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Immunobiology 228 (2023) 152364

Contents lists available at ScienceDirect

Immunobiology

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

Preferential production and secretion of the complement regulator factor H-like protein 1 (FHL-1) by human myeloid cells

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ARTICLE INFO

Keywords:
Complement
Factor H/FHL-1
Human
Inflammation
Monocyte/Macrophages

ABSTRACT

Factor H is a pivotal complement regulatory protein that is preferentially produced by the liver and circulates in high concentrations in serum. There has been an increasing interest in the extrahepatic production of complement factors, including by cells of the immune system, since this contributes to non-canonical functions of local complement activation and regulation. Here we investigated the production and regulation of factor H and its splice variant factor H-like protein 1 (FHL-1) by human myeloid cells. As validation, we confirmed the predominant presence of intact factor H in serum, despite a strong but comparable mRNA expression of *CFH* and *FHL1* in liver. Comparable levels of *CFH* and *FHL1* were also observed in renal tissue, although a dominant staining for FHL-1 was shown within the proximal tubules. Human *in vitro* generated pro- and anti-inflammatory macrophages both expressed and produced factor H/FHL-1, but this was strongest in pro-inflammatory macrophages. Production was not affected by LPS activation, but was increased upon stimulation with IFN-γ or CD40L. Importantly, in both macrophage subsets mRNA expression of *FHL1* was significantly higher than *CFH*. Moreover, production of FHL-1 protein could be confirmed using precipitation and immunoblotting of culture supernatants. These data identify macrophages as producers of factor H and FHL-1, thereby potentially contributing to local complement regulation at sites of inflammation.

1. Introduction

Macrophages are potent phagocytic cells playing an important role in homeostasis, tissue repair and immune responses (Tang et al., 2019; Wynn et al., 2013; Wynn and Vannella, 2016). In the injured tissue, the local production and release of cytokines, chemokines and complement factors (anaphylatoxins C3a/C5a) is responsible for the recruitment of immune cells, including monocytes (Tang et al., 2019; Wynn and Vannella, 2016). Proinflammatory cytokines like interferon gamma (IFN-γ), pathogen-associated molecular patterns (PAMPs) like lipopolysaccharide (LPS) and damage-associated molecular patterns (DAMPs) can polarize monocytes into proinflammatory macrophages. Recruited monocytes can also adopt an anti-inflammatory phenotype, involved in suppressing inflammation and inducing tissue repair (Tang et al., 2019). In addition, these pro- and anti-inflammatory macrophages function as antigen-presenting cells (APCs), directing T cell differentiation into T helper 1 (Th_1) and Th_2 cells, respectively (Mills and Ley, 2014; Tang et al., 2019).

Abbreviations: APC, antigen-presenting cells; CCP, complement control protein; CUBN, cubilin; DAF, decay-accelerating factor; DAMP, damage-associated molecular pattern; FHL-1, factor H-like protein 1; GM-CSF, granulocyte/macrophage colony-stimulating factor; IFN-γ, interferon gamma; LPS, lipopolysaccharide; M−CSF, macrophage colony-stimulating factor; PAMP, pathogen-associated molecular pattern; PBMC, peripheral blood mononuclear cells; PNA, peanut agglutinin; RA, rheumatoid arthritis; RPE, retinal pigment epithelium; Th, T helper.

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https://doi.org/10.1016/j.imbio.2023.152364
Received 19 July 2022; Received in revised form 23 February 2023; Accepted 24 February 2023
Available online 28 February 2023

The complement system plays an important role in the defense against microorganisms and the opsonization of dead cells (Roos et al., 2004; Walport, 2001). Most complement factors are predominantly produced by the liver, however extrahepatic sources like production by myeloid cells have also been described (Lubbers et al., 2017). Some complement factors are mainly produced extrahepatically, like C1q (Castellano et al., 2004) and properdin (Dixon et al., 2017; van Essen et al., 2022b). Negative complement regulator factor H, a ~ 155 kDa soluble factor, is involved in complement regulation by causing the decay of the C3 convertase and in addition contains cofactor activity (Parente et al., 2016; Schmidt et al., 2008). Alternative splicing of the factor H gene results in the generation of ~ 42–49 kDa factor H-like protein 1 (FHL-1), containing complement control protein (CCP) domains 1–7 similar to factor H, but lacks the C-terminal domains 8–20 (Estaller et al., 1991; Hellwage et al., 1997; Józsi and Zipfel, 2008; Mannes et al., 2020; Schwaeble et al., 1987). Factor H and FHL-1 are produced by the liver, and information on extrahepatically production is rather limited. Factor H and/or FHL-1 were detected in some non-hepatic cells, amongst others in monocytes, dendritic cells, endothelial cells, keratinocytes and skin fibroblasts ((Brooimans et al., 1989; Dixon et al., 2017; Katz and Strunk, 1988; Ripoche et al., 1988; Schwaeble et al., 1991; Timár et al., 2006) and reviewed by (Friese et al., 1999)). FHL-1 is also expressed by retinal pigment epithelium (RPE) cells, contributing to local complement regulation in the eye (Clark et al., 2014). Besides these findings, the physiological role for FHL-1 remains largely unknown (Mannes et al., 2020). Whether cells produce equal amounts of factor H and FHL-1 is currently unknown and also the impact of differential expression is unresolved. However, it is conceivable that the bulky factor H will have different functional properties as compared to the slim FHL-1 especially in the context of tissues outside the blood compartment and in cell–cell contact. Recently, it was shown that immune cells are able to produce various complement factors and express complement receptors like CRIg, and C3aR and C5aR to respond to anaphylatoxins C3a and C5a (Helmy et al., 2006; Lubbers et al., 2017). In addition, C3a and C5a generated locally were shown to enhance local T cell proliferation and activation in APC - T cell interaction (Cravedi et al., 2013). Downregulation of the decay-accelerating factor (DAF) also increased T cell proliferation in APC-T cell interaction (Heeger et al., 2005).

We investigated systemic and local production and secretion of soluble complement factors factor H and FHL-1. Comparable *CFH* and *FHL1* expression was observed in the liver, however, FHL-1 levels were much lower in normal human serum. Analysis in renal biopsies showed deposition of FHL-1 in proximal tubules where no factor H was present, despite slightly higher *CFH* expression levels. Human monocyte-derived pro- and anti-inflammatory macrophages expressed both *CFH* and *FHL1*, in which *FHL1* levels were higher. Factor H and FHL-1 protein levels were detected in the macrophage supernatant, and levels were altered upon stimulation. Preferential expression and protein secretion of FHL-1 by cells from the myeloid cell compartment was observed, suggesting a role for FHL-1 in local complement regulation.

2. Materials and methods

2.1. Generation of macrophages

Macrophages were derived from monocytes as described previously (van Essen et al., 2022a; Verreck et al., 2004; Xu et al., 2006). In brief, peripheral blood mononuclear cells (PBMCs) were isolated from healthy blood donors (buffy coats, permission: NVT0126.01, Sanquin, Amsterdam, The Netherlands) using Ficoll density gradient centrifugation (Pharmacy, LUMC, Leiden, The Netherlands). Monocytes were isolated by positive magnetic cell sorting using anti-CD14 MACS microbeads (130-050-201, Miltenyi Biotec, Leiden, The Netherlands). Cultures were supplemented with medium (RPMI 1640 (524000-025, Gibco/Life technologies, Breda, The Netherlands) + 10% heat-inactivated fetal calf serum (FCS; Bodinco, Alkmaar, The Netherlands), 90 U/mL penicillin and 90 μg/mL streptomycin (Gibco/Life technologies)) containing 10 ng/mL granulocyte–macrophage colony-stimulating factors (GM-CSF, Premium grade, 130-093-868, Miltenyi Biotec) or 100 ng/mL macrophage colony-stimulating factor (M-CSF, Premium grade, 130-096-493, Miltenyi Biotec) for the generation of pro- and anti-inflammatory macrophages, respectively. Cultures were refreshed at day three and five, and cells were harvested at day seven using trypsin (T4174, Sigma-Aldrich, Saint Louis, MO, USA). Differentiation was determined by flow cytometry (LSR-II 4L Full, BD Biosciences, San Jose, CA, USA) using mouse anti-human CD163 (PE, 1:20, Clone GHI/61, 333606, Biolegend, San Diego, CA, USA) and mouse anti-human CD14 (APC, 1:200, Clone MφP9, cat no. 345787, BD). Results were analyzed using FlowJo Software version 10.6.1 (Tree Star, Ashland, OR, USA). In addition, differentiation was analyzed by microscopy (DMI6000 B, Leica, Wetzlar, Germany) using LAS X image software.

