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Multifaceted role of the complement system in health and disease: a focus on properdin

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Properdin produced by dendritic cells contributes to the activation of T cells

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Abstract

The complement system does not only play an important role in the defence against microorganism and pathogens, but also contributes to the regulation of innate and adaptive immunity. Especially activation fragments C3a and C5a and complement activation at the interface of antigen presenting cell (APC) and T cell, were shown to have a role in T cell activation and proliferation. Whereas most complement factors are produced by the liver, properdin, a positive regulator of the C3 convertase, is mainly produced by myeloid cells. Here we show that properdin can be detected in myeloid cell infiltrate during human renal allograft rejection. *In vitro*, properdin is produced and secreted by human immature dendritic cells (iDCs), which is further increased by CD40-L-matured DCs (mDCs). Transfection with a specific properdin siRNA reduced properdin secretion by iDCs and mDCs, without affecting the expression of co-stimulatory markers CD80 and CD86. Co-culture of properdin siRNA-transfected iDCs and mDCs with human allogeneic T cells resulted in reduced T cell proliferation, especially under lower DC-T cell ratio's (1:30 and 1:90 ratio). In addition, T cell cytokines were altered, including a reduced TNF-α and IL-17 secretion by T cells co-cultured with properdin siRNA-transfected iDCs. Taken together, these results indicate a local role for properdin during the interaction of DCs and allogeneic T cells, contributing to the shaping of T cell proliferation and activation.

Introduction

The complement system, as part of the innate immune system, is important in our first line of defense. This system comprises of three pathways, the classical (CP), lectin (LP) and alternative (AP) pathway and activation results in the formation of C3 and C5 convertases. These convertases cleave C3 and C5 which results in the generation of anaphylatoxins C3a and C5a, involved in the recruitment of immune cells. At the end of the cascade, a membrane attack complex is formed, responsible for the lysis of cells (1,2). AP activation can occur spontaneously via “tick-over”, generating fluid-phase C3 convertases, contributing to complement activation. In addition, the AP functions as an amplification pathway, also enhancing complement activity when triggered by CP or LP activation. Negative regulation of the AP is important and is mediated both by fluid phase and membrane bound regulators (3–5).

Properdin, the only known positive regulator, is able to stabilize the AP C3 convertase, thereby increasing its half-life five to ten times, resulting in enhanced C3 cleavage (6). Crystal structures of properdin have contributed to the understanding of its role in C3 convertase stabilization (7,8). More recently, the role of properdin in stabilizing the AP C5 convertases was assessed (9). Properdin is also able to function as a pattern-recognition molecule (PRM) by direct interaction with several surfaces, for example with proximal tubular epithelial cells, early and late apoptotic cells, dead cells, and the surface of bacteria (10–17), thereby directing the location of complement activation. However, this PRM-function of properdin remains a matter of debate, since others showed that properdin binding depends on initial C3b deposition (18,19). Still, properdin binding was observed in the glomeruli of C3 knockout mice with induced anti-glomerular basement membrane disease (20).

Next to these classical roles of properdin, also non-complement related roles have been described. Properdin was able to inhibit the phagocytosis of *Mycobacterium bovis* BCG, thereby affecting the cytokine production by the myeloid cell line THP-1 (21). In addition, properdin was shown to modulate Influenza A infection of lung epithelial cells, where the immunomodulatory effects of properdin depended on the Influenza A subtype (22).

Complement factors are able to direct adaptive immune responses. Immune cells, including antigen presenting cells, produce and secrete complement factors (23). During antigen-presenting cell (APC) and T cell interaction, absence or siRNA knockdown of decay-accelerating factor (DAF), involved in the dissociation of the C3 convertase, resulted in enhanced T cell activation (24,25). APCs and T cells were shown to produce and secrete complement factors which contribute to T cell proliferation and activation,

in which C3a and C5a play an important role (23–31). Also intracellular C3 activation has been described to be important for T cell homeostasis and directing effector cell differentiation (32,33). More recently, it was shown that germinal center B cells were able to lower the expression of DAF, thereby allowing local complement activation. Generation of C3a and C5a was necessary for positive selection. Interestingly, inhibitor CD59 was upregulated, preventing the formation of the membrane attack complex during these processes (34).

Since properdin is mainly produced by monocytes, (tolerogenic) dendritic cells and neutrophils ((35–37) and reviewed by (38)) and local roles for C3a and C5a have been addressed, we questioned whether properdin could play a role locally during APC-T cell interaction. Therefore, we further investigated the regulation of properdin production by dendritic cells and its role in T cell activation.

