Focus on Complement

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Dear Readers,

Welcome to the December 2017 issue of '*Focus on Complement*'. This 48th issue of FoC contains the following:

- Viviana Ferreira reviews publications for the **News Flash**, which provide evidence for the identification of novel receptors for C4a and Properdin.
- **The Complement research teams around the world** series featuring Dr. Deborah Fraser, in California, USA, as well as Dr. Lourdes Isaac and Dr. Angela Barbosa**,** in São Paulo, Brazil.
- A meeting report from the **16th European Meeting on Complement in Human Disease,** which took place in Copenhagen, Denmark, in September 2017.
- Three upcoming **complement meeting announcements**.

If you would like to contribute with an article to a future issue or have suggestions for a subject theme, please contact Trent Woodruff (t.woodruff@uq.edu.au) or Michael Holers (Michael.Holers@ucdenver.edu).

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NEWS FLASH (reported by Dr. Viviana Ferreira, USA)

News Flash 1:

Complement-activation fragment C4a mediates effector functions by binding as untethered agonist to protease-activated receptors 1 and 4. Wang HB, Ricklin D, Lambris JD. *PNAS*. 2017. 114(41):10948

C4a, an ~9,000 Da protein that shares high homology with C3a and C5a, is produced by the activation of the classical and lectin pathways of complement. The authors of this study set out to define a previously unidentified receptor for C4a, and its biological functions and signal transduction mechanisms. Given the previous finding that C4a can decrease intracellular cAMP levels by acting on Gi protein-coupled pathways in mast cells, the authors hypothesized that the biological function of C4a is mediated through a yet to be defined G protein-coupled receptor (GPCR). Using a GPCR cell-based reporter assay, they identified that C4a is a specific ligand for protease-activated receptors (PAR) 1 and 4. The family of PARs are GPCRs that are expressed predominantly in vascular, immune and epithelial cells, astrocytes and neurons and transmit cellular responses to coagulant proteases as well as other proteases expressed in distinct tissues. PARs are critical mediators of hemostasis, thrombosis, and inflammation. PARs are traditionally activated by proteases such as thrombin, that will cleave an N-terminal fragment of the PAR receptors, allowing the shortened terminus to bind the active site as a tethered ligand. In the case of C4a, however, the authors showed that PAR1/PAR4 activation appeared to be mediated by direct binding to the receptor. Their results also demonstrate that C4a induces distinct signaling patterns for PAR1 and PAR4, induces calcium mobilization through the PAR1/Gαq/PLCβ signaling axis, and induces enhanced endothelial permeability. In addition, unlike previous studies, C4a did not activate anaphylatoxin receptors (C3aR, C5aR1, or C5aR2). These findings contribute to the relevant growing knowledge of complement-derived effector molecules that serve as a link between complement, coagulation and endothelial barrier systems. It remains to be determined whether MASP-1, which has been shown to proteolytically activate PAR4 and modulate endothelial cell functions, works in a coordinated or in an independent manner with C4a-related mechanisms. In addition, studies aimed at defining the consequences of the PAR1,4/C4a interactions in pathophysiological conditions, including pro-thrombotic diseases such as atypical hemolytic uremic syndrome, among others, are warranted.

News Flash 2:

Complement factor P is a ligand for the natural killer cell-activating receptor NKp46. Narni-Mancinelli E, Gauthier L, Baratin M, Guia S, Fenis A, Deghmane A, Rossi B, Fourquet P, Escalière B, Kerdiles YM, Ugolini S, Taha M, Vivier E. *Sci. Immunol.* 2017. 2(10): doi: 10.1126/sciimmunol.aam9628