2.2. Stimulation of macrophages

Harvested pro- and anti-inflammatory macrophages were stimulated with lipopolysaccharide (LPS, final concentration of 200 ng/mL, *E.coli* Serotype EH100, Enzo Life Sciences, Zandhoven, Belgium), irradiated (7500 Rad, Gammacell 1000 Elite, Best Theratronics, Kanata, ON, Canada) mouse fibroblast L-cells stably transfected with CD40L (1:10 ratio, (Garrone et al., 1995)) or control cells (L-Orient) or with IFN-γ (100 ng/mL, PeproTech, London, UK) diluted in medium containing GM-CSF or M−CSF. After 48 h, the supernatants were harvested and kept frozen at −20℃ until further usage.

2.3. Cytokine and factor H/FHL-1 measurements

Secretion of IL-6 and IL-10 by macrophages was analyzed by ELISA following manufacturer's instructions (IL-6: M9316, Sanquin, IL-10: M9310, Sanquin, or 555157, BD Biosciences). Absorbance was measured at 450 nm (iMark Microplate Reader, Bio-Rad, Hercules, CA, USA).

Factor H/FHL-1 levels were also measured by ELISA. In brief, mouse monoclonal anti-human factor H (19.5 μg/mL, OX23) was coated overnight. Plates were blocked (PBS-1% BSA) and supernatants were diluted in dilution buffer and incubated for 1 h at 37 °C. Next, plates were washed and incubated with rabbit anti human-factor H-Digoxin (DIG, 1:10.000, in-house derived) for 1 h at 37 °C. After washing, plates were incubated with peroxidase-conjugated IgG fraction monoclonal mouse anti-DIG (0.1 μg/mL, 200–032-156, Jackson ImmunoResearch) for 1 h at 37 °C and developed using TMB. The reaction was stopped with 1 M H_2SO_4 and the absorbance was measured at 450 nm.

2.4. Gene transcription levels

mRNA was isolated from ~ 0.5×10^6 pro- and anti- inflammatory macrophages using the RNeasy Micro kit (Qiagen) following the manufacturer's instructions. mRNA was isolated from ~ 3×10^6 monocytes, HuH-7 (gift from L.J.A.C. Hawinkels/M.A.C. Mieremet-Ooms) and HepG2 cells (gift from J. Kroon/H.C.M. Sips) using the RNeasy Mini kit (Qiagen) following the manufacturer's instructions. mRNA from liver and renal tissue was isolated using Trizol followed by the RNeasy Mini Kit (Qiagen). On column genomic DNA digestion and cDNA generation was performed as described previously (van Essen et al., 2020).

For qPCR analysis, 2 ng cDNA was pipetted into a 384 hard-shell PCR plate (thin wall, Bio-Rad) in combination with primers diluted in IQ SYBR green mix (Bio-Rad). Specific primers were used to determine human *GAPDH* (Dixon et al., 2017), *CFH* and *FHL1* expression (Table 1, final primer concentration of 1.25 μM, Biolegio, Nijmegen, The Netherlands). Samples were run (CFX384 touch real-time PCR detection system, C1000 Thermal Cycler, (Bio-Rad)) and gene expression levels were determined by calculating the mean values of triplicates followed

Table 1
Oligonucleotide sequences used for real-time PCR.

Target gene	GenBank accession no.	Product size (bp)	Forward primer (5′- 3′)	Reverse primer (5′-3′)
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	NM_001357943.2	175	TTCCAGGAGCGAGATCCCT	CACCCATGACGAACATGGG
CFH	NM_000186.4	128	GGACACAGTGAAGTGGTGGAAT	CCACAGGTACTCTCCTCCACA
FHL1	NM_001014975.3	99	TCCTGGCTACGCTCTTCCAA	CAGAGGGTAAAGCTGACACGG

by the ΔCT (gene of interest – *GAPDH*). Copies per *GAPDH* were calculated using $2^{-\Delta CT}$.

2.5. Factor H and FHL-1 staining on renal tissue

Pretransplant biopsies of donor kidneys and tissue not suitable for transplantation due to technical reasons (Eurotransplant, obtained with Research Consent) were used for staining of factor H and FHL-1 and represent healthy kidney. In brief, biopsies were sectioned (3 μm), acetone fixed and frozen. Sections were thawed and endogenous peroxidase (PBS-0.1% NaN_3-0.3% H_2O_2) was blocked for 30 min at room temperature. Next, sections were washed and blocked in PBS-1 % BSA-1% heat inactivated normal goat serum (NGS). Slides were incubated with OX24 (0.1 μg/mL), staining both factor H and FHL-1, and with FH.16 (0.4 μg/mL, clone anti-FH.16, binding factor H domains 16/17, gift from I. Jongerius (Pouw et al., 2019)) diluted in PBS-1 %BSA (PB) overnight at room temperature. Next day, slides were washed, incubated for 1 h with goat anti-mouse HRP (5 μg/mL, P0447, DAKO) diluted in PB. All slides were washed and developed using Nova RED (SK-4800, Vector Labs, Peterborough, UK). Slides were washed (PBS) and counterstained with Mayers hematoxylin (1.09249.0500, Merck, Darmstadt, Germany). Slides were dried overnight at room temperature before being covered using entellan (1.07961, Merck, Darmstadt, Germany). Slides were imaged (Pannoramic Midi scanner, 3DHISTECH, Budapest, Hungary) and images were taken (CaseViewer from 3DHISTECH).

In addition, immunofluorescent staining was performed on frozen tissue. After blocking, slides were incubated with OX24 (0.1 μg/mL) or FH.16 (0.4 μg/mL) in combination with cubilin (CUBN, 0.33 μg/mL, PA5-83684, Invitrogen, Waltham, MA, USA), staining proximal tubules, in PB. Next day, slides were washed and incubated with horse anti-mouse Dylight 549 (7.5 μg/mL, DI-2549, Vector Labs) and horse anti-rabbit Dylight 488 (7.5 μg/mL, DI-1088) in PB. Slides were washed, stained with Hoechst (1 μg/mL, H3569, Invitrogen) and mounted with ProLong gold (P36930, ThermoFisher Scientific, Waltham, MA, USA). Slides were also stained with OX24 or FH.16 in combination with lectin peanut agglutinin (PNA, 20 μg/mL, FL-1071–5, Vector Labs), staining distal tubules. Images were captured with the white light laser confocal microscope TCS SP8 (Leica) using LAS X software (Leica).