Materials and methods

Human renal rejection tissue

Human renal rejection tissue was sectioned (3 μm), aceton fixed and frozen until further usage. Kidney sections were defrosted and air dried before fluorescent staining was performed. In brief, slides were blocked for 1 h at room temperature (PBS containing 1% bovine serum albumin (BSA; Sigma-Aldrich, Saint Louis, MO, USA) and 2% normal horse serum (S-2012, Vector Labs)) followed by incubation with mouse anti-human properdin monoclonal antibody (2 μg/mL, A235, Quidel) in combination with either rabbit-anti human CD3 (2 μg/mL, A0452, DAKO) or rabbit anti human CD11b (0.28 μg/mL, AB52478, Abcam, Cambridge, United Kingdom) diluted in PBS-1%BSA, overnight at room temperature. Next, slides were washed in PBS and the staining was visualized by incubation with horse-anti-mouse IgG Dylight 488 (7.5 μg/mL, DI-2488, Vector Labs) or horse-anti-rabbit IgG Dylight 594 (7.5 μg/mL, DI-1094, Vector Labs) for 1 h at room temperature (prior to dilution, antibodies were centrifuged for 10 min at 3000 rpm). Sections were washed with PBS and incubated with hoechst (1 μg/mL, H3569, Invitrogen, Carlsbad, CA, USA) for 2 min at room temperature. Sections were washed with PBS and covered using Dabco glycerol (0.2 M Dabco (D2522, Sigma-Aldrich), 90% glycerol (1.04094, Merck) and 10% PBS) and imaged using the AxioCamMRc5 (Zeiss, Oberkochen, Germany, 40x objective) with the AxioVision software (special release 4.9.1).

Monocyte isolation and monocyte-derived dendritic cells (MoDC) generation

Peripheral blood mononuclear cells (PBMCs) were isolated from healthy blood donors (Sanquin, Amsterdam, The Netherlands) using Ficoll density gradient centrifugation

(Pharmacy, LUMC, Leiden, The Netherlands). Monocytes were isolated, dendritic cells were generated and differentiation was examined as described before (39).

Stimulation of immature MoDCs

Immature MoDCs (iDCs) were harvested and stimulated with irradiated (7500 Rad, Gammacell 1000 Elite, Best Theratronics, Kanata, ON, Canada) mouse fibroblast L-cells stably transfected with CD40L (1:10 ratio) or control cells (L-Orient) (40). After 48 h, supernatants were harvested and kept frozen (-20˚C) until further usage.

Gene transcription levels

mRNA was isolated from ~0.5 x 10^6 iDCs and mDCs using the RNeasy Micro kit (Qiagen) following the manufacturer's instructions. Genomic DNA digestion and cDNA generation was performed as described previously (39). Primers were used to determine human *GAPDH* and *CFP* expression (Table 1, final primer concentration of 1.25 μM, Biolegio, Nijmegen, The Netherlands) (37). Samples were run (CFX384 real-time system, C1000 Thermal Cycler, Bio-Rad) and analysis was performed as described previously (39). Gene expression levels were determined by calculating the mean values of triplicates followed by the ΔCT (gene of interest – *GAPDH*). Copies per *GAPDH* were calculated using $2^{-\Delta CT}$.

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
CFP	**NM_001145252.3**	181	TTGCGGCTTCGTGTCTCC	GTAATCACCCTGCTCCCAAG

siRNA transfection

IDCs were transfected as described previously (39). In brief, transfection complexes were generated containing 100 nM control siRNA (siGENOME Non-Targeting siRNA Pool #2, D-001206-14-20, Dharmacon) or properdin siRNA (siGENOME CFP siRNA M-012648-02-0010, Dharmacon). MoDCs were harvested and plated in IMDM medium containing 10% heat-inactivated fetal calf serum (FCS, Bodinco, Alkmaar, The Netherlands) in the presence of 20 ng/mL IL-4 (PHC0041, Invitrogen) and 10 ng/mL GM-CSF (Premium grade, 130-093-868, Miltenyi Biotec, Leiden, The Netherlands; 250.000 cells/well, 500μL, 12 wells plate, and two-five wells of each condition were plated per donor). To immature cell (iDCs) cultures, control cells (L-Orient, 1:10 ratio) were added. For the generation of mature MoDCs (mDCs), L-CD40L expressing cells (1:10 ratio) were added. Supernatants

were harvested and pooled (two-five wells of each condition) after 48 h and kept frozen until further usage (-20˚C). In addition, cells from the corresponding wells were pooled and used for subsequent experiments.

