tolerance. This notion is supported by genetic SLE population studies (Nath et al. 2008). A decline in CD4<sup>+</sup> Foxp6<sup>high</sup>Tregs (Valencia et al. 2007) may be pegged to defects in peripheral innate immune cell tolerance, such as of monocytes and DCs. In contrast to altered-self antigen tolerance, in viral infections, CR3 promotes TLR3 pro-inflammatory signalling by recognizing extracellular dsRNA and triggers independently oxidative burst in infected macrophages (Zhou et al. 2013).

A strong susceptibility to SLE, owing to C1q and C4 insufficiency, is suggestive of a constant homeostatic need for classical and lectin pathway activation in self-tolerance maintenance. In comparison, C3 deficiency confers a lower risk for SLE development (Carroll 2004). In addition to FH effects on C3, C4-C2 proteins contribute to C3 convertase amplification cycle, to C5 convertase formation and C5a production, as regulated by C4b binding protein (C4bp) (Walport 2001a). C4bp, as a cofactor of FI, may contribute to iC4b/C4d covalent opsonisation of apoptotic cells for their silent monocyte and DC uptake, similarly to their iC3b opsonisation. In these events, both leukocyte CR3 and CR4 (CD11c/CD18) integrins as well as macrophage CR1 and CR2 proteins may be involved (Ahearn and Fearon 1989; Bajic et al. 2013; Chen et al. 2012).

Recently, it has been revealed that iC3b interacts differentially with CR3 and CR4 integrin  $\alpha$ I domains *via* their thioester containing (TED) C3d (for CR3) and C3c domains (for CR4). They are expressed on macrophages and DCs with similar apoptotic cell/PS clearance function, in which CR3 is more prominent. However, they differ in macrophage and DC

antigen presentation. In addition to its associated SLE susceptibility, CR3 is a known anti-inflammatory-tolerogenic receptor in iC3b mediated phagocytosis of apoptotic cells (Amarilyo et al. 2010). In contrast to CR4, CR3 is upregulated in stromal tissue DCs in their tolerogenic effects, potentially mediated by their secreted paracrine FH together with MSCs (Corcione et al. 2006). This finding may suggest that such potent broad tolerogenic effects of paracrine FH may be mediated via its receptor CR3, a marker of myeloid cell lineage. The innate cell immunosuppressive TAM receptor tyrosine kinases and apoptotic cell/PS specific Tim receptors may be recruited, all associated with SLE, may co-operate in these events (Ding et al. 2013; Nakayama et al. 2009). MSC paracrine FH operates in various tissue microenvironment including lymph nodes, in presence of ubiquitous apoptotic cell turnover (Corcione et al. 2006).

In lymph organ B cell antigen presentation, CR3 and B cell co-receptor CR2 ligate iC3b, capturing self and pathogen antigens as complexes (CR3-CR2-iC3b-antigen), which they present to antigen specific mIg-BCRs (Bajic et al. 2013). Lymph node resident macrophages and FDCs may secrete these receptors and are platforms for their assembly and captured antigens (Bajic et al. 2013) CR3 may at the same time negatively regulate B cell proliferation signalling *via* ligating inhibitory CD22 receptor, involved in self-reactive B cell tolerance, averting a risk for SLE. CR3 R77H mutation is linked to SLE susceptibility (Ding et al. 2013; Nath et al. 2008).

C4 comes in two isoforms, C4A (acidic) and C4B (basic), encoded by linked genes, which are both highly polymorphic and have copy number variants, and ineffective null alleles (Carroll 2004; Yang et al. 2004). C4 allele structure such as gene size, long and short, function, and plasma level varies in ethnic populations. As studied with European American patients and healthy people, a C4 partial deficiency, such as a low copy number confers a risk for SLE. In contrast, people with a higher allele number from that of normal range, were protected from SLE (Wu et al. 2008; Yang and Xu 2007). C4A isoform deficiency conferred a SLE risk in all ethnic groups worldwide, while deficiency in C4B was prevalent in Spanish, Mexican and Australian Aborigines (Yang et al. 2004).

SLE inflammatory organ-tissue lesions are associated with complement C1, C4 and C3 activation and deposition, and deposition of immune complexes (Walport 2002). These include autoantibodies binding dsDNA and anti-C1q autoantibodies. Anti-C1q autoantibodies are associated with lupus nephritis, a severe renal disease with high morbidity and mortality

rate, found in 25-50 % of SLE patients. It has been shown using a murine model that anti-C1q autoantibody mediated lupus nephritis develops in two steps. Complement activation-glomerular injury-inflammation is elicited by anti-basement membrane protein autoantibodies, which *per se* are not sufficient to fully develop lupus nephritis. This feature is augmented by C1q/Ab complexes formed on basement membrane and anchored by C1q-collagen domain-ligation. Pathogenic anti-C1q autoantibodies then ligate specifically C1q of this complex to develop the disease (Holers 2008; Trouw et al. 2004). C1q autoantibodies have been found to be of diverse specificities, also ligating globular C1q domain epitopes, in addition to those binding to its collagen domain epitopes. These autoantibodies may potentially inhibit activation of gC1qR, expressed on Th1 cells, mediating a C1q regulatory pathway (Ghebrehiwet et al. 2014; Nayak et al. 2010; Radanova et al. 2012; Tsacheva et al. 2007; Walport 2002).