2.6. Immunoblot analysis

Purified factor H (75 ng, A410, Quidel), recombinant FHL-1 (75 ng, (Harder et al., 2016)), and normal human serum (3 μl) were prepared for separation using SDS PAGE (24 μl sample + 12 μl blue protein loading dye, (B7703S, New England BioLabs Inc., Ipswich, MA, USA) and boiled for 5 min). All samples were loaded onto a mini-Protean 10% gel (4568033, Bio-Rad) with a marker (10 μl, PageRuler Plus Prestained Protein Ladder, 10–250 kDa, 26619, ThermoFisher Scientific). Next, samples were blotted using the trans-blot turbo transfer system (PVDF, Bio-Rad, 1704156). Blots were blocked (PBS-5% skim milk (70166, Sigma-Aldrich)) overnight at 4 °C and incubated for 1 h at room temperature with either rabbit anti-human factor H-DIG (1:5000, in-house DIG labelled) or OX24 (5 μg/mL) diluted in PBS-5% skim milk. Blots were washed (PBS-0.05% Tween-20) and incubated with either peroxidase-conjugated IgG fraction monoclonal mouse anti-DIG (1:1000, Jackson) or goat-anti-mouse-HRP (50 ng/mL, P0447, DAKO) for 1 h at room temperature. Blots were washed and developed (SuperSignal West Pico PLUS Chemiluminescent Substrate, 34580, ThermoFisher Scientific) and imaged with the Chemidoc Touch (Bio-Rad). Figures were prepared using Image Lab software (Bio-Rad).

2.7. Immunoprecipitation

Factor H and FHL-1 were immunoprecipitated from the supernatant from pro- and anti-inflammatory macrophages (stimulated with IFN-γ for 48 h) following the protocol. In brief, per sample 50 μl magnetic Dynabeads Protein G (30 mg/mL, 10004D, Invitrogen) were mixed with 5 μg OX24 or an isotype control (5 μg, mouse IgG1, DAKO, X0931) diluted in PBS-0.02% Tween-20 and incubated for 10 min at room temperature rotating. Magnetic bead-antibody complexes were washed (placed tube on magnet, removed supernatant, added PBS-0.02% Tween-20) and cross-linked using the cross-linking immunoprecipitation (CLIP) protocol. In brief, the antibody-bead complexes were washed twice with 200 μl conjugation buffer (20 mM sodium phosphate, 0.15 M NaCl, pH 7.4). The complexes were resuspended in 250 μl 5 mM BS^3 (dissolved in 20 mM sodium phosphate, 0.15 M NaCl, pH 7.4) and incubated for 30 min at room temperature rotating. The reaction was quenched using 12.5 μl/sample (1 M Tris HCl, pH 7.5) and incubated for 15 min at room temperature rotating. The cross-linked antibody-beads complexes were washed three times with 200 μl PBS-0.02% Tween-20. Next, the antigen was immunoprecipitated. Supernatant (1 mL containing ~ 15 ng factor H/FHL-1) was incubated for 15 min at room temperature rotating. Supernatant was removed (placing the tube containing the magnetic beads-antibody-antigen complexes on the magnet with consecutive removal of the fluid) and the beads were incubated again with 1 mL supernatant. The magnetic beads-antibody-antigen complexes were washed 3 times using PBS by putting the tube on the magnet. The mixture was transferred to a new tube and the antigen was eluted by incubating the complexes with 20 μl elution buffer (50 mM glycine pH 2.8) for 4 min at room temperature. The eluted protein was transferred to a clean tube and 4 μl 1 M Tris (pH 7.5) was added to adjust the pH of the sample. The eluted samples were prepared for separation using SDS PAGE (to 24 μl elution buffer 12 μl blue protein loading dye (B7703S) was added). In addition, purified factor H (15 ng, A410, Quidel) and recombinant FHL-1 (15 ng) were prepared and all samples were loaded and blotted as described above. Factor H and FHL-1 were detected using rabbit anti-human factor H-DIG (1:5000, in-house DIG labelled) and peroxidase-conjugated IgG fraction monoclonal mouse anti-DIG (1:1000, Jackson). This protocol was also used to immunoprecipitate factor H and FHL-1 from supernatant from unstimulated pro- and anti-inflammatory macrophages generated from two donors (after concentrating the supernatant, factor H/FHL-1 levels as determined by ELISA: pro-inflammatory ~ 100 ng/mL, anti-inflammatory ~ 15 ng/mL). Figures were prepared using Image Lab software (Bio-Rad) and factor H/FHL-1 levels were estimated using volume tools after global background subtraction. In addition, immunoprecipitation of factor H and FHL-1 from these supernatants (both pro- and anti-inflammatory macrophages, n = 1 stimulated with IFN-γ and n = 2 supernatant from unstimulated macrophages) was repeated and subsequently analyzed by immunoblotting in parallel with various concentrations of purified factor H (50, 10, 2 ng) and recombinant FHL-1 (50, 25, 12.5 ng) to determine factor H and FHL-1 protein levels.

2.8. Statistics

Statistical analysis between two groups was assessed by a paired, Wilcoxon matched-pairs signed rank test. Significance was defined as $P \leq 0.05$. For statistical analysis and graphical representations, GraphPad Prism v.9.3.1 was used (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Generation of monocyte derived macrophages

Polarization of monocytes into either pro- or anti-inflammatory macrophages was analyzed using microscopy. Proinflammatory macrophages, generated with GM-CSF, showed a "fried-egg" phenotype, whereas anti-inflammatory macrophages, generated with M−CSF, showed a spindle-shaped phenotype (Fig. 1a) (Xu et al., 2013). Polarization was also followed using flow cytometry, showing the intermediate expression of CD14 and CD163 on proinflammatory macrophages, whereas anti-inflammatory macrophages express homogeneous and higher levels of CD14 and CD163 (Fig. 1B-C). Functional polarization of macrophages was confirmed by significantly higher levels of IL-6 secretion by proinflammatory macrophages following LPS stimulation (Fig. 1D). Anti-inflammatory macrophages were able to secrete IL-6, however, to a ~ 65-fold lower extent when compared to proinflammatory macrophages (Fig. 1D). Both pro- and anti-inflammatory macrophages produced similar amounts of IL-10 upon stimulation with LPS.

3.2. Distribution of factor H and FHL-1

Since most circulating complement factors are produced by the liver, we validated our tools by examining *CFH* and *FHL1* expression in unstimulated liver cells and tissue. Using primers to specifically discriminate between factor H and its splice variant FHL-1, it was observed in mRNA isolated from two liver biopsies that expression of *CFH* and *FHL1* was high and no difference was observed. Also, in the HuH-7 liver cell line there was no difference in expression between *CFH* and *FHL1*, albeit that the expression was lower when compared to the expression in liver tissue. In the HepG2 cell line, *CFH* and *FHL1* expression were not detected, as was reported for factor H before (Lappin et al., 1992) (Fig. 2A).

Immunoblot analysis validated the detection of purified factor H and recombinant FHL-1 both by a monoclonal (OX24) and polyclonal antibody (Fig. 2B). Despite similar levels of mRNA for *CFH* and *FHL1* in the liver, systemically, as measured in serum, only minute amount of FHL-1 protein could be detected (Fig. 2B). Staining of tissue sections of human kidneys with OX24, a monoclonal antibody detecting both factor H and FHL-1, resulted in a very prominent staining at the tubular brush border (Fig. 2C, arrowheads). In contrast, when using an antibody specific for factor H (FH.16), no staining was observed in the tubular compartment (arrowheads). However, some staining in the glomeruli was observed (arrow, biopsy 2), most likely representing intravascular factor H. In renal tissue, low but comparable levels of *CFH* and *FHL1* mRNA expression were detected (Fig. 2D). Immunofluorescent staining of renal tissue indicated the presence of FHL-1 in proximal tubules, but not in distal tubules (Fig. 2E, left and middle panel). Factor H staining (using FH.16) was not observed in tubular structures, but was primarily observed within the glomeruli (Fig. 2E).