Properdin secretion measured by ELISA

Properdin production was analysed by ELISA. In brief, mouse monoclonal anti-human properdin (0.3 μg/mL, A233, Quidel, San Diego, CA, USA) was coated overnight at room temperature. Plates were blocked (PBS-1%BSA) and samples (pooled supernatant was measured in duplicate) were diluted in dilution buffer (PBS-0.05%Tween20 (Sigma-Aldrich)-1%BSA) and incubated for 1 h at 37°C. Next, plates were washed with wash buffer (PBS-0.05%Tween-20) and incubated with rabbit anti human-properdin-Digoxigenin (DIG; in-house, 1:2500) for 1 h at 37°C. After washing, plates were incubated with anti-Digoxigenin-POD Fab fragments (1:2500, 16620900, Roche Diagnostics, Indianapolis, IN, USA) for 1 h at 37°C and developed using 3,3',5,5'-tetramethylbenzidine (TMB, Sigma-Aldrich). The reaction was stopped using 1 M H_2SO_4. Absorbance was measured at 450nm (iMark Microplate Reader, Bio-Rad, Hercules, CA, USA).

Analysis of maturation

Effect of properdin siRNA on iDCs and mDCs maturation was determined using flow cytometry (LSR-II, BD Biosciences). In brief, pooled cells were stained for 30 min with mouse anti-human HLA-DR (APC, 1:75, Clone L243, cat no. 347403, BD), mouse anti-human CD80 (FITC, 1:20, Clone L307(4), cat no. 557226, BD Pharmingen), mouse anti human-CD83 (PeCy7, 1:40, Clone HB15e (RUO), cat no. 561132, BD Pharmingen) and mouse anti-human CD86 (V450, 1:40, Clone 2331 (FUN-1; RUO), cat no. 560357 BD Pharmingen). Data was analysed using FlowJo Software version 10.6.1 (Tree Star, Ashland, OR, USA).

Mixed leukocyte reactions: co-culture of dendritic cells and T cells

Total T cells were obtained from peripheral blood lymphocytes (PBL, remaining fraction after monocyte-isolation) by negative selection using a Pan T cell isolation kit (130-096-535, Miltenyi Biotec) and kept frozen (-80˚C, IMDM containing 10% heat-inactivated FCS, 10% DMSO, 90 U/mL penicillin and 90 μg/mL streptomycin) until further usage. T cells were thawed on the day L-Orient and L-CD40L stimulated and/or siRNA-transfected MoDCs were harvested for co-culture experiments. T cells were labelled with 1 μM carboxyfluorescein succinimidyl ester (CFSE; cat no. C34554, Thermo Fisher Scientific) diluted in PBS. MoDCs were co-cultured with allogeneic T cells (10^5 T cells vs 10^4, $3.3x10^3$, $1.1x10^3$ MoDCs, for each condition a technical triplicate was plated) in IMDM containing 10% heat-inactivated FCS and 90 U/mL penicillin and 90 μg/mL streptomycin. After six days, supernatants were harvested and kept frozen (-20˚C). Cells were stained

using mouse anti-human CD3 (PE; 1:50, cat no. 555333, BD Biosciences), mouse anti-human CD4 (V450; 1:50, Clone RPA-T4 (RUO), cat no. 560345, BD Biosciences) and mouse anti-human CD8 (APC; 1:50, SK1 clone, cat no. 345775 BD Biosciences) and analysed using flow cytometry (LSR-II; BD Biosciences). Gating strategy and analysis of T cell proliferation was similar as described before (39). Acquired flow cytometric data was analysed using FlowJo Software. Furthermore, T cell cytokines were determined using the Bio-Plex human Cytokine Th1/Th2 assay according to the manufacturer's instructions (Bio-Rad, Hercules, CA, USA). The following cytokines were measured: GM-CSF, IFN-γ, TNF-α, IL-2, IL-4, IL-5, IL-10, IL-12(P70) and IL-13. Magnetic beads for IL-6 and IL-17 cytokines were added to the Th1/Th2 Bio-Plex assay.