It is well known that individuals with properdin deficiency (as well as terminal complement component deficiencies) are highly susceptible to neisserial infections. In addition, it has been shown previously by others that the convertase-stabilizing function of properdin is required for effective complement-mediated killing of *Neisseria* and that treatment of mice with exogenous properdin have significantly reduced bacteremia and increased survival rates. The authors of this manuscript have discovered a novel function for properdin as well as for innate lymphoid cells (ILC) that are NKp46^{+} (which include NK cells, subsets of ILC1, and natural cytotoxicity receptor^{+} ILC3). In their quest to identify cellular ligands of NKp46, they discovered that properdin: (i) binds to isolated NKp46 as well as to NKp46 on cell surfaces. (ii) The interaction initiates a transduction pathway on NK cells, which is distinct from the canonical pathway induced by anti-NKp46 mAbs (i.e. does not induce classical NK cell activation). (iii) Recognition of *Neisseria meningitidis* by NKp46 required serum opsonization, presumably mediated by the presence of properdin on the opsonized cells. Bacteria in the presence of properdin depleted serum, which would *not* effectively opsonize the bacteria and thus not bind properdin, did not bind NKp46. (iv) Group 1 innate lymphoid cells (i.e. NK cells and/or NKp46^{+} ILC1) are involved in controlling *N. meningitidis* infection in mice. (v) Finally, they determined that primary NKp46+ innate lymphoid cells contribute, at least in part, to the previously reported therapeutic benefit of exogenous properdin treatment for control of *Neisseria mengitidis in vivo*. The molecular mechanisms involved in properdin-mediated signal transduction via NKp46 remain to be determined as well as whether the reported binding of NKp46 to multiple ligands, including several microbial components, involves properdin.

COMPLEMENT TEAMS AROUND THE WORLD

Complement Research in Long Beach, California, USA: Dr. Deborah Fraser's Research Group

The Fraser lab is based at California State University in the city of Long Beach, California. The overall research interest of the group is to investigate the role of complement in the recognition and removal of damaged or altered-self such as "bad" (oxidized) cholesterol, apoptotic cells, amyloid plaques and cancer cells. The group is currently funded by NIH (SCORE) to investigate the role of complement protein C1q in programming macrophage responses in atherosclerosis. The central hypothesis is that complement-independent actions of C1q program protective, anti-atherosclerotic cellular responses in atherosclerosis. Students in the lab have identified a number of potentially beneficial outcomes when phagocytes encounter targets opsonized directly with C1q. For example, it was demonstrated that C1q modulates phagocyte cytokine responses toward a more anti-inflammatory, M2-biased state and dampened pro-inflammatory M1 responses during the clearance of oxidized lipoproteins and apoptotic cells, which may limit inflammation early in disease. Several signaling pathways were shown to be involved. This includes C1q modulation of inflammasome, NFκB, JAK-STAT, PPARγ and LXR signaling. A novel role was also identified for C1q in modulation of macrophage lipid metabolism. These studies showed that C1q binds and enhances clearance of modified, atherogenic, lipoproteins, such as oxLDL, and increases cholesterol efflux in cholesterol-loaded primary human monocytes and macrophages. This leads to increased survival of macrophage foam cells through dampening activation of apoptosis and enhancing autophagy. Investigations of the effect of C1q on the macrophage lipidome are currently underway. The overall goal of the group is to identify C1q molecular mechanisms of action that may elucidate opportunities for therapeutic intervention. Dr. Fraser's group have ongoing collaborations related to their interest in investigating non-complement associated roles of C1q with Dr. Andrea Tenner (UC-Irvine, California), Dr. Suzanne Bohlson (Des Moines University, Iowa), and Dr. Nicole Thielens (University Joseph Fourier, Grenoble). The Fraser lab also collaborates with marine biology colleagues at CSULB to investigate innate immune activation in bat sea stars (*Patiria miniata)* and the California round stingray (*Urobatis halleri*).

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The Fraser lab currently comprises 8 undergraduate and 3 MSc graduate students. (Top row) Marc Pulanco, John Guerrero, Viky Espericueta, Paulina Pardo, Lizette Curiel. (Bottom row) Christian Masia, Ashley Wong, Zach Wagoner, Deborah Fraser, Jessica Huynh, Emmeline Cosman, Laurel Lam.