3.3. Preferential expression of FHL1 in pro- and anti-inflammatory macrophages

Next to the important systemic role of complement, there is an increasing interest in the local contribution of complement production, activation and regulation, with a prominent role for myeloid cells (Lubbers et al., 2017). Therefore, we examined the expression of *CFH* and *FHL1* by different myeloid cells. Freshly isolated human peripheral blood monocytes only showed very low levels of *CFH* and *FHL1* expression (Fig. 3A). In contrast, culture and differentiation of monocytes into both pro- and anti-inflammatory macrophages showed clear expression of *CFH* and *FHL1*. Despite individual donor variation, in all fourteen donors tested, *FHL1* expression was significantly higher compared to *CFH* expression (Fig. 3A, proinflammatory 4.5-fold increase and anti-inflammatory macrophages 5.2-fold increase). Preferential *FHL1* expression was also observed in monocyte-derived dendritic cells (Suppl. Fig. 1).

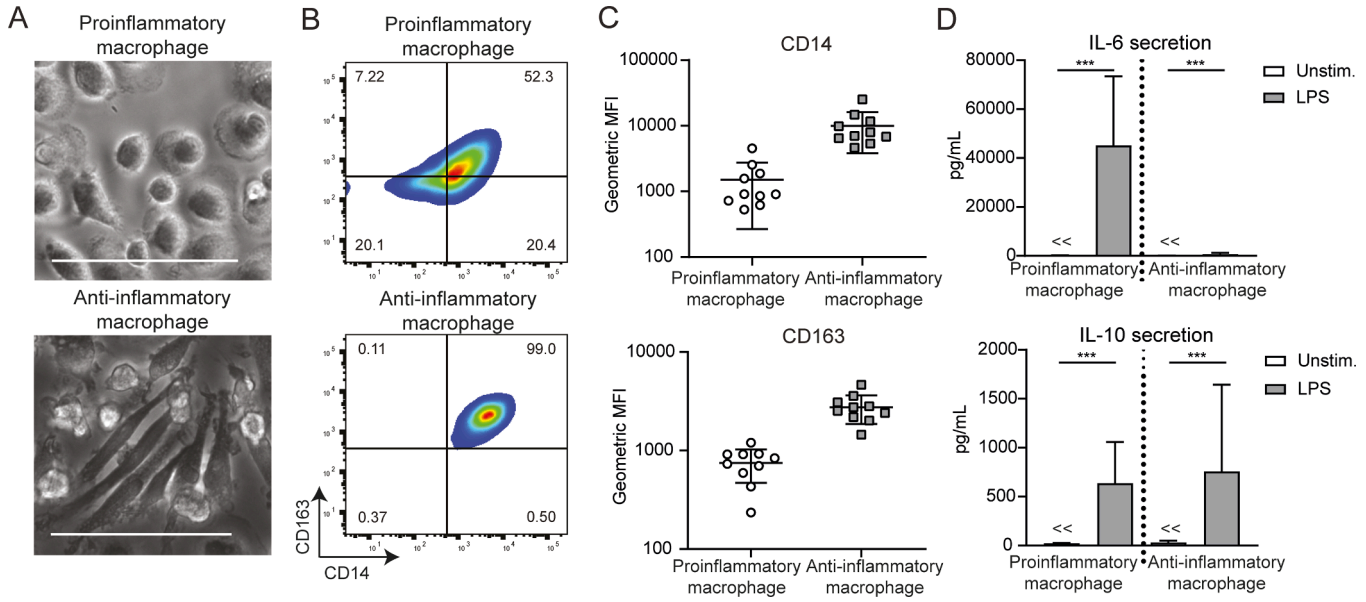

Fig. 1. Phenotypic analysis of monocyte-derived pro- and anti-inflammatory macrophages. Monocytes were isolated and stimulated for 7 days with GM-CSF or M−CSF for the generation of proinflammatory or anti-inflammatory macrophages, respectively. (A) Microscopic analysis of pro- and anti-inflammatory macrophages at day 7. Scale bar: 100 μm. (B) Representative FACS plots for the phenotypic analysis of CD163 and CD14 expression. (C) Quantification of CD14 and CD163 expression on pro- and anti-inflammatory macrophages (n = 10). (D) Analysis of IL-6 and IL-10 production by unstimulated and LPS stimulated pro-and anti-inflammatory macrophages (n = 11). Data are presented as mean ± SD. Wilcoxon matched-pairs signed rank test, *** $P \leq 0.001$.