Statistical analysis

Statistical analysis was performed using a two-tailed Wilcoxon matched-pairs signed rank test. Significance was defined as $P \leq .05$. For statistical analysis and graphical representations GraphPad Prism v.9.0.1 was used (San Diego, CA, USA).

Results

Presence of properdin in renal biopsies with allograft rejection

To link properdin production to the activation of allogeneic T cells, we performed immunofluorescent staining on biopsies from rejected renal transplants. Properdin staining was observed at the tubular brush borders and in cellular infiltrates (Figure 1). Properdin was detected in close proximity to $CD3^+$ T cells (Figure 1A), and colocalized with $CD11b^+$ myeloid cells (Figure 1B), which are the main producers of properdin. To confirm properdin expression by myeloid APC, monocytes were isolated and differentiated into immature DCs (iDCs) and differentiation was examined using flow cytometry. As expected, iDCs were positive for DC-SIGN (Figure 1C, upper panel) and CD1a (Figure 1C, lower panel), while lacking CD14 (Figure 1C) (41,42). iDCs expressed properdin mRNA (*CFP*), and *CFP* levels were upregulated by L-CD40L stimulation (Figure 1D). In addition, iDCs were able to secrete properdin spontaneously (white bars, average production 2.5±1.5 ng/mL) and levels were significantly upregulated after stimulation with L-CD40L (Figure 1E, average production 16.8±7.7 ng/mL). These data indicate that properdin is produced by $CD11b^+$ cells like DCs and levels are upregulated by CD40-receptor engagement.

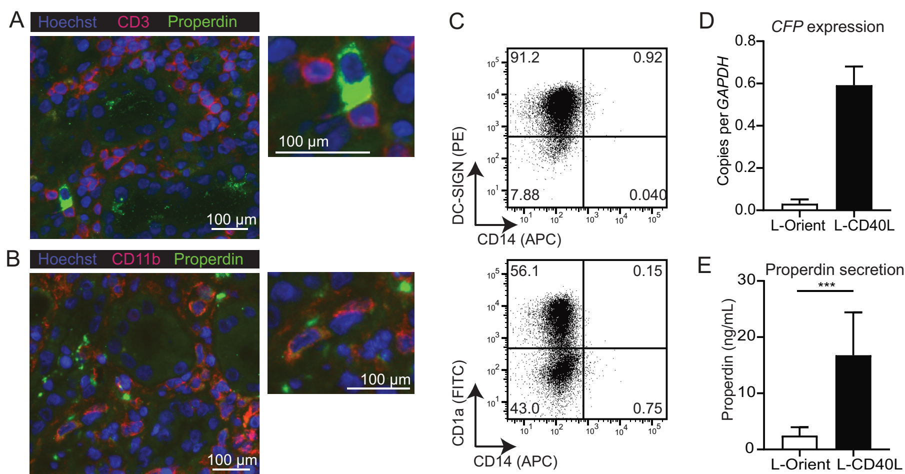

Figure 1: Properdin is produced and secreted by dendritic cells. (A) Renal biopsies were sectioned and stained for the presence of CD3⁺ cells (red) and properdin (green) or **(B)** CD11b⁺ cells (red) and properdin (green). Representative of n=2. Scale bar: 100 µm. **(C)** Monocytes were isolated from PBMCs and iDCs were generated by the addition of IL-4 and GM-CSF to the cultures. Differentiation was assessed by flow cytometry. Numbers represent the percentage of cells which express the indicated markers. **(D)** Expression of properdin mRNA (*CFP*) in DCs stimulated with L-Orient and L-CD40L. Data are presented as mean ± SD of independent experiments performed on DCs generated from three different monocyte-donors. **(E)** Supernatants were harvested 48 h after L-Orient and L-CD40L stimulation and properdin production was determined by ELISA. Data are presented as mean ± SD of independent experiments performed on DCs generated from 20 different monocyte-donors. Wilcoxon matched-pairs signed rank test, *** $P < .001$.

Reduction of properdin levels using a specific siRNA

To investigate the role of properdin during APC-T cell interaction, we followed our optimized protocol for transfection of DCs (39). MoDC were transfected with a control siRNA or a properdin siRNA in the presence of either L-Orient (iDCs) or L-CD40L (generation of mDCs). After 48h, supernatant was harvested and properdin levels were determined by ELISA. Secreted properdin levels were reduced upon transfection of iDCs with properdin siRNA (Figure 2A, mean fold change 0.51, varying from 0.11 to 1.51). Also, in mDCs, despite the significantly higher levels of properdin production, a similar reduction in the level of properdin secretion was obtained (Figure 2B, mean fold change 0.39, varying from 0.30 to 0.48). Transfection of iDCs and mDCs with properdin siRNA did not affect CD80 (Figure 2C) and CD86 (Figure 2D) surface expression when compared to untransfected cells.