A recent study revealed that SLE can appear like a glycolipid lysosomal storage disease, accumulating in CD4<sup>+</sup> T cells glycosphingolipids (GSLs), a metabolic defect which may be a further facet of SLE syndrome (McDonald et al. 2014). Such a process distorted T cell lipid metabolism, trafficking and lipid raft signalling. It was accompanied by an unhealthy serum lipoprotein fraction shift, i.e. toward low density lipoprotein LDLs. The T cell lipid increment was partially due to an enhanced GSL synthesis, recycling, and internalisation. The patients' T cells exhibited an accelerated proliferation, and in homologue B cells an overproduction of anti-dsDNA autoantibodies. The metabolic changes were linked to an over-expression of the liver nuclear X receptor  $\beta$  (LXR $\beta$ ), regulating lipid metabolism. It is thought that the dyslipidaemia occurring in the SLE patients may have largely distorted T-cell GSLs production and trafficking. The cell lysosome function in lipid degradation was normal, but was seemingly overwhelmed by the GSL quantum, even though vesicle number was increased. GSLs overproduction can be normalised by an inhibitor of its biosynthesis (McDonald et al. 2014).

In such SLE like-disorders, lipoproteins and LDL may be subjected to oxidative processes generating oxidation-specific epitopes (OSEs) such as LDL-MDAs. These neo-epitopes ligate FH, which may thus mitigate tissue cell oxidative stress. This process is in addition to apoptotic and necrotic cell debris modified by MDAs. It has been shown that surface MDA-bound FH enhances iC3b production for cellular debris clearance. This mechanism has been suggested in protection against retinal membrane drusen accumulation and oxidative damage

of retinal cells, as it occurs in AMD development (Benz and Amann 2009; Weismann et al. 2011; Zipfel and Skerka 2009). C3NeF, an autoantibody which stabilises C3bBb and promotes C3 turnover, can be also detected in SLE patients enhancing complement activation and inflammation, but the FH may partially counteract this mechanism (Devine and Rosse 1987; Zipfel and Skerka 2009).

In RA, FH and its alternative splicing product FHL1 were found to protect synovial cells from complement damage. FHL1 consists of FH CCP modules 1-7, is produced by synovial cells (and many other cell types) and has a spreading surface property (Friese et al. 2003). An international study with patients suffering with SLE, RA, lupus LA+ thrombosis, in comparison with aHUS patients, revealed a significant increase in anti-FH autoantibodies associated with rheumatic diseases. In FH autoantibody positive patients, there was a substantial elevation in frequency of homozygous deletion of the FHR-1 gene. FH autoantibodies in SLE, RA and LA+ targeted many FH-CCP domains, in contrast to its known CCP 19-20 C-terminal domains in aHUS syndrome (Zadura et al. 2012). Anti-FH autoantibodies were also detected in APS patients (Guerin et al. 1997).

Thus, SLE syndrome appears to be a variable composite of many diseases, based on multiple gene polymorphism risks, and environmental factors. In common are complement activation, tissue inflammation, cellular debris accumulation, and a reduction of peripheral tolerance (Goodnow et al. 2005). FH may overall contribute to regulation of inflammation and to a broad tolerance via its signalling CR3 receptor, in conjunction with PS recognition by PS specific Tim receptors, and by immunosuppressive TAM receptors, expressed on macrophages and dendritic cells.

### **Anti-phospholipid syndrome (APS)**

Primary APS, also called Hughes syndrome, is a thrombophilia condition associated with recurrent arterial-venous thrombosis and foetal loss (Hughes 1983). APS has been characterised by anti-phospholipid autoantibodies such as to cardiolipin (CL) and by lupus anticoagulant (LAs), termed anti-phospholipids (aPLs). Lately, it has been revealed that in APS, the main pathogenic autoantibodies are targeting  $\beta$ 2-GP1 glycoprotein, which adheres to CL and PS exposed on damaged cell surfaces, including on vascular endothelial cells and platelets, a feature which is specific for primary APS (Kertesz et al, 1995; Guerin et al. 1997; Pierangeli et al. 2005; Willis and Pierangeli 2013). FH and  $\beta$ 2-GP1 are homologues, and have

very similar binding specificity for anionic phospholipids. Since FH is more abundant in plasma, it would be expected that it would significantly modulate the binding of  $\beta$ 2-GP1 to cardiolipin and PS (Kertesz et al. 1995; Tan et al. 2010). Anti- $\beta$ 2-GP1 autoantibodies are the main effectors in SLE thrombotic events, such as deep venous thrombosis, pulmonary embolism, and arterial cardiac or cerebral infarction. APS can be associated with thrombocytopenia (de Groot and Urbanus 2012; Hughes 1983; Tripodi et al. 2011). aPL can also be found secondarily in SLE, and in other thrombotic disorders. Other autoantibodies include anti-coagulation factors such as anti-prothrombin (de Groot and Urbanus 2012) aPLs, targeting CL, are also associated with certain viral and bacterial infections such as of skin, HIV-1 infection, pneumonia and urinary infections, found in around 15% of such patients. aPL may also arise by pathogen molecular mimicry to an amino acid motif in  $\beta$ 2-GP1-5<sup>th</sup> CCP domain (Guerin et al. 1997; Willis and Pierangeli 2013). Lately, a global APS score (GAPSS) has been introduced to assess its thrombotic risk. By point value these are anti-cardiolipin IgG/IgM, 5, anti- $\beta$ 2-GP1, IgG/IgM, 4, lupus anticoagulant, 4, anti-prothrombin/phosphatidyl serine complex (aPS/PT) IgG/IgM, 3, hyperlipidaemia, 3 and arterial hypertension, 1 (Sciascia and Bertolaccini 2014).

Physiological function of  $\beta$ 2-GP1, and that of FH, appears to be a protection from thrombosis associated with anionic phospholipids (Nimpf et al. 1986). Anti- $\beta$ 2-GP1 and anti-FH autoantibodies may impair this protection. Phospholipids are required for coagulation factor complex assembly (de Groot and Urbanus 2012).