Complement Research in São Paulo, Brazil: The Team of Dr. Lourdes Isaac and Dr. Angela Barbosa

Lourdes Isaac and Angela Barbosa's team is dedicated to study the role of the complement system in controlling leptospirosis and the immune evasion strategies used by pathogenic *Leptospira* to evade complement activation. Leptospirosis is an important human and veterinary health problem and one of the most widespread zoonosis in the world. The Caribbean, Central and South America, as well as Southeast Asia and Oceania, are highly endemic for the disease. Flood-prone regions lacking proper sanitation facilities deeply contribute to epidemics of leptospirosis in tropical areas. Approximately, one million cases and 60,000 deaths are reported each year.

While saprophytic *Leptospira* are rapidly eliminated in the serum, pathogenic *Leptospira* have evolved multiple strategies to escape the host complement system, which is important for innate and acquired immunity. In the past ten years, our main interests have been focused in understanding how *Leptospira* avoid complement-mediated killing. Leptospiral strategies include recruitment of host complement regulators (Factor H; FH-like-1, C4BP and Vitronectin); acquisition of host proteases (plasminogen/plasmin) that degrade C3b, C4b and C5 on the bacterial surface; and, secretion of leptospiral proteases that inactivate C3, Factor B, C4 and C2 in the *Leptospira* surroundings.

The combination of host-derived and endogenous factors enables these spirochetes to successfully establish the infection and colonize target organs of the host. Therefore, *Leptospira* ligands of host regulators and secreted proteases constitute potential sites for immune interference, either as vaccine candidates or as targets for therapeutic agents in the development of new treatments and prophylactic approaches in leptospirosis.

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Angela Barbosa's lab (from left to right): Danielle Courrol, Matilde Costa Lima de Souza, Angela Silva Barbosa, Thais Akemi Amamura, Ludmila da Silva

EMCHD 2017 Meeting Report

Concluding Remarks from the 16th European Meeting on Complement in Human Disease (EMCHD), Copenhagen, 2017

Dear fellow complementologists,

It was a great pleasure and honor to host the 16th European Meeting on Complement in Human Disease (EMCHD2017), from September 8th - 12th, 2017 in Copenhagen. Around 200 attended the C3G satellite symposium on Friday 8th. The regular EMCHD2017 meeting opened at the Copenhagen City Hall the same evening with speeches, the famous Rådhus pancakes and a Tivoli tour. The next day we had a very well attended teaching day about complement to introduce newcomers and those who needed a brush up about the field. At Sunday morning the 10th, we learned about the latest development in microbiome research from the keynote speaker professor Oluf Borbye Pedersen, before the scientific sessions started. During lunch we were updated about use of complement inhibitors in refractory Myasthenia Gravis by dr. Camille Bedrosian from Alexion. Next day in a keynote speech professor Piet Gros showed us exciting new possibilities in structural biology using cryo-electron microscopy. On Tuesday 12th, the last keynote speech was given by professor Dror Mevorach who reported the exciting discovery that loss of CD55 is the molecular origin behind inherited protein-losing enteropathy. Apart from the lively discussions after each oral presentation it was really rewarding to see the interest and discussions at the poster sessions. EMCHD2017 was combined with different social gatherings, industry meetings, prize ceremonies, Danish food, parties and a mentalist that I think all of us could not figure out how he could trick us. An interesting observation was the interest in EMCHD2017 from the pharmaceutical and diagnostic industry underscoring that the complement field is approaching the clinic. 440 participated at EMCHD2017 and we had attendees from 28 nations showing the true international interest for the complement field.