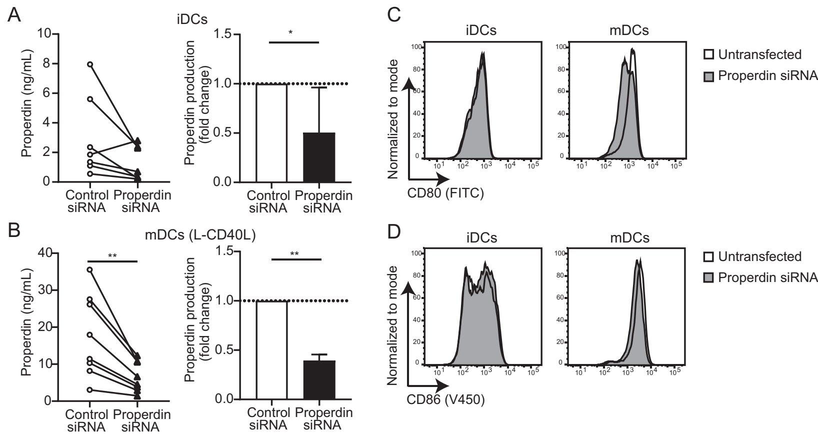

Figure 2: Reduction of properdin secretion in both iDCs and mDCs upon transfection with properdin siRNA. MoDCs were transfected with either 100 nM control or properdin siRNA in the presence of L-Orient or L-CD40L cells ($2.5x10^5$ MoDCs vs 2.5×10^4 L-Orient or L-CD40L). After 48 h, supernatants were harvested and pooled and properdin levels were analyzed (in duplicate) by ELISA. **(A)** Measurement of properdin levels in iDCs transfected with control or properdin siRNA. Data are obtained from independent experiments performed on DCs generated from seven different monocyte-donors. **(B)** Measurement of properdin levels in mDCs transfected with either control or properdin siRNA. Data are obtained from independent experiments performed on DCs generated from eight different monocyte-donors. **(C-D)** Representative histograms of the effects of transfection iDCs and mDCs with properdin siRNA on **(C)** CD80 and **(D)** CD86 expression (grey histogram) compared to untransfected cells (white histograms). Data are presented as the mean ± SD. Wilcoxon matched-pairs signed rank test, * $P \le .05$, ** $P < .01$.

Reduced allostimulatory capacity of iDCs upon reduced properdin secretion

T cell proliferation upon allogeneic stimulation was examined by flow cytometry. Viable, single cells were gated and $CD3^+$ cells were selected. Next, $CD4^+$ or $CD8^+$ cells were selected and their proliferation was examined (Figure 3A). Co-culture of T cells with iDCs resulted in $CD4^+$ and $CD8^+$ T cell proliferation, which further increased upon co-cultured with mDCs. No T cell proliferation was observed when T cells were cultured in the absence of DCs (Figure 3A). In both cases, proliferation of $CD4^+$ and $CD8^+$ T cells dose-dependently increased with increasing numbers of iDCs or mDCs (Figure 3B).

To assess the role of properdin during DC-T cell interaction, control siRNA- or properdin siRNA-transfected iDCs and mDCs were co-cultured with allogeneic T cells. A reduction in $CD4^+$ T cell proliferation was observed when co-cultured with properdin siRNA-transfected iDCs (Figure 3C), which was also the case when $CD4^+$ T cells were co-cultured with properdin siRNA-transfected mDCs, however, only in suboptimal conditions (1:90 and 1:30 DC-T cell ratio's). Similar effects were observed for $CD8^+$ T cell proliferation, when co-cultured with properdin siRNA-transfected iDCs (Figure 3E) or mDCs (Figure 3F). These results show that lowering the properdin production in DC reduces the capacity to stimulate $CD4^+$ and $CD8^+$ T cell proliferation, especially under suboptimal stimulatory conditions (lower DC-T cell ratio's).