$\beta$ 2-GP1 is composed of 5 CCP modules of which the CCP5 module with an extended loop has cationic regions for anionic phospholipid interaction, such as a cluster of lysyl residues (Guerin et al. 1997; Sheng et al. 1996; Steinkasserer et al. 1991). Damaged vascular endothelial cells, platelets and monocytes expose PS on the outer membrane leaflet, a surface which is inflammatory and pro-coagulant. According to a model,  $\beta$ 2-GP1 circulates as closed rings which have been visualised. It is only upon its attachment to PS or CL that the ring assumes a stretched lipid bound conformation. This glycoprotein form exposes cryptic immunogenic epitopes mainly on CCP-1 domain which are targets for  $\beta$ 2-GP1 autoantibodies. Upon binding, autoantibodies to  $\beta$ 2-GP1 form dimeric complexes, and can attach via its 5th domain, to vascular endothelial cell, monocyte and platelet adhesion molecules such as annexin A2, apolipoprotein endothelial receptor 2 (ApoER2), Toll-like receptor, platelet factor 4, and glycoprotein Ib-V-IX (GPIb-V-IX). Such receptors may

mediate cell binding, inflammatory and thrombotic effects (de Groot and Urbanus 2012; Guerin et al. 1997; Tripodi et al. 2011; Willis and Pierangeli 2013).  $\beta$ 2-GP1-autoantibody\_binding immunogenic epitopes, located on its domain 1, are heterogeneous across different animal species  $\beta$ 2-GP1, and of recognition by sera from APS patients (Guerin et al. 2000).

Anti-FH autoantibodies have also been found in 14 out of 19 APS patients examined, as well as autoantibodies against C4bp in 8 patients, which may contribute to the disease episodes (Guerin et al. 1997). FH appears to have anti-complement activation and anti-coagulant properties when interacting with anionic phospholipid surfaces, including PS (Ferluga et al. 2014; Ricklin et al. 2010; Tan et al. 2010). FH/autoantibody immune complexes might remove such FH protection. Their deposition on vascular endothelial cells or on platelets may contribute to inflammatory responses and thrombosis, similarly to those mediated by anti- $\beta$ 2-GP1/complex receptors, though this possibility seems to be unexplored. Apparently, FH upon phospholipid binding also exposes cryptic immune epitopes, since fluid phase FH does not bind auto-IgG antibodies (Guerin et al. 1997). This finding may be in agreement with a compact FH form in plasma (Oppermann et al. 2006).

In a recent study, APS patients with thrombotic disease history were screened for the disease associated loci. A SNP and a gene copy number variation (CNV) of the TNF receptor adaptor SH2B3 protein regulating endothelial cell cytokine signalling, was strongly associated with this disease. SH2B3 gene is located on chromosome 12q24.12 and its polymorphism together with that of an adjacent gene, inherited as a haplotype, conferred this pathology risk. In aPL carriers and in general population, these gene defects are also present, but their copy number variation may apparently not be a significant susceptibility risk for thrombotic phenotype (Ochoa et al. 2013). A significant reduction in plasma  $CD4^+CD25^+Foxp3^+$ Tregs and  $CD3^-CD19^+$  B cells was detected, suggesting a decline in peripheral self-tolerance (Dal Ben et al. 2013). Thus, susceptibility to primary APS inflammatory and thrombotic phenotype appears to be multigenic, including the complement system. It may overlap with SLE and other disorders. APS is dominated by autoantibodies against  $\beta$ 2-GP1, a homologue of FH, with a common anti-coagulant function (de Laat et al. 2009; Guerin et al. 1997; Pierangeli et al. 2005).

## **Atypical haemolytic uremic syndrome (aHUS)**

Atypical haemolytic uremic syndrome (aHUS) is a rare heterogeneous disease, defined by alternative pathway dysregulation with complement hyperactivation, inflammation, thrombosis and C3 consumption. aHUS is characterised by non-immune haemolytic anaemia due to abnormal red cell fragmentation, thrombocytopenia, and renal impairment (Kavanagh and Goodship 2011; Kavanagh et al. 2013; Noris and Remuzzi 2009). It may be accompanied by anti-FH autoantibodies (Noris and Remuzzi 2009). Typically, glomerular arterioles and capillaries exhibit complement C3 fragment deposits and platelet rich thrombi formation designated thrombotic microangiopathy (TMA), and ensuing ischaemia lesions (Ruggenenti et al. 2001). Other organs such as brain and heart can be affected (Hofer et al. 2014). aHUS susceptibility may be inherited as a familial trait, or be sporadic. Its acute episodes can be precipitated by glomeruli endothelial cell damage, such as in pregnancy, by trauma, drugs and infection. Pregnancy aHUS susceptibility is associated with mutation of FH and/or of MCP in over half the number of aHUS cases, but in other aHUS patients, complement genetic background appears to be unknown. Even in some people carrying alternative complement pathway defects, aHUS may not develop, suggesting complexity and redundancy in the genes (Kavanagh and Goodship 2011; Kavanagh et al. 2013; Noris and Remuzzi 2009; Noris et al. 2010; Richards et al 2003). aHUS pathology may be due to genetic deficiency of expression, or structural defects of C3 regulators such FH, MCP and of the protease FI, all of which normally modulate complement activation on cell surfaces for self-protection (Caprioli et al. 2006). On the other hand, gain-of-function mutations of C3 or zymogen FB may also over-activate complement by stabilizing C3bBb convertase in resisting its decay by negative regulators (Meri 2013; Roumenina et al. 2009).

A familial homozygous FH deficiency in aHUS was first studied with two infant brothers. The 8-month old suffered from an acute aHUS episode, and the 3-year old remained symptomless, but both having very low FH, and low C3 plasma level, suggesting its consumption (Thompson and Winterborn 1981). However, most of genetic HUS cases are heterozygous for FH pathogenic mutations, conferring a low risk for the disease. In hereditary aHUS, up to 45% of patient's cases are associated with FH loss-of-function mutations, and around 10% of such patients develop the autoimmune aHUS form with anti-FH autoantibodies (Blanc et al. 2012; Maga et al. 2011; Meri 2013; Noris and Remuzzi 2009; Pickering et al. 2007; Roumenina et al. 2009).