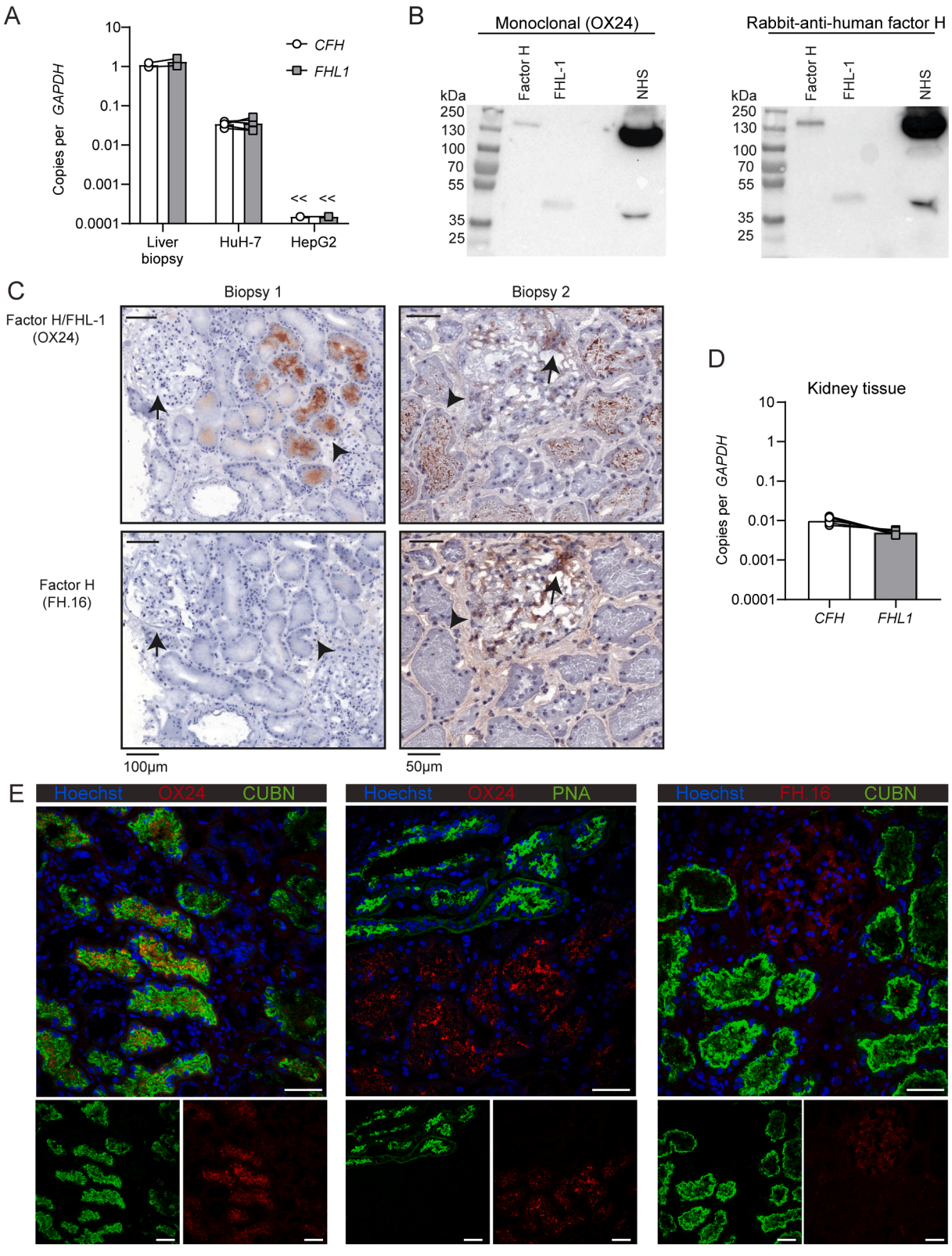

Fig. 2. Distribution of factor H and FHL-1. (A) Comparison of *CFH* and *FHL1* expression in tissue (liver biopsy n = 2) and liver cell lines (HuH-7 n = 5, HepG2 n = 4). (B) Immunoblot analysis of purified factor H (~75 ng), recombinant FHL-1 (~75 ng) and factor H and FHL-1 in normal human serum (NHS, 3 µl). Factor H and FHL-1 were detected using both a monoclonal (OX24) and polyclonal (rabbit-anti-human factor H-DIG) antibody. (C) Staining for factor H/FHL-1 (OX24) or factor H specifically (FH.16) in biopsies of healthy renal tissue. Representative images representing the same location of factor H/FHL-1 staining in two biopsies of healthy renal tissue. Arrowheads indicate presence of tubular factor H/FHL-1, arrows indicate factor H/FHL-1 protein in glomeruli. (D) *CFH* and *FHL1* expression in renal tissue (n = 5). (E) Immunofluorescent staining of renal tissue for factor H and FHL-1 (OX24) or factor H specifically (FH.16) in combination with either cubilin (CUBN), staining proximal tubules, or with peanut agglutinin (PNA), staining distal tubules. Scale bar: 50 µm.

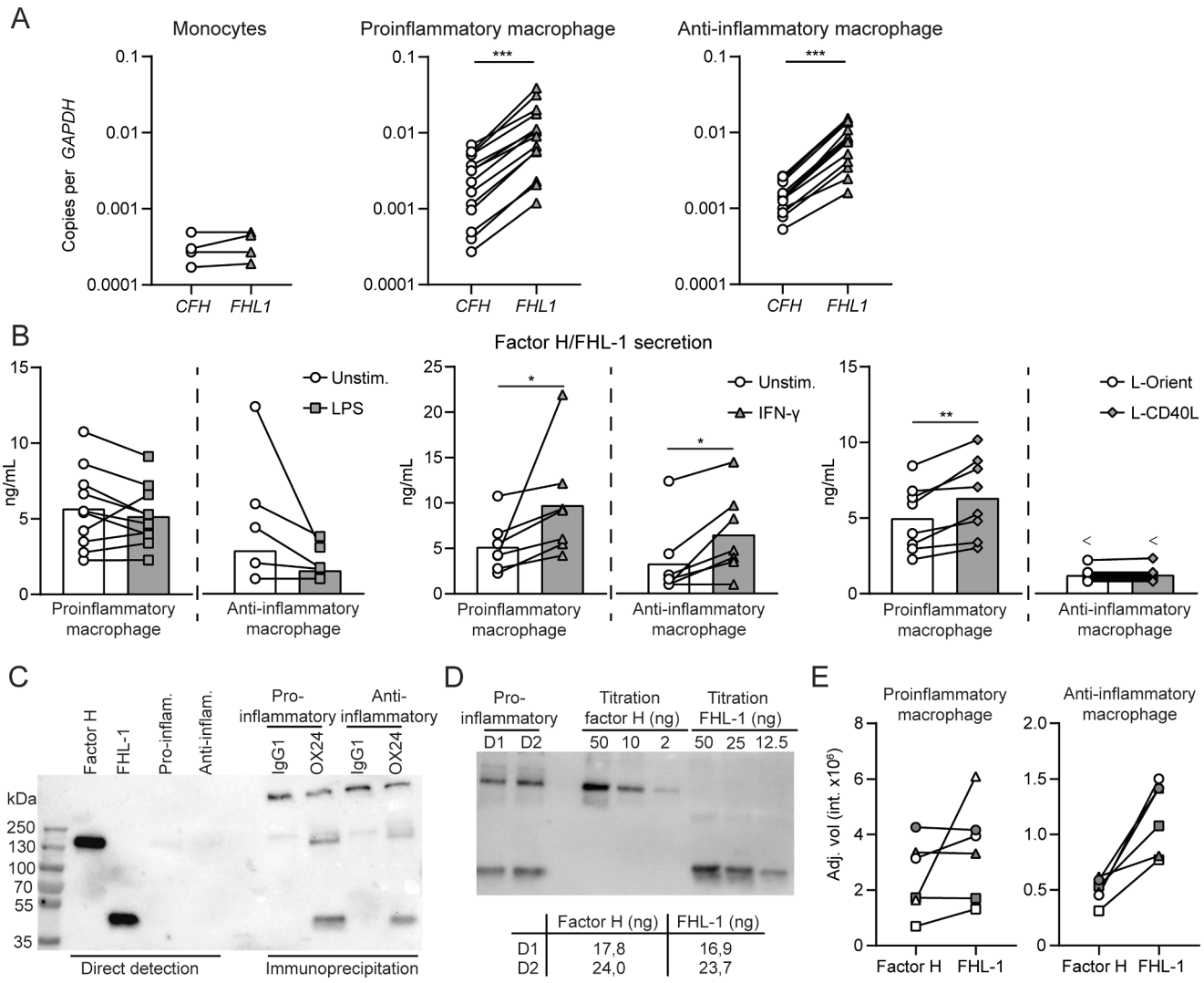

Fig. 3. Expression and secretion of factor H and FHL-1 by myeloid cells. (A) *CFH* and *FHL1* expression by monocytes (*CFH* n = 4/4 CT > 32; *FHL1* n = 2/4 CT > 32, CT = 32 used for calculations), proinflammatory macrophages (n = 14; in 2/14 *CFH* CT > 32, CT = 32 used for calculations) and anti-inflammatory macrophages (n = 14; in 2/14 *CFH* CT > 32, CT = 32 used for calculations). (B) Factor H/FHL-1 secretion by unstimulated and LPS stimulated pro- (n = 10) and anti-inflammatory macrophages (n = 11, no secretion detected in 7/11 donors), unstimulated and IFN-γ stimulated pro- (n = 7) and anti-inflammatory macrophages (n = 7, no secretion detected in 3/7 unstimulated and 1/7 IFN-γ stimulated anti-inflammatory macrophages). Factor H/FHL-1 secretion by L-Orient and L-CD40L stimulated pro- (n = 8) and anti-inflammatory macrophages (n = 8, no secretion detected in 7/8 stimulated anti-inflammatory macrophages). (C) Direct detection of purified factor H (~15 ng loaded), recombinant FHL-1 (~15 ng loaded) or factor H and FHL-1 in supernatant from IFN-γ stimulated pro- and anti-inflammatory macrophages. In addition, factor H and FHL-1 were immunoprecipitated from supernatant of IFN-γ stimulated pro- and anti-inflammatory macrophages, using either OX24-coupled or mouse IgG1-coupled control beads, and analyzed by immunoblot. (D) Immunoprecipitation of factor H and FHL-1 from concentrated pro-inflammatory macrophage supernatant derived from two different monocyte donors (Donor 1 (D1): 100 ng/mL. donor 2 (D2): 106 ng/mL, 1 mL incubated with OX24 antibody-coupled beads). Concentration was determined using volume tools (ImageLab) based on the titration of purified factor H (50, 10, 2 ng) and FHL-1 (50, 25 and 12.5 ng). (E) Factor H and FHL-1 secretion by pro- and anti-inflammatory macrophages. Factor H and FHL-1 in supernatant from unstimulated (n = 2) and IFN-γ stimulated (n = 1) pro- and anti-inflammatory macrophages were immunoprecipitated twice in independent experiments, as indicated by the corresponding symbols. Densitometry analysis was performed with Image Lab using the volume tools after global background subtraction. Wilcoxon matched-pairs signed rank test, * P <.05, ** P <.01, *** P <.001.