The local organizing committee would like to thank all teachers, chairmen, speakers and poster presenters for their great work. A special thank shall go to those who wrote very nice review papers for the special EMCHD2017 issue of Molecular Immunology. They have been really fun to read. We also like to thank the board of the European Complement Network for scoring the abstracts and posters, our sponsors, The University of Copenhagen and not least the Meeting Planners and Hotel Scandic Copenhagen for their great job putting the pieces together. I am looking forward to the next ICS meeting in Santa Fe in 2018 and the EMCHD2019 to be arranged in Madrid. On the next pages you will find a condensed summary of the EMCHD2017 oral presentations from the chairmen of the scientific sessions and pictures of the winners of this year ECN medal, the outstanding abstract achievement awards and poster prizes.

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COPENHAGEN 2017
16th European Meeting on
COMPLEMENT
IN HUMAN DISEASE

Session: *Function* **Chairs:** Anne Rosbjerg, Seppo Meri

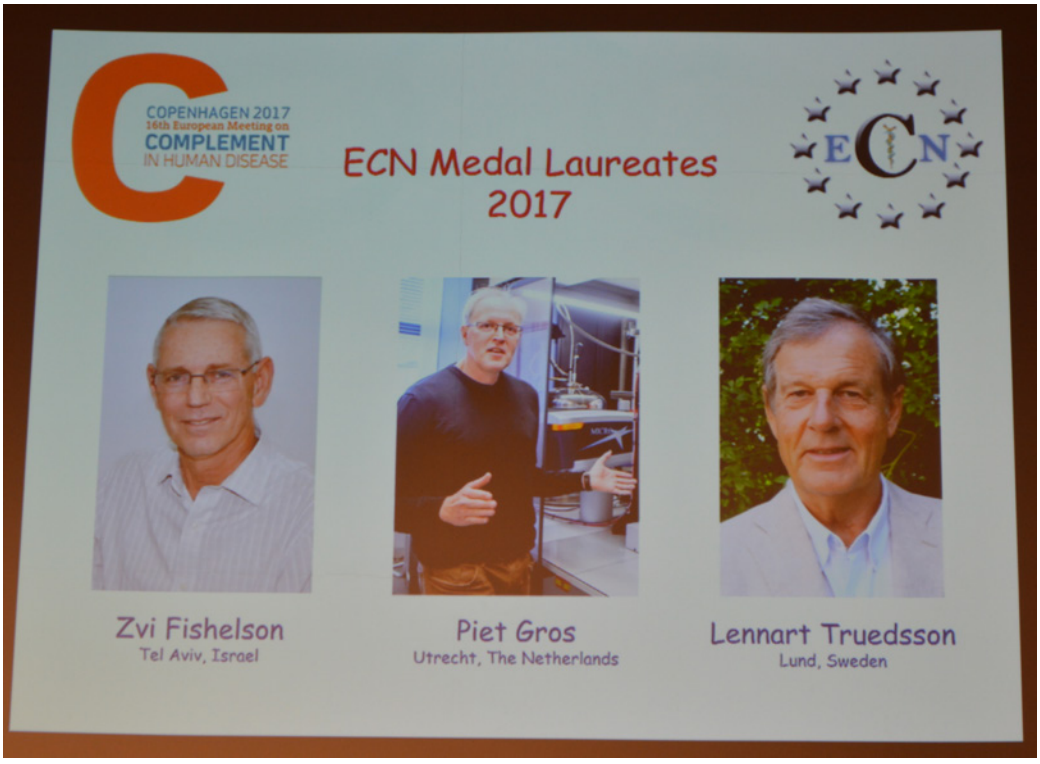
This session highlighted the complement activating function of various complement proteins. **Rasmus Pihl** (Copenhagen) showed that MASP-3 is an important activator of circulating pro-factor D, however not the exclusive activator. Active versus pro factor D was differentiated by isoelectric focusing enabling separation of the proteins despite the small difference in molecular size. The source of factor D was also discussed in this session by **Xiabo Wu** (St. Louis). A lipodystrophy mouse model was used to show that the main part of circulating factor D derives from adipose tissue. The experiments also showed that only a small fraction of factor D was necessary to mediate complement activation, which could be a concern regarding factor D targeted therapeutics. Next, a study by **Sarah Irmscher** (Jena) showed that kallikrein is a player in the alternative pathway amplification loop. Through direct cleavage of C3, kallikrein was shown to produce C3b that could form new C3 convertases. Since kallikrein can cleave factor B, it was suggested that the contact system can activate complement solely dependent on kallikrein. Factor H-related proteins also have a positive effect on alternative pathway. **Eva Karpati** (Budapest) showed that FHR-1 and -5 inhibit factor H regulation of complement via competition for the shared ligands. This enhanced the complement activation on late apoptotic/necrotic cells. Interaction of FHRs and CRP/PTX3 on the dead cells augmented complement activity. Another member of the factor H related proteins, FHR-3, was explored in another study by **Nicole Schäfer** (Regensburg) with regard to age-related macular degeneration. The effect of locally produced FHR-3 in the retina was investigated *in vitro*. Human retinal pigment epithelial cells were found to respond to FHR-3 by upregulating *C3, CFB, CR3* and *NLRP3* and pro-inflammatory cytokines. Furthermore, the study indicated that FHR-3 is transported in the RPE cells from the apical to the basal site.