Patient genomic and pedigree studies also revealed that variants of complement regulators FH and MCP inherited together as haplotypes, or of FI gene mutation variants, were significantly linked with severity of aHUS disease. Mutation of other regulators such as CR1, DAF and C4bp included in haplotypes, spanning their encoded region in RCA locus, were less influential. Thus normally, a combination of fully functional FH-MCP cofactors for adequately expressed and fully functional FI, appears to be required for protection of endothelial cells, such as glomeruli from complement damage (Esparza-Gordillo et al. 2006).

aHUS associated complement protein mutants may have altered function or impaired expression. Patients relating to MCP mutation had a milder form of the disease, as compared with those associated with FH defects (Caprioli et al. 2006). Certain mutations of FH-CCP 19-20 domains can disable its dual attachment to GAGs on cell surfaces as well as to C3b/C3d deposits and increase the risk for aHUS development (Ferreira et al. 2009; Manuelian et al. 2003; Noris and Remuzzi 2009; Pickering et al. 2007). Anti-FH IgG autoantibodies in aHUS patients, specific to CCP19-20 domains also prevented normal FH binding to endothelial cells (Blanc et al. 2012; Jozsi et al. 2007). In 10% of children suffering with acute aHUS, anti-FH autoantibodies can be a diagnostic marker (Dragon-Durey et al. 2013).

Large comparative studies with aHUS patients relating to FH, MCP and FI mutation, found their frequency to be of around 30%, 13% and 5%, respectively. Mutations impaired their C3b binding, C3 convertase decay, and for FI, secretion. About half of aHUS patients showed no connection with these complement factors. On the other hand, half of people with a penetrant FH mutation never developed aHUS (Caprioli et al. 2006). In an extended study, familial and sporadic aHUS patients were examined for disease treatment, kidney transplantation and outcome. C3 and thrombomodulin abnormalities as well as anti-FH autoantibodies were included (Noris et al. 2010). Most patients were heterozygous for FH mutations, which are located on the CCP20 domain. Children with mutation in FH, Thrombomodulin or with anti-FH autoantibodies tended to have the earliest onset of aHUS, from birth to one year, and the worst prognosis. In a few patients, aHUS was associated with membranoproliferative glomerulonephritis (MPGN). In approximately 70% of patients, the disease was precipitated by viral or bacterial infection. Combined factor deficiencies are also presented (Noris et al. 2010). Familial HUS patients have an unfavourable prognosis, as compared with sporadic cases, with 25 % mortality due to renal failure (Noris and Remuzzi

2009; Noris et al. 2012). Other studies have revealed that anti-FH autoantibodies occurred in 4 to 13% of aHUS cases (Kavanagh and Goodship 2011). In some children suffering with aHUS, anti-FH autoantibodies were found to coincide with CFHR1/CFHR3 deletion. Such autoantibodies interacted with FH C-terminal recognition sites (Jozsi et al. 2008).

C5a and its receptor are mediators of inflammatory leukocyte attraction to an injury, but also a pro-thrombotic stimulator *via* tissue factor activation. Monoclonal antibody Eculizumab has been highly successful in treatment of aHUS patients, but to some degree also in other thrombotic diseases including SLE. It prevents C5 convertase splitting C5 into C5b and C5a. (Wong et al. 2013). FH and MCP are important in protection of glomerular endothelia against aHUS development, in C3 convertase regulation, and in FI-mediated C3b and C4b processing. They have anti-inflammatory and tolerogenic properties of their own, engaging the CR3 integrin tolerogenic pathway, and Treg-1, respectively. By being a transmembrane molecule, MCP may also transmit anti-inflammatory signals. This has been indicated by its cytoplasmic domain tyrosine phosphorylation by src kinases, and by their cross-linking with MV (measles virus) haemagglutinin in human monocytes, down-regulating IL-12 production (Wang et al. 2000).

Typical HUS (tHUS) can appear as a secondary feature to gastroenteritis caused by *Escherichia coli* 0175:H7 Shiga-like toxin (Stx) (STEC-HUS), or to other bacterial toxins. aHUS is, thus, regarded as the same renal phenotype appearing in the absence of such toxins. tHUS is about 10 times more common than aHUS and prevails in children under 5 years, with incidence of 6 cases per 100,000 children (Noris et al. 2012). tHUS is an acute gastroenteritis disease which can be haemorrhagic. However, in most cases, such symptoms are cleared spontaneously or with drug treatment. A familial trait in tHUS appears to be unknown. Acute tHUS disease, which combines complement activation with renal vascular thrombosis, has been studied using a mouse STEC-HUS model that is induced upon Stx and LPS injection. Stx binds to its receptor Gb3 on glomerular vascular endothelial cells (Morigi et al. 2011). Such treated mice exhibited in their glomerular blood vessels occlusive platelet-fibrin thrombosis.

In STEC-HUS patients, as in mice, FB product Bb was increased, and thrombomodulin was decreased in their blood, indicating complement activation. Thrombomodulin as a cofactor enhances FH/FI-C3b degradation, and accelerates C3a and C5a inactivation *via* carboxypeptidase B (Delvaeye et al. 2009). There have been sporadic outbreaks of tHUS,

such as caused by *E. coli* serotype 0104:H4 Shiga toxin in Germany in May 2011, transmitted by contaminated fruit (Muniesa et al. 2012).