Using an ELISA to examine factor H and FHL-1 protein levels, it was confirmed that proinflammatory macrophages secreted higher levels of both factor H and FHL-1 compared to anti-inflammatory macrophages. Stimulation with LPS did not have an effect (Fig. 3B, left graph). Stimulation with IFN-γ resulted in increased factor H/FHL-1 secretion in both cell-types, whereas L-CD40L only increased factor H/FHL-1 secretion by proinflammatory macrophages (Fig. 3B).

Since this ELISA is not able to discriminate between factor H and FHL-1, we performed immunoblotting on these supernatants. However, sensitivity of this assay was not sufficient to directly detect factor H/FHL-1 (Fig. 3C). Therefore, we performed immunoprecipitation on supernatants from IFN-γ-stimulated pro- and anti-inflammatory macrophages, followed by immunoblot analysis of the eluate. Factor H and FHL-1 were immunoprecipitated from the supernatant after incubation with OX24-coupled beads, but not after incubation with mouse IgG1-coupled beads (Fig. 3C). To determine the quantity of factor H and FHL-1, we immunoprecipitated factor H and FHL-1 from concentrated supernatant from unstimulated pro-inflammatory macrophages which were analyzed by immunoblot along with varying amounts of factor H and FHL-1 (Fig. 3D). Both factor H and FHL-1 were detected, and densitometry analysis showed that both proteins were present in similar amounts (Fig. 3D). Densitometry analysis of immunoprecipitated factor H and FHL-1 levels from the macrophage supernatants showed that secreted FHL-1 levels were higher when compared to factor H, especially in anti-inflammatory macrophages (Fig. 3E).

4. Discussion

Most complement factors are produced by the liver, generating the pool of systemic complement factors. However, also production of complement factors by extrahepatic sources are increasingly recognized, e.g., the local production and secretion by cells of the immune system (Lubbers et al., 2017). In this study, we investigated and compared the expression and secretion of factor H and FHL-1 by different myeloid cells. Importantly, we showed that both pro- and anti-inflammatory macrophages, generated from human monocytes, are potent producers of factor H and FHL-1. Direct comparison showed preferential expression and secretion of FHL-1 by these myeloid cells, which could suggest a role in the local regulation of complement activation.

In normal human serum, mainly containing complement factors produced by the liver, preferentially the full length ~ 155 kDa factor H protein is observed, whereas FHL-1 levels are much lower (Fig. 2B) (Dopler et al., 2019; Schwaeble et al., 1987). Despite this, *CFH* and *FHL1* levels have a similar expression in the liver and HuH-7 liver cells (Fig. 2A). This might be expected since *CFH* and *FHL1* are derived from the same gene and thereby share their promotor and transcription start site (Friese et al., 2000; Mannes et al., 2020).

Pharmacokinetic analysis previously showed that, when both human factor H and FHL-1 were injected in mice, the FHL-1 clearance rate is ~ 6-fold higher compared to factor H clearance (Dopler et al., 2019). In our hands, we observed FHL-1 in the proximal tubules of healthy human renal tissue, whereas at the same location factor H was absent (Fig. 2C and 2E). This difference was not explained by an increased renal *FHL1* expression (Fig. 2D). These data are compatible with the notion that the smaller ~ 42–49 kDa FHL-1 is subject to renal clearance while the larger factor H is not. Additional research is needed to address whether this is indeed the case, e.g. by examination of factor H and FHL-1 levels in urine. In addition, dedicated tracing experiments would be required to experimentally establish this point.

We observed a preferential expression of *FHL1* in cells from the myeloid compartment, including monocyte-derived dendritic cells and macrophages (Fig. 3A and Suppl. Fig. 1). Currently, it is not known how this differential splicing within myeloid cells is regulated. Therefore, we aimed to translate this to protein expression. Since discrimination of factor H/FHL-1 by ELISA is difficult and protein levels were not directly detected in the macrophage supernatant by immunoblot (Fig. 3C), we combined this with immunoprecipitation. Factor H and FHL-1 were both detected and, in comparison with factor H levels, anti-inflammatory macrophages secreted more FHL-1. The production of complement regulators by myeloid cells indicates a potential role in regulating complement activation locally. A local role for FHL-1 has been described in the eye where, in contrast to factor H, the smaller FHL-1 protein was able to diffuse through Bruch's membrane, contributing to local complement regulation (Clark et al., 2014). In addition, an increased ratio of *FHL1* expression has been observed in synovial fluid in the context of rheumatoid arthritis (RA) (Friese et al., 2000).

Interestingly, expression of *CFH* and *FHL1* was very low in monocytes, the precursor cells we used for the *in vitro* generation of macrophages and dendritic cells. This suggests that monocytes, when present in blood, do not seem to contribute to factor H/FHL-1 production. However, when monocytes migrate into tissue, thereby differentiating into macrophages, they do become potent produces of predominantly FHL-1. Levels of factor H and FHL-1 can be further increased upon stimulation IFN-γ and L-CD40L, representing T-cell mediated activation, whereas production was not affected by LPS. Interestingly, both pro- and anti-inflammatory macrophages produced comparable levels of factor H/FHL-1. It might be counterintuitive that a proinflammatory cytokine enhances the production of the complement regulatory proteins factor H/FHL-1 by proinflammatory macrophages. It has been shown that the addition of factor H during the generation of monocyte-derived dendritic cells resulted in the generation of tolerogenic dendritic cells (Olivar et al., 2016). Therefore, this could act as a negative feedback mechanism and underscores the complex role that complement activation plays in inflammation and immunity (Hajishengallis et al., 2017). However, a limitation of our study is that *in vitro* polarized monocyte-derived macrophage subsets are a strong oversimplification. *In vivo* macrophages are very plastic and more heterogeneous and represent a spectrum of different functional cells (Martinez and Gordon, 2014; Sica and Mantovani, 2012; Tang et al., 2019).

The small FHL-1 protein contains all domains needed for complement regulation and was shown to display a regulatory activity overall comparable to factor H (Harder et al., 2016). The important difference is that FHL-1 contains less recognition domains (e.g. domain 19 and 20) for cell attachment and discriminates less between host and foreign surfaces compared to full length factor H (Dopler et al., 2019). This highlights the importance of FHL-1 in regulating AP activity at specific compartments where FHL-1 molecules outnumber factor H. In tissues, when macrophages predominantly produce and secrete the small FHL-1, this might still be sufficient to be locally retained and regulate complement activation. In line with this, also other complement factors, including C1q (Castellano et al., 2004), properdin (Dixon et al., 2017; van Essen et al., 2022b) and C7 (Würzner, 2000), have been described to be predominantly produced by myeloid cells. Properdin and factor H derived from antigen-presenting cells were shown to modulate T-cell activation during APC-T cells interaction (Dixon et al., 2017; van Essen et al., 2022b). This supports a role for the local AP activation and regulation in the context of both innate and adaptive immune responses.

In conclusion, we have demonstrated that, whereas the systemic compartment is mainly characterized by full length liver-derived factor H, macrophages show a bias towards the expression and production of the splice variant FHL-1. It will be important to further delineate the distribution of these two proteins and to understand different functional properties, for instance based on the differential expression of cell recognition domains.