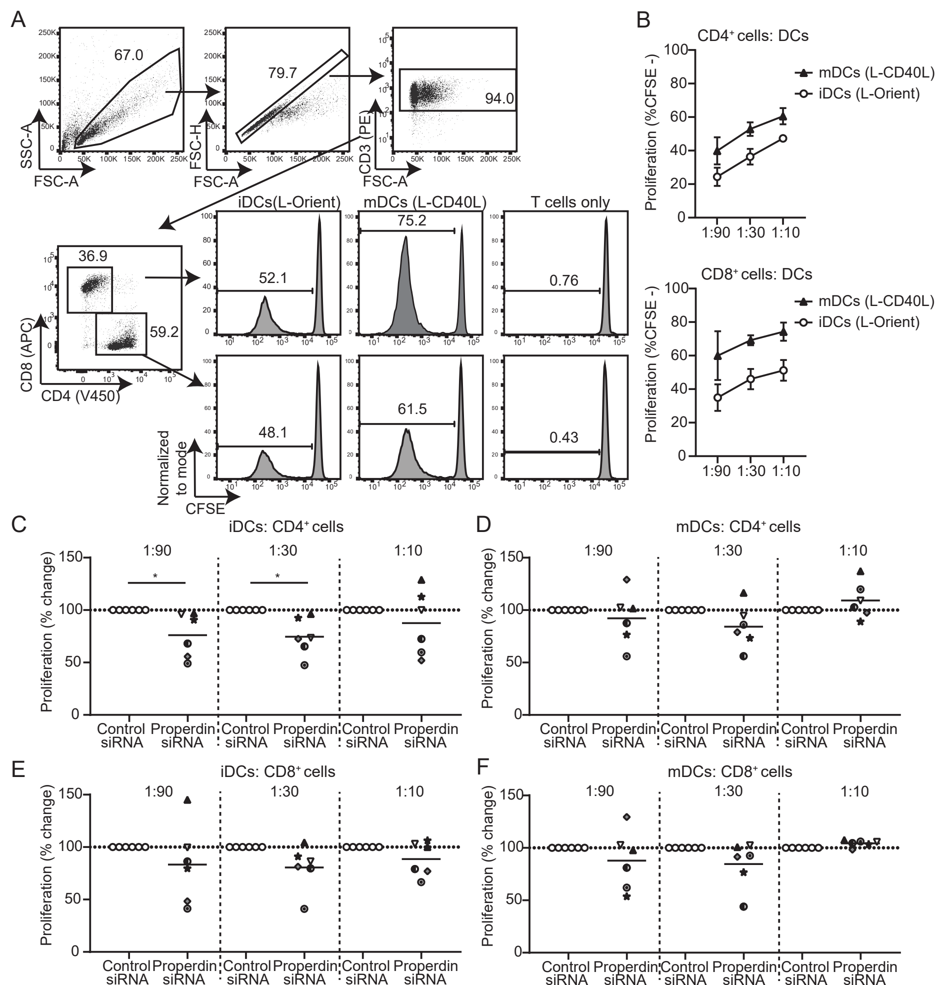

Figure 3: Reduced allostimulatory capacity of iDCs and mDCs with reduced properdin levels. (A) MoDCs were stimulated with either L-Orient or L-CD40L expressing cells (1:10 ratio) for 48 h before being co-cultured with carboxyfluorescein diacetate succinimidyl ester (CFSE)-labelled allogeneic T cells in different ratio's (10^5 Pan T cells vs 10^4, $3.3x10^3$, $1.1x10^3$ MoDCs). After six days, proliferation was examined using flow cytometry. CFSE dilution of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells was used to determine T cell proliferation. **(B)** $CD4^+$ and $CD8^+$ T cell proliferation when co-cultured with iDCs (L-Orient) and mDCs (L-CD40L). Data points are a triplicate of one out of six representative donors. **(C)** Effect of properdin reduction in iDCs on the allogeneic $CD4^+$ T cell proliferation compared to control siRNA-transfected iDCs. **(D)** Effect of properdin reduction in mDCs on the allogeneic $CD4^+$ T cell proliferation compared to control siRNA-transfected mDCs. **(E)** Effect of properdin reduction in iDCs on the allogeneic $CD8^+$ T cell proliferation compared to control siRNA-transfected iDCs. **(F)** Effect of properdin reduction in mDCs on the allogeneic $CD8^+$ T cell proliferation compared to control siRNA-transfected mDCs. DCs were generated from four different monocyte-donors and co-cultured with allogeneic T cells (six independent experiments). Each data point represents the average T cell proliferation (which was measured in triplicate) and similar donors are indicated by the same symbols. 1:90 ratio, 10^5 T cells vs $1.1x10^3$ DCs; 1:30 ratio: 10^5 T cells vs $3.3x10^3$ DCs; 1:10 ratio: 10^5 T cells vs 10^4 DCs. Wilcoxon matched-pairs signed rank test, * $P < .05$.