### **Conditions related to aHUS, TTP, MPGN, AMD pathogenesis and a possible FH protective role**

It has been suggested that aHUS, TTP and MPGN are a spectrum of the same disease regarding their microvascular complement over-activation and thrombosis (Noris et al. 2012; Pickering and Cook 2008; Skerka et al. 2009). Their development entails glomerular microvascular endothelial cell damage, complement activation and adherence of activated platelets to such cells in thrombus formation. All can be associated with variant FH polymorphism (Zipfel and Lauer 2013). TTP shares TMA lesions with aHUS and tHUS, although its pathogenesis entails an inherited or acquired deficiency of metalloproteinase-13 (ADAMTS13). This suggested a common thrombotic mechanism for TMA formation under fast blood flow conditions (Noris et al. 2012; Reininger et al. 2006; Ruggenenti et al. 2001; Skerka et al. 2009; Tsai 2009).

aHUS glomerular histological findings, which are indistinguishable from those of tHUS and TTP, show TMA lesion with swelling and thickening of arteriolar and capillary walls, and their partial and occlusive platelet rich thrombosis. Endothelial cells recede from basement membrane, with the space filled with fragmented cell debris material. There can be ischemic tissue damage with necrosis. Other organs such as brain, lungs, and gastro-enteric tract may be affected (Hofer et al. 2014). Red blood cell damage and haemolysis, as well as that of platelets results from their very fast shear flow through narrowing arteriole and capillary lumen, partially obstructed by thrombi. In such conditions, platelet arrest depends on vWF adhesion (Savage et al. 1996). Platelets can become activated by shear stress, and may release their thrombin, and membrane micro particles (PMPs) involved in TTP-TMA pathogenesis (Benz and Amann 2009; Noris and Remuzzi 2009; Reininger et al. 2006). Microvascular endothelial cells seem to be highly vulnerable to toxins such as *E. coli* Stx, immune complex deposits, to complement activation fragments and other triggers, not the least by being fenestrated for a vast volume/rate of plasma filtration. Normally, endothelia are protected against complement activation by membrane regulators such as cell-bound MCP and cell-adherent FH (Jozsi et al. 2004). However, basement membrane lacks MCP molecules, where there is only the complement FH protection available (Clark et al. 2006; Jozsi et al. 2004). This fragile status can be perturbed by a local injury and complement over-reaction (de Groot

et al. 2012). In such conditions complement becomes over-activated on endothelial cell membranes and on platelets, damaging cells and precipitating TMA lesion formation in various organs (Noris et al. 2012). Since C5-C5a strongly contributes to microvascular thrombosis, monoclonal anti-C5 Eculizumab has been used to ameliorate pathology in these syndromes. In aHUS patients it can resolve thrombocytopenia, normalizing anaemia with improvement of renal function in 15 of 17 cases (Noris et al. 2012; Tsai and Kuo 2014)

### **Thrombotic Thrombocytopenic Purpura (TTP)**

The role of vWF in microvascular platelet adhesion in haemostasis, and in TMA formation, is more evident in TTP. vWF exhibits its pathogenic ultra large multimer over-expression on damaged endothelia and on ECM, able to capture activated platelets in their thrombus formation. This vWF-size and function shift is due to deficiency of its negative regulator plasma metalloproteinase-13 (ADAMTS13) (Sadler 2008). Normally, the enzyme cleaves such hyper-adhesive large vWF multimeres, in keeping a thrombotic balance. Its deficiency may be hereditary, associated with a severe TTP form. In most cases, mainly in adult patients, susceptibility to TTP is acquired by their production of inhibitory autoantibodies to ADAMTS13. Approximately 80% of such patients respond to plasma exchange therapy (de Groot and Urbanus 2012; Noris et al. 2012; Sadler 2008; Tsai 2009). As compared with aHUS, TTP-TMA lesions are more disseminated in various organs and primarily in brain microvasculature, causing its damage and mental symptoms. Consequently, thrombocytopenia and haemolysis-anaemia are more pronounced. Purpura appears from shortcomings in platelet haemostasis maintenance, in plugging small vessel constant leakage (Noris et al. 2012; Ruggerenti et al. 2001; Sadler 2008).

As indicated in their adhesion studies, ADAMTS13 may only cleave large vWF multimers under microvascular fast flow shear stress, which causes their conformational activation, stretching and unfolding the proteinase substrate residues, as well as various platelet binding regions. It may thus limit vWF-mediated haemostatic thrombosis function, and that in pathogenic thrombosis, to microvascular structures such as in glomeruli, brain and of other organs in TTP, aHUS and MPGN (Sadler 2008; Savage et al. 1996; Tsai 2009). In these conditions, e.g. at shear rate  $1500\text{ s}^{-1}$ , vWF, as bound to ECM type 1 collagen, can promptly initiate platelet tethering and rolling on damaged microvascular endothelial cells. This is achieved through its A1 domain interaction with its platelet ligand GPIIb $\alpha$  of GPIb-V-IX receptor complex (Bergmeier and Hynes 2012; Savage et al. 1996). This high dissociation

reversible binding can be stabilized by vWF ligation of its specific platelet  $\alpha_{IIb}\beta_3$  integrin receptor, concomitantly with that of platelet  $\alpha_2\beta_1$  integrin receptor, under high flow shear rate conditions (Savage et al. 1998). At lower shear rates, below  $600\text{ s}^{-1}$  as occurring in wider vessels, thrombin activated platelets may directly firmly bind to vascular, or ECM fibrinogen, through their  $\alpha_{IIb}\beta_3$  integrin in thrombus formation, a condition which is not permissive for vWF- $\alpha_{IIb}\beta_3$  receptor interaction (Savage et al. 1998).

Potentially, FH together with TSP-1, may partially inhibit these prothrombotic ligands by competition for  $\alpha_{IIb}\beta_3$  binding, perhaps in both shear rate conditions. FH has a high affinity for this integrin (Vaziri-Sani et al. 2005). ADAMTS13 activity has been studied in patients with various connective tissue diseases containing TMA lesion. Its autoantibodies were associated with RA and SLE (Matsuyama et al. 2009). FH S890I mutation was found in a patient with chronic renal failure, in a family associated with V88M and G1238V mutations of ADAMTS13 deficiency and with TTP cerebral disease, suggesting an aHUS disease combination (Noris et al. 2005). To distinguish clinically between these two diseases, a normal or 10% higher ADAMTS13 plasma level in renal disease patients has been recommended to exclude genetic and acquired TTP (Tsai and Kuo, 2014).