Session: *Complement Cross-Talk* **Chairs:** Robert Rieben and Ying Jie Ma

The first talk in this session was by **Marina Noris** from Ranica, Italy. She talked about interaction between multimeric VWF and complement, and presented data showing that the activation of the complement system is affecting blood coagulation. Her results suggest in particular that MASP-1 is able to enhance clot formation in whole blood. **Daniel Ricklin** from Basel, Switzerland, then presented a role for C4a – finally! He showed that C4a signals via PAR1/4 and may lead to endothelial leakage. **Bálint Kovács** from Lübeck, Germany, went on with a talk on the C5a/C5aR1 axis in autoimmune Epidermolysis bullosa acquisita (EBA), which is an autoimmune blistering disease caused by auto-antibodies against collagen VII. C5aR1$^{-/-}$ BL-6S mice were protected from EBA, supposedly because of decreased levels of pro-inflammatory agalactosylated IgG auto-antibodies. **Nathalie Niyonzima** from Trondheim, Norway, presented data on the intracellular C5 system, which controls sterile inflammation in human monocytes. She showed evidence for intracellular C3/C5 convertase-driven C5 activation, required for DAMP sensing of human monocytes. **Erin E. West** from Bethesda, United States talked about intracellular C5. She showed that Th1 responses are auto-regulated through carboxypeptidase M-generated C5a-desArg. These data support the idea that immune cells have adopted an autonomous complement activity, allowing them to sustain their effector functions in tissues. Finally, **Ben C King** from Lund, presented a novel function for CD59. He found a non-GPI anchored isoform of CD59, which is required for insulin secretion from beta cells in mice.