As an additional read-out for the T cell activation, cytokine secretion was analyzed by screening the supernatant from the co-culture experiments by Luminex. Co-culture of T cells with iDCs transfected with properdin siRNA showed a tendency that most cytokines were produced at lower levels (Figure 4A). Although not statistically significant, this effect appeared strongest for the production of TNF-α and IL-17. In contrast, co-culture of T cells with mDCs transfected with properdin siRNA did not result in such inhibitory effects, and even showed an increased IL-17 secretion (Figure 4B).

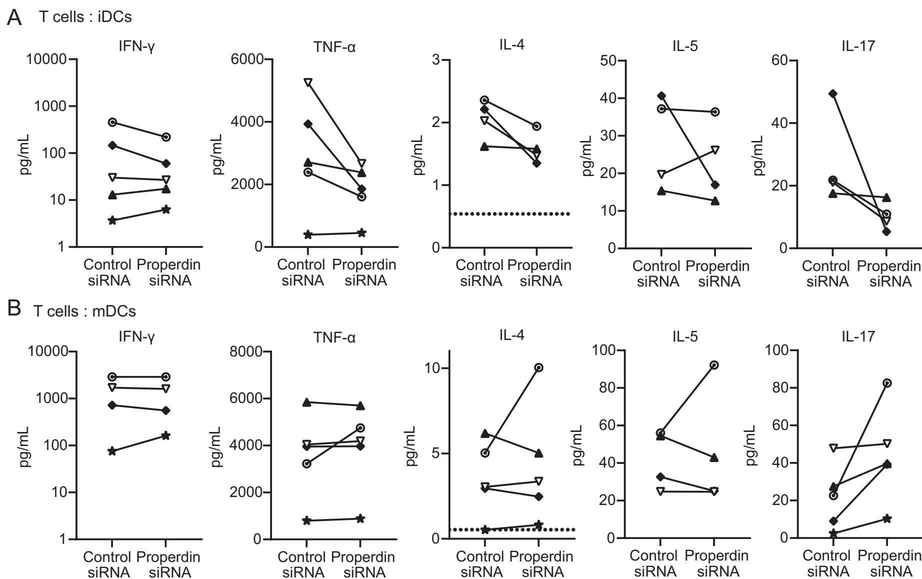

Figure 4: Cytokine secretion by T cells co-cultured with iDCs and mDCs having reduced properdin levels. Supernatants were harvested at day six of the mixed leukocyte reaction and T cell cytokines were measured by Luminex. **(A)** Cytokine levels for T cells co-cultured with properdin siRNA-transfected iDCs compared to control siRNA-transfected iDCs. IFN- γ (n=5), TNF- α (n=5), IL-4 (n=4), IL-5 (n=4), IL-17 (n=4). **(B)** Cytokine levels for T cells co-cultured with properdin siRNA-transfected mDCs compared to control siRNA-transfected mDCs. IFN- γ (n=4), TNF- α (n=5), IL-4 (n=5), IL-5 (n=4), IL-17 (n=5). Dotted lines indicate the detection limit of the assay. Each data point represents the average cytokine level (which was measured in triplicate) for 5 independent experiments.

Discussion

Properdin is the only known positive regulator of the complement system, stabilizing the half-life of the C3 convertase 5-10 times (6). Since local roles for C3a and C5a have been addressed, we questioned whether properdin, as stabilizer of the convertases, thereby facilitating the generation of C3a and C5a, could play a role locally during APC-T cell interaction. Here we showed that properdin was produced and secreted by

immature DCs. Stimulation with CD40L, a molecule specifically expressed on activated T cells, which was used to mimicking cognate DC-T cell interaction, resulted in increased properdin secretion. Secretion of properdin by both iDCs and mDCs was reduced for ~50% using a properdin siRNA. This properdin-specific siRNA did not affect the CD80 and CD86 expression on iDCs and mDCs. Co-culture of allogeneic $CD4^+$ and $CD8^+$ T cells with properdin siRNA treated iDCs and mDCs, thus the APCs produce less properdin, resulted in reduced T cell proliferation under suboptimal culture conditions. When examining cytokines, it was observed that IL-17 levels were decreased in properdin siRNA-transfected iDCs when co-cultured with T cells. However, IL-17 levels were increased in co-cultures of properdin siRNA-transfected mDCs with T cells, affecting T cell skewing.