### **Membranoproliferative glomerulonephritis type 2 (MPGN type 2)**

MPGN type- 2 (DDD) represent a heterogeneous and complex inflammatory renal disease often of unknown causes. In children and in adults, DDD frequency is approximately 4% and 7 %, respectively, of general glomerular kidney disease (Appel et al. 2005; Benz and Amann 2009). It is characterized by hyperplasia of mesangial cells, thickening of basement membrane, and by electron-dense deposits. Deposition of C3, C9 and vascular clots are also seen (Appel et al. 2005; Sethi et al. 2009). These lesions may impair glomerular plasma filtration as detected by albuminuria. Approximately in 10% of DDD patients, electron dense deposits are also found in their eye Bruch's membrane with drusen, causing visual impairment. There is a cell-tissue structural similarity between glomerular and retinal accessory cells and membranes (Appel et al. 2005). MPGN type 2 lacks immune complex glomerular depositions, in contrast to MPGN types-1 and 3 (Benz and Amann 2009). Prognosis is poor, as in half of patients the disease progresses to terminal stage (Appel et al. 2005).

DDD is associated with complement alternative pathway and with terminal pathway over-activation (Appel et al. 2007). Most DDD cases appear to be acquired, i.e., mediated by C3-nephritic factor (C3NeF) autoantibodies, which bind to a neoepitope formed when C3 and FB form the C3bBb complex. Similarly to FB gain-of-function mutation (Roumenina et al. 2009) C3NeF stabilizes C3bBb convertase, and thus hyper-activating the alternative pathway. A lower number of DDD patients are associated with inherited FH mutation, impairing its C3 convertase decay acceleration, self-protection, and its FI cofactor capacity. These FH dysfunctions may also contribute to the alternative pathway over-activation on injured glomerular endothelial cells and on ECM basement membrane (Botto et al. 2009; Noris and Remuzzi 2009). In the renal microvascular thrombotic environment, the platelet granule FH, stored at a high concentration, may upon its degranulation overcome the C3NeF stabilization of C3bBb convertase complex formed on glomerular endothelia (Devine and Rosse 1987; Weiler et al. 1976).

MPGN type 2 phenotype, as seen in a large kidney biopsy registry, is more common in African and Eastern European populations. Its frequency can reach 30% of kidney disease, although nephrotic pathology cases prevail. In Western Europe MPGN frequency varies between 4-7%, and in USA can be less than 1% in young adults (Benz and Amann 2009). DDD pathogenesis, like aHUS, may in susceptible people, be precipitated by an injury to basement membrane, which may not be sufficiently protected against complement by a defective FH adherence (Botto et al. 2009). FH is the only complement protector on such ECM structures (Clark et al. 2013; Jozsi et al. 2004).

### **Age related macular degeneration (AMD) and FH in the clearance of apoptotic cellular debris**

FH, apart from its cell membrane protective function, is capable of functioning in the removal of oxidised apoptotic cell debris such as drusen in AMD (Weismann et al. 2011). AMD is a major cause of blindness in the western world, and a FH susceptibility gene variant is a strong risk factor for AMD (Klein et al. 2005, Clark et al. 2006). It is characterised by deposition of cellular debris (drusen) on retinal pigmented epithelial cells and their Bruch's membrane (Figure 3), and by degeneration of macular retinal cells. These cells produce FH/FHL-1 proteins. In FH CCP7 and FHL-1 CCP7 domain a point mutation results in replacement of Tyr by His at residue 402, designated as Y402H (Day et al. 1988, Klein et al. 2005; Clark et al. 2006; Skerka et al. 2007; Zipfel and Lauer 2013) and this replacement

substantially increases the risk of AMD. A large cohort genetic haplotype analysis of AMD patients found several additional common FH susceptibility SNPs (Hageman et al. 2005). Retinal Bruch's membrane differs from renal glomerular basement membrane in its GAGs: heparin, heparan sulphate, and dermatan sulphate, which interact with FH *via* its CCP7 and CCP20 domains (Clark et al., 2013). Such FH differential GAG binding, and that of FH-FHL-1 interaction with Y402H residue and other polymorphism sites together suggest, FH to be vital in retinal epithelia protection (Clark et al. 2013). AMD and aHUS associated amino acid polymorphisms have been compared, positioned on FH CCP7 and CCP20 domains, respectively (Rodriguez et al. 2014; Zipfel and Lauer 2013). FHR1-3 negatively regulate FH and FHL-1 effects. Their loss may enhance FH eye protection and decrease the risk for AMD development (Fritsche et al. 2010).

Retinal environment is strongly exposed to light and lipid peroxidation, and prone to produce pathogenic amounts of reactive oxygen species (ROS) (Weismann et al. 2011). It would appear that FH has a dual role in protecting retinal membranes and cells from complement attack, and from oxidative damage-stress, by binding such debris, tagged by OSEs or GAGs for their clearance (Clark et al. 2013; Handa 2012; Weismann et al. 2011).