CRediT authorship contribution statement

Mieke F. van Essen: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Emma T.M. Peereboom:** Data curation, Formal analysis, Writing – review & editing. **Nicole Schlagwein:** Data curation, Formal analysis, Writing – review & editing. **Daniëlle J. van Gijlswijk-Janssen:** Data curation, Formal analysis, Writing – review & editing. **Tessa Nelemans:** Data curation, Formal analysis, Writing – review & editing. **Jivan V. Joeloemsingh:** Data curation, Formal analysis, Writing – review & editing. **Cathelijne W. van den Berg:** Writing – review & editing. **Jurriën Prins:** Writing – review & editing. **Simon J. Clark:** Writing – review & editing. **Christoph Q. Schmidt:** . **Leendert A. Trouw:** . **Cees van Kooten:** Conceptualization, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We would like to thank Dr. I. Jongerius for providing the factor H antibody (clone anti-FH.16). Flow cytometry was performed with BD FACS LSR-II 4L Full (BD Biosciences) at the Flow cytometry Core Facility (FCF) of Leiden University Medical Center (LUMC) in Leiden, the Netherlands. The COMBAT consortium is sponsored by the Dutch

Kidney Foundation (grant 13OCA27). The COMBAT consortium is an inter-university collaboration in the Netherlands that is formed to study basic mechanisms, assay development and therapeutic translation of complement-mediated renal diseases. Principal investigators are (in alphabetical order): S. Berger (Department of Internal Medicine-Nephrology, University Medical Center Groningen, The Netherlands), J. van den Born (Department of Internal Medicine-Nephrology, University Medical Center Groningen, The Netherlands), P. Gros (Department of Chemistry, Utrecht University, The Netherlands), L. van den Heuvel (Department of Pediatric Nephrology, Radboud University Medical Center, Nijmegen, The Netherlands), N. van de Kar (Department of Pediatric Nephrology, Radboud University Medical Center, Nijmegen, The Netherlands), C. van Kooten (Department of Internal Medicine-Nephrology, Leiden University Medical Center, The Netherlands), M. Seelen (Department of Internal Medicine-Nephrology, University Medical Center Groningen, The Netherlands), A. de Vries (Department of Internal Medicine-Nephrology, Leiden University Medical Center, The Netherlands).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.imbio.2023.152364.

References

Brooimans, R.A., Hiemstra, P.S., van der Ark, A.A., Sim, R.B., van Es, L.A., Daha, M.R., 1989. Biosynthesis of complement factor H by human umbilical vein endothelial cells. Regulation by T cell growth factor and IFN-gamma. J Immunol. 142 (6), 2024-2030.

Castellano, G., Woltman, A.M., Nauta, A.J., Roos, A., Trouw, L.A., Seelen, M.A., Schena, F.P., Daha, M.R., van Kooten, C., 2004. Maturation of dendritic cells abrogates C1q production in vivo and in vitro. Blood 103 (10), 3813–3820. https://doi.org/10.1182/blood-2003-09-3046.

Clark, S.J., Schmidt, C.Q., White, A.M., Hakobyan, S., Morgan, B.P., Bishop, P.N., 2014. Identification of factor H-like protein 1 as the predominant complement regulator in Bruch's membrane: implications for age-related macular degeneration. J. Immunol. 193 (10), 4962–4970. https://doi.org/10.4049/jimmunol.1401613.

Cravedi, P., Leventhal, J., Lakhani, P., Ward, S.C., Donovan, M.J., Heeger, P.S., 2013. Immune cell-derived C3a and C5a costimulate human T cell alloimmunity. Am. J. Transplant. 13 (10), 2530–2539. https://doi.org/10.1111/ajt.12405.

Dixon, K.O., O'Flynn, J., Klar-Mohamad, N., Daha, M.R., van Kooten, C., 2017. Properdin and factor H production by human dendritic cells modulates their T-cell stimulatory capacity and is regulated by IFN-γ. Eur. J. Immunol. 47 (3), 470–480. https://doi.org/10.1002/eji.201646703.

Dopler, A., Guntau, L., Harder, M.J., Palmer, A., Höchsmann, B., Schrezenmeier, H., Simmet, T., Huber-Lang, M., Schmidt, C.Q., 2019. Self versus Nonself Discrimination by the Soluble Complement Regulators Factor H and FHL-1. J. Immunol. 202 (7), 2082–2094. https://doi.org/10.4049/jimmunol.1801545.

Estaller, C., Schwaeble, W., Dierich, M., Weiss, E.H., 1991. Human complement factor H: two factor H proteins are derived from alternatively spliced transcripts. Eur. J. Immunol. 21 (3), 799–802. https://doi.org/10.1002/eji.1830210337.

Friese, M.A., Hellwage, J., Jokiranta, T.S., Meri, S., Peter, H.H., Eibel, H., Zipfel, P.F., 1999. FHL-1/reconectin and factor H: two human complement regulators which are encoded by the same gene are differently expressed and regulated. Mol. Immunol. 36 (13–14), 809–818. https://doi.org/10.1016/s0161-5890(99)00101-7.

Friese, M.A., Hellwage, J., Jokiranta, T.S., Meri, S., Müller-Quernheim, H.J., Peter, H.H., Eibel, H., Zipfel, P.F., 2000. Different regulation of factor H and FHL-1/reconectin by inflammatory mediators and expression of the two proteins in rheumatoid arthritis (RA). Clin. Exp. Immunol. 121 (2), 406–415. https://doi.org/10.1046/j.1365-2249.2000.01285.x.

Garrone, P., Neidhardt, E.M., Garcia, E., Galibert, L., van Kooten, C., Banchereau, J., 1995. Fas ligation induces apoptosis of CD40-activated human B lymphocytes. J. Exp. Med. 182 (5), 1265–1273. https://doi.org/10.1084/jem.182.5.1265.

Hajishengallis, G., Reis, E.S., Mastellos, D.C., Ricklin, D., Lambris, J.D., 2017. Novel mechanisms and functions of complement. Nat. Immunol. 18 (12), 1288–1298. https://doi.org/10.1038/ni.3858.

Harder, M.J., Anliker, M., Höchsmann, B., Simmet, T., Huber-Lang, M., Schrezenmeier, H., Ricklin, D., Lambris, J.D., Barlow, P.N., Schmidt, C.Q., 2016. Comparative Analysis of Novel Complement-Targeted Inhibitors, MiniFH, and the Natural Regulators Factor H and Factor H-like Protein 1 Reveal Functional Determinants of Complement Regulation. J. Immunol. 196 (2), 866–876. https://doi.org/10.4049/jimmunol.1501919.

Heeger, P.S., Lalli, P.N., Lin, F., Valujskikh, A., Liu, J., Muqim, N., Xu, Y., Medof, M.E., 2005. Decay-accelerating factor modulates induction of T cell immunity. J. Exp. Med. 201 (10), 1523–1530. https://doi.org/10.1084/jem.20041967.

Hellwage, J., Kühn, S., Zipfel, P.F., 1997. The human complement regulatory factor-H-like protein 1, which represents a truncated form of factor H, displays cell-attachment activity. Biochem. J 326 (Pt 2) (Pt 2), 321–327. https://doi.org/10.1042/bj3260321.

Helmy, K.Y., Katschke, K.J., Gorgani, N.N., Kljavin, N.M., Elliott, J.M., Diehl, L., Scales, S.J., Ghilardi, N., Van Lookeren Campagne, M., 2006. CRIg: A macrophage complement receptor required for phagocytosis of circulating pathogens. Cell 124 (5), 915–927. https://doi.org/10.1016/j.cell.2005.12.039.

Józsi, M., Zipfel, P.F., 2008. Factor H family proteins and human diseases. Trends Immunol. 29 (8), 380–387. https://doi.org/10.1016/j.it.2008.04.008.

Katz, Y., Strunk, R.C., 1988. Synthesis and regulation of complement protein factor H in human skin fibroblasts. J Immunol. 141 (2), 559–563.