Our findings show that there is heterogeneity in the responses and effects. First, a difference was observed in the level of properdin secretion by dendritic cells. This is probably due to donor variability, since these DCs are generated from different monocyte donors. Second, differences in the efficiency of properdin suppression were observed. Third, there were clear differences in the outcome of T cell proliferation, both the strength of the allogeneic response as well as the inhibitory effect of properdin siRNA. Finally, large differences were observed in the basal cytokine production between the different donors, for example shown by the IFN-γ produced in T cells co-cultured with iDCs, in which there is a ~100-fold difference between the lowest and highest levels produced. We have tried to correlate the different results of proliferation and cytokine production with the level of properdin suppression, but did not observe this (data not shown). It is most likely that in these co-culture conditions, also other signals like co-stimulatory molecules and immunogenic cytokines will be present, which could potentially overrule effects induced by the reduction of properdin levels. Therefore, it might be possible that the effects of the contribution of the complement system will be only visible under specific circumstances, e.g. under suboptimal stimulatory conditions, like observed with lower DC-T cell ratio's.

Our results confirm the findings described previously on the production of properdin by DC and the functional contribution to T cell activation (37). We extend on these data by demonstrating an increased properdin secretion upon stimulation with CD40L, mimicking APC-T cell interaction. In our current study, secreted properdin levels were reduced using siRNA using an optimized transfection method (39) and effects on T cell proliferation were examined using CFSE dilution instead of 3H-thymidine incorporation, allowing the analysis of both $CD4^+$ and $CD8^+$ T cell proliferation. Furthermore, effects on cytokine secretion were examined more extensively.

Activation of the complement system has previously been shown to be important during APC-T cell interaction. It was observed that C3 synthesis by DCs was needed for full T cell activation (43). In addition, downregulation of complement regulator DAF resulted in an enhanced T cell proliferation (24,25). C3a and C5a signaling induce phenotypic changes of DCs, and blockage of C3aR and/or C5aR altered T cell alloreactivity, as was observed by less IFN-γ production and less proliferation. This indicates that the T cell proliferation partially depends on activation of the complement system, suggesting local roles for C3a and C5a (25,27,28). Additional investigations are necessary to pinpoint the exact mechanism on how properdin affects APC-T cell interaction, and to determine whether this could be a therapeutic target of interest.

Properdin staining in the renal tissue has been described before, in various locations and in various (renal) diseases (reviewed by (44)). Using immunofluorescence, we showed that properdin was mainly present at sites of infiltrating $CD11b^+$ immune cells, and in close proximity of $CD3^+$ T cells. This, together with the *in vitro* experiments, indicates local production of properdin in the kidney might contribute to the local immune activation. In addition, various ligands for properdin binding have been described, including glycosaminoglycan structures which are present on the cell surface (10–12), the surface of early apoptotic, late apoptotic and necrotic cells (13,14,16,17), and CHFR5 (45), potentially also contributing to the local AP activation. Apart from this, properdin was also shown to have complement-independent roles, being able to inhibit the phagocytosis of *Mycobacterium bovis* BCG, which affected the cytokine production by THP-1 cells (21), and modulating Influenza A infection of lung epithelial cells (22). Whether such activities play a role in APC-T cell interaction and renal inflammation remains to be established. Various experimental models using properdin deficient mice have resulted in seemingly opposing effects (46–49). In a mouse model for renal ischemia and reperfusion injury (IRI), mice deficient for complement regulators DAF and CD59 were protected when deficient for properdin or other components of the alternative pathway. Blocking properdin with an anti-properdin antibody 24h before induction of ischemia and reperfusion resulted in ameliorated renal injury (50).

Altogether, from these models, the precise mechanism of action of properdin remains not fully uncovered. An important distinction will be the different role that complement factors play in the systemic compartment versus a local contribution of production, activation and regulation. Further investigations are needed to determine the systemic or solid-phase regulatory process of properdin. Our findings add to the knowledge on the production of properdin by dendritic cells and confirm a local role for properdin and complement in adaptive immunity, contributing to the shaping of T cell proliferation and activation.

Acknowledgments
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