### **Immune thrombocytopenia Purpura (ITP)**

ITP is a heterogeneous and diverse disease associated with autoantibodies to various platelet antigens, with platelet destruction, their reduced production, and with tendency for bleeding. Most cases of ITP are idiopathic, but can be secondary to diverse causes, including infections and autoimmune conditions. ITP can be complicated with venous and arterial thrombosis (Cines et al. 2009). When activated upon vascular injury, e.g. with ECM collagen, thrombin, adenosine diphosphate (ADP), or shear stress, platelets seem to have an intrinsic propensity to stage complement activation on their surface. They may thus through their adherence largely contribute to a local vascular inflammation, and in haemostatic and pathogenic thrombosis (Peerschke et al. 2006; Peerschke et al. 2008). Clinical ITP studies showed that patients' sera (complement) enhanced C1q, C4d, C3b and of C5b-C9 component deposition on platelets, which correlated with a decrease of immature platelets in their blood, and with thrombocytopenia. Such features suggested an activated complement cascade participation in this disease (Peerschke et al. 2009). Moreover, such platelet complement pattern was found in blood of patients suffering from SLE and APS. These include anti-phospholipid antibodies (aPL), anti- $\beta$ 2-GPI autoantibodies, and a risk for arterial thrombosis. C1q may induce pro-

inflammatory signalling and cytokines in platelets via its globular C1q receptor (gC1qR) (Peerschke et al. 2006; Peerschke et al. 2008; Peerschke et al. 2009).

## Conclusions

Systemic autoimmune diseases such as SLE and APS, associated with complement component dysfunction, are complex, heterogeneous and variable in symptoms, including variability in autoantibody production. However, such diseases have in common pathogenic features in tissue inflammation, thrombosis and decline of peripheral tolerance to self-antigens. FH contributes substantially to regulation of these events. These include C3 and C5 convertase decay acceleration. FH may also potentially influence a silent apoptotic cell clearance by phagocytes. This event has been suggested by tests in which C1q mediated apoptotic cell engulfment by human monocytes was modulated by FH *via* CR3 (Kang et al. 2012).

Apoptotic cell turnover process is vital for development and homeostasis. It is thought to be conserved in innate immune cells, providing a broad peripheral self-tolerance. There appear to be several partially redundant pathways. One is apoptotic cell-iC3b opsonisation for their phagocyte CR3/CR4 integrin ligation (Amarilyo et al. 2010). Complement protein FH can also recognise PS, a marker of apoptotic cells to be engulfed via various scavenger receptors, and by PS specific Tim receptors. Apoptotic cells (ACs) also present TSP-1, a ligand of FH, capable of interacting with several receptors, including its own CD36 receptor, beta-1,-3 integrins and CD47 (integrin associated protein, IAP) expressed on dendritic cells (DCs) and macrophages. This TSP-1 receptor binding was PS independent. TSP-1 thus induced in DCs their apoptotic cell phagocytosis, as well as their self-tolerogenic state (Krispin et al. 2006). IAP may recruit on the cell membrane its immuno-inhibitory receptor SIRP $\alpha$ . In this way IAP can assemble, in cell plasma membrane glycosphingolipid and cholesterol enriched domains, the signalling supra-molecular complex, comprising TSP-1/CD36/ $\alpha$ v $\beta$ 3/ISP/SIRP $\alpha$  receptors. Such a receptor complex is apparently involved in a silent apoptotic cell clearance by phagocytes, due to the presence of activated SIRP $\alpha$ , mediating tolerogenic cell responses (Green et al. 1999). FH is a strong ligand of TSP-1, and may influence apoptotic cell clearance in this way, in addition to acting via the complement integrins for which FH is also a ligand.

A paracrine, high-gradient FH produced constitutively by pluripotent MSCs has been shown to mediate immunosuppressive and self-tolerogenic response in DCs and macrophages. MSCs are found in various local tissue microenvironments, including lymph nodes, conditions where systemic liver produced FH is at low concentration (Tu et al. 2010). CR3, upregulated in DCs in stromal organ tissues of spleen, lungs and liver, has also been associated with self-tolerance (Zhang et al. 2004). A paracrine FH may potentially, through its receptor CR3, engage Mer-tyrosine kinases TAM as an immunosuppressive component. Tim-receptor family, specific for PS ligation, may also be engaged. Such a suggested model would be compatible with findings in peritoneal resident macrophages, which co-express Tim-4, MerTK, and CR3, in a silent apoptotic cell clearance, comparable to that by DCs, which is dependent on MerTK activation (Nishi et al. 2014; Sen et al. 2007). A further complement connection is a co-expression of the negative regulator gC1qR, together with tolerogenic Tim-3 expressed on Th1 cells, both inhibiting Th1 cell proliferation (Ghebrehiwet et al. 2014).

Deficiency and polymorphism of FH increase a risk for developing aHUS in susceptible people, and of MPGN type II. Often accompanied by anti-FH autoantibodies, aggravating the disease, aHUS may partially arise due to a decline of broad tolerogenic effects. FH autoantibodies have been detected in patients suffering with APS, and in those with RA. FH Tyr402His mutation variant confers susceptibility for AMD. On the other hand, the inherited CR3 variant (Arg77His) is a risk factor for SLE in ethnic populations.

FH discriminates between self and pathogen response of immune cells by protecting mainly self-antigens from complement attack. This includes joint synovial cell covering, as indicated in RA patients. In addition, pathogens lack a surface PS and become bound to induce inflammatory responses in phagocytic cells. FH inhibits PS-contact activation of the pro-coagulant Hageman factor system, the initial part of the intrinsic blood coagulation pathway. FH is also a ligand of platelet  $\alpha$ IIB $\beta$ 3 integrin, potentially reducing its pro-thrombotic effects. Thus, FH may be part of innate immune cell regulatory balance, with its anti-inflammatory and self-tolerogenic effects.

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## Figure legends.

**Figure 1: Factor H as an essential Regulator of the Alternative pathway.** (A) A general structure of Complement Factor H (FH) and the CCP binding sites. CCP1-4 are involved in the interaction of C3b and TED for the cleavage of C3b. CCP 10-13 are positively charged and so may interact with negative groups. The CCP 19-20 are involved in binding to GAGs or C3b/d (B) FH binds to C3b on the cell surface and the membrane of host cells is equipped with complement receptors, including CR3 and CR4 which are integrin receptors for FH. CR3 and CR4 can also bind iC3b, C3c, C3dg and C3d and mediate phagocytosis of iC3b-opsinized cells. FH blocks the formation of C3 convertase and C5 convertase, thus inhibiting the formation of MAC and cell lysis.