Lappin, D.F., Guc, D., Hill, A., McShane, T., Whaley, K., 1992. Effect of interferon-γ on complement gene expression in different cell types. Biochem. J 281 (Pt 2) (Pt 2), 437–442. https://doi.org/10.1042/bj2810437.

Lubbers, R., van Essen, M.F., van Kooten, C., Trouw, L.A., 2017. Production of complement components by cells of the immune system. Clin. Exp. Immunol. 188 (2), 183–194. https://doi.org/10.1111/cei.12952.

Mannes, M., Dopler, A., Huber-Lang, M., Schmidt, C.Q., 2020. Tuning the functionality by splicing: factor h and its alternative splice Variant FHL-1 share a gene but not all functions. Front. Immunol. 11, 596415 https://doi.org/10.3389/fimmu.2020.596415.

Martinez, F.O., Gordon, S., 2014. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep. 6, 13. https://doi.org/10.12703/p6-13.

Mills, C.D., Ley, K., 2014. M1 and M2 macrophages: the chicken and the egg of immunity. J. Innate Immun. 6 (6), 716–726. https://doi.org/10.1159/000364945.

Olivar, R., Luque, A., Cárdenas-Brito, S., Naranjo-Gómez, M., Blom, A.M., Borràs, F.E., Rodriguez de Córdoba, S., Zipfel, P.F., Aran, J.M., 2016. The Complement Inhibitor Factor H Generates an Anti-Inflammatory and Tolerogenic State in Monocyte-Derived Dendritic Cells. J. Immunol. 196 (10), 4274–4290. https://doi.org/10.4049/jimmunol.1500455.

Parente, R., Clark, S.J., Inforzato, A., Day, A.J., 2016. Complement factor H in host defense and immune evasion. Cell. Mol. Life Sci. 74 (9), 1605–1624. https://doi.org/10.1007/s00018-016-2418-4.

Pouw, R.B., Brouwer, M.C., de Gast, M., van Beek, A.E., van den Heuvel, L.P., Schmidt, C. Q., van der Ende, A., Sánchez-Corral, P., Kuijpers, T.W., Wouters, D., 2019. Potentiation of complement regulator factor H protects human endothelial cells from complement attack in aHUS sera. Blood Adv. 3 (4), 621–632. https://doi.org/10.1182/bloodadvances.2018025692.

Ripoche, J., Mitchell, J.A., Erdei, A., Madin, C., Moffatt, B., Mokoena, T., Gordon, S., Sim, R.B., 1988. Interferon gamma induces synthesis of complement alternative pathway proteins by human endothelial cells in culture. J. Exp. Med. 168 (5), 1917–1922. https://doi.org/10.1084/jem.168.5.1917.

Roos, A., Xu, W., Castellano, G., Nauta, A.J., Garred, P., Daha, M.R., van Kooten, C., 2004. Mini-review: a pivotal role for innate immunity in the clearance of apoptotic cells. Eur. J. Immunol. 34 (4), 921–929. https://doi.org/10.1002/eji.200424904.

Schmidt, C.Q., Herbert, A.P., Hocking, H.G., Uhrín, D., Barlow, P.N., 2008. Translational Mini-Review Series on Complement Factor H: structural and functional correlations for factor H. Clin. Exp. Immunol. 151 (1), 14–24. https://doi.org/10.1111/j.1365-2249.2007.03553.x.

Schwaeble, W., Zwirner, J., Schulz, T.F., Linke, R.P., Dierich, M.P., Weiss, E.H., 1987. Human complement factor H: expression of an additional truncated gene product of 43 kDa in human liver. Eur. J. Immunol. 17 (10), 1485–1489. https://doi.org/10.1002/eji.1830171015.

Schwaeble, W., Schwaiger, H., Brooimans, R.A., Barbieri, A., Möst, J., Hirsch-Kauffmann, M., Tiefenthaler, M., Lappin, D.F., Daha, M.R., Whaley, K., Dierich, M.P., 1991. Human complement factor H Tissue specificity in the expression of three different mRNA species. Eur. J. Biochem. 198 (2), 399–404. https://doi.org/10.1111/j.1432-1033.1991.tb16028.x.

Sica, A., Mantovani, A., 2012. Macrophage plasticity and polarization: in vivo veritas. J. Clin. Invest. 122 (3), 787–795. https://doi.org/10.1172/jci59643.

Tang, P.M., Nikolic-Paterson, D.J., Lan, H.Y., 2019. Macrophages: versatile players in renal inflammation and fibrosis. Nat. Rev. Nephrol. 15 (3), 144–158. https://doi.org/10.1038/s41581-019-0110-2.

Timár, K.K., Pasch, M.C., van den Bosch, N.H., Jarva, H., Junnikkala, S., Meri, S., Bos, J. D., Asghar, S.S., 2006. Human keratinocytes produce the complement inhibitor factor H: Synthesis is regulated by interferon-γ. Mol. Immunol. 43 (4), 317–325. https://doi.org/10.1016/j.molimm.2005.02.009.

van Essen, M.F., Schlagwein, N., van Gijlswijk-Janssen, D.J., Anholts, J.D.H., Eikmans, M., Ruben, J.M., van Kooten, C., 2020. Culture medium used during small interfering RNA (siRNA) transfection determines the maturation status of dendritic cells. J. Immunol. Methods 479, 112748. https://doi.org/10.1016/j.jim.2020.112748.

van Essen, M.F., Schlagwein, N., van den Hoven, E.M.P., van Gijlswijk-Janssen, D.J., Lubbers, R., van den Bos, R.M., van den Born, J., Ruben, J.M., Trouw, L.A., van Kooten, C., 2022a. Initial properdin binding contributes to alternative pathway activation at the surface of viable and necrotic cells. Eur. J. Immunol. 52 (4), 597–608. https://doi.org/10.1002/eji.202149259.

van Essen, M.F., Schlagwein, N., van Gijlswijk-Janssen, D.J., Ruben, J.M., van Kooten, C., 2022b. Properdin produced by dendritic cells contributes to the activation of T cells. Immunobiology 227 (4), 152246. https://doi.org/10.1016/j.imbio.2022.152246.

Verreck, F.A.W., de Boer, T., Langenberg, D.M.L., Hoeve, M.A., Kramer, M., Vaisberg, E., Kastelein, R., Kolk, A., de Waal-Malefyt, R., Ottenhoff, T.H.M., 2004. Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco)bacteria. PNAS 101 (13), 4560–4565. https://doi.org/10.1073/pnas.0400983101.

Walport, M.J., 2001. Complement. First of two parts. N. Engl. J. Med. 344 (14), 1058–1066. https://doi.org/10.1056/NEJM200104053441406.

Würzner, R., 2000. Modulation of complement membrane attack by local C7 synthesis. Clin. Exp. Immunol. 121 (1), 8–10. https://doi.org/10.1046/j.1365-2249.2000.01263.x.

Wynn, T.A., Chawla, A., Pollard, J.W., 2013. Macrophage biology in development, homeostasis and disease. Nature 496 (7446), 445–455. https://doi.org/10.1038/nature12034.

Wynn, T.A., Vannella, K.M., 2016. Macrophages in tissue repair, regeneration, and fibrosis. Immunity 44 (3), 450–462. https://doi.org/10.1016/j.immuni.2016.02.015.

Xu, W., Roos, A., Schlagwein, N., Woltman, A.M., Daha, M.R., van Kooten, C., 2006. IL-10 producing macrophages preferentially clear early apoptotic cells. Blood 107 (12), 4930–4937. https://doi.org/10.1182/blood-2005-10-4144.

Xu, W., Zhao, X., Daha, M.R., van Kooten, C., 2013. Reversible differentiation of pro- and anti-inflammatory macrophages. Mol. Immunol. 53 (3), 179–186. https://doi.org/10.1016/j.molimm.2012.07.005.