Session: *Inflammation* **Chairs:** Katrine Pilely and Leendert Trouw

The session is opened by a presentation by **Myriam Martin**, from Malmo, Sweden on the development and use of an ELISA based assay for the detection of C4d. Antibodies specific for a neo-epitope in C4d allowed specific detection of activated C4 in plasma samples of SLE patients and controls. The data revealed increased levels of C4d especially in patients in active disease with nephritis. The assay could potentially be used in the clinic to predict flares, allowing the clinician to modify treatment. Next **Ninette Genster**, a local from Copenhagen, presented data on Ficolins and LPS-mediated inflammation. Although initial in-vitro data clearly indicated that purified and serum derived Ficolins bind to LPS, in-vivo experiments using ficolin deficient mice and wild-type mice displayed a similar dose dependent (lethal) immune activation. The data suggest that ficolins do not play an essential role in LPS-mediated immune activation in mice. **Nicolas Merle**, from Paris, France, reported on intravascular hemolysis mediated by complement activation. Patients suffering from sickle cell anemia may present with hemolysis and release free heme. This heme is activating complement resulting in deposition of activated complement fragments in target organs, especially the kidney. Using hemopexin in the in-vitro experiments could completely prevent the heme-mediated complement activation. **Corinna Lau**, from Bodo, Norway, provided an overview of her work on the analysis of whole blood stimulated with E.coli. Anti-coagulated whole blood was incubated with E.coli in the presence or absence of C5-inhibitors or CD14 inhibitors. New in this analysis was the specific focus on the phosphokinetic response in the cells present in whole blood. FACS analysis of phosphorylated signalling molecules revealed highly effective therapeutic effects of both complement and CD14 mediated immune cell activation. Finally, to the best of our knowledge as a premier at an EMCHD meeting, we enjoyed a presentation by **Jose Halperin**, Boston, USA, via Skype. Following the identification of glycated-CD59 in sera and tissues of patients suffering from diabetes the team of Dr. Halperin now focussed on quantitatively analysing the presence of gCD59 in the context of pregnancy-induced diabetic complications. The presented data suggest that the current cumbersome testing for glucose intolerance may be replaced by an assay based on detecting blood levels of gCD59. High levels of gCD59 were reported to be associated with clinical complications such as 'large for gestational age'-babies.



Session: *Therapy* **Chairs:** Mikkel-Ole Skjoedt and Carl-Wilhelm Vogel

This session comprised the latest attempts on complement related therapeutic interventions. The first presentation from **Nirmal Banda** (Univer. of Colorado) used the collagen antibody induced arthritis mouse model (CAIA) to show that specific inhibition of the isoform transcript *MASP1v2* (MASP-3) attenuated the arthritis disease activity. Liver expression of MASP-3 protein was reduced to around half without affecting the MASP-1 levels. The second presentation by **Alonso Ricardo** from RA Pharmaceuticals addressed the anti-hemolytic effect in vitro of the cyclic RA101495 peptide C5 inhibitor. Compared to eculizumab, the C5 inhibiting peptide was more efficient in inhibiting C5 activation and hemolysis in various in vitro assays. The third presentation from **Bärbel Rohrer** described treatment approaches to age-related macular degeneration (AMD). An AAV vector based strategy was used to deliver a fusion construct of the binding domain from the complement receptor 2 and factor H (CR2-fH). The CR2-fH chimeric construct reduced choroidal neovascularization and C3a generation suggesting a potential local treatment strategy for AMD. The next presentation was from **Mingjun Huang** (Achillion Pharmaceuticals) who presented an orally administered factor D inhibitor in a clinical phase 1 PNH study. The factor D inhibitor ACH-4471 displayed an approximately 3-fold inhibition improvement over eculizumab and has been included in a phase 2a study in PNH patients. **Yi Yang** (Newcastle University) presented the last report in this session and disclosed a dimerized mini factor H (HDM-FH) with improved half-life and with a better functional efficacy in terms of complement inhibition in vitro and a reduction in C3 deposition in the glomerular membrane in vivo. She proposed that the HDM-FH construction might be a candidate for treatment of C3 glomerulopathy.

Session: *Structure and Regulation* **Chairs:** Rafael Bayarri Olmos and Santiago Rodriguez de Cordoba

Dr. J. Dobó, from Péter Gál's Group (Budapest, Hungary), addressed the question of how MASP-3 gets activated. He presented kinetics' simulations suggesting the involvement of zymogenic MASP-1 to the activation of MASP-3, and discussed the contribution of auto- and MASP-2-mediated activation. **R. K. Jensen** (J.R. Andersen group; Aarhus, Denmark) reported the generation of a camelid nanobody against C3 with potential applications in therapeutics and basic research. Extensive structural and functional data indicate that it binds to C3 and C3b, blocking both formation of the AP C3-proconvertase and activation of C3 by the AP C3 convertase. Interestingly this nanobody also blocks cleavage of C3b by FH/FI. **Dr. C.Q. Schmidt** (Ulm, Germany) revisited the AP regulatory activities of FHL