**Figure 2: Regulation of Alternative pathway.** (A) Domain Alignment and Mechanism of Complement Factor H- Related (CFHR) Proteins. CFHR1, CFHR2, and CFHR5 share high homology in their two N-terminal domains, forming homo- and heterodimers. These CFHR proteins, most particularly CFHR1 act in opposition to Factor H, where they compete with Factor H for C3b binding on the target cell surface, resulting in the formation of C3 convertase and MAC. (B) Factor H can bind to C3b and GAGs found on the cell surface and the level of complement activation depends on architecture of the complex and the density of C3b deposited. FH can bind to C3b via CCP1-4, and if subsidiary interactions can be formed with GAGs or C3d via CCP6-8 or 19-20, this increases the apparent avidity of FH for C3b, thereby preventing FB binding and resulting in breakdown by FI of C3b in the C3b-FH complex, to form iC3b. FH is also thought to regulate the complement classical pathway by competing with C1q for binding to some targets (Tan et al, 2010; 2011). (Figure adapted from Merle et al, 2015)

**Figure 3: An overview of Factor H functions and their implication in pathological conditions.** (A) Factor H can control complement activation on target cell surfaces, and prevent host cells from undergoing complement attack. Factor H may bind to target cells and basement membranes by interacting with deposited C3b and glycosaminoglycans. Pentraxins CRP and PTX3, have a role in mediating the binding of factor H to some targets. Factor H may also interact with specific receptors, and modulate cellular functions, such as cell adhesion and phagocytosis. (B) Mutations and SNPs in factor H, and anti-FH autoantibodies, are associated with numerous diseases, including Dense Deposit Disease (DDD), Atypical hemolytic-uremic syndrome (aHUS) and Age-related macular degeneration (AMD)



Figure 2:

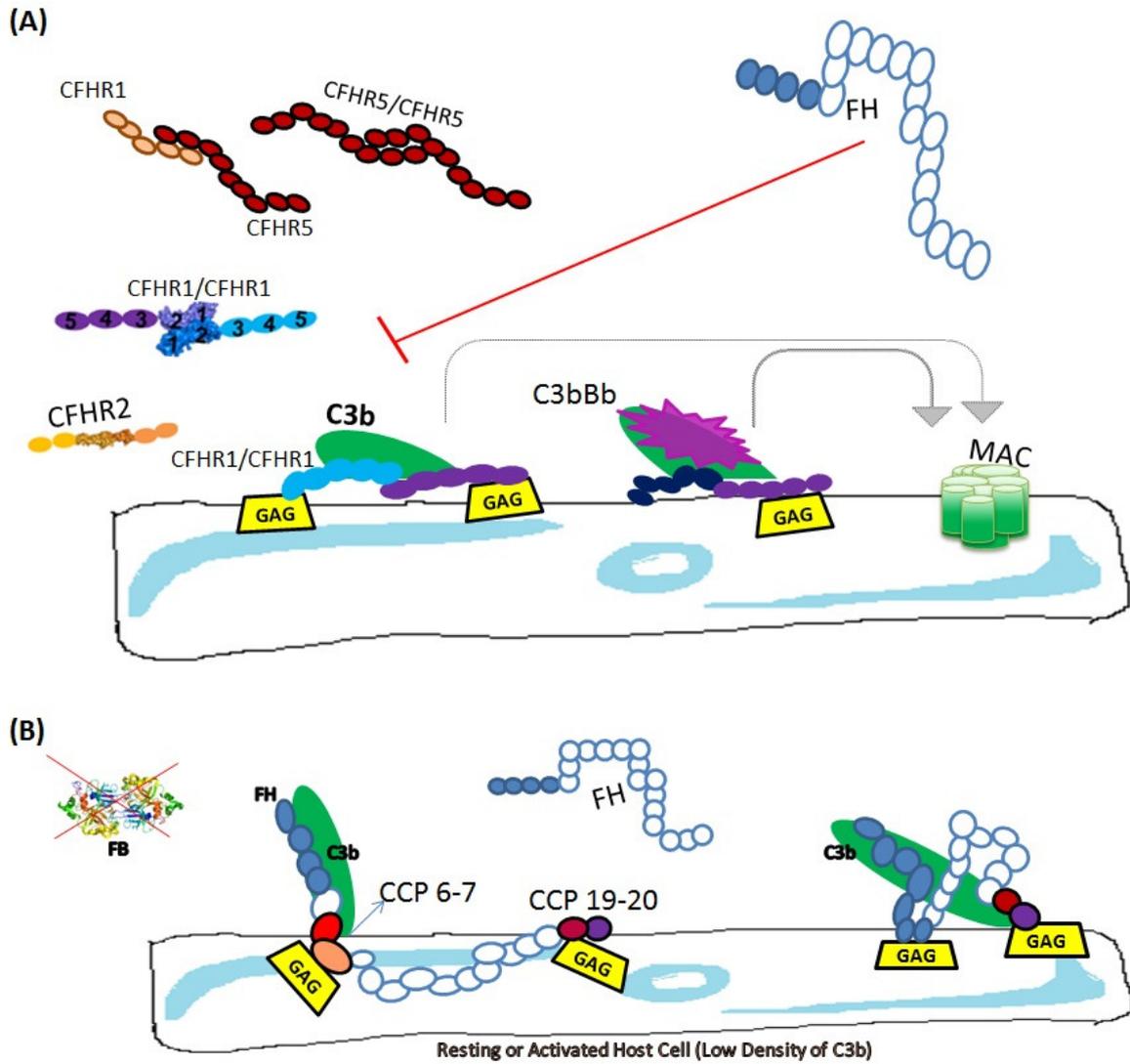


Figure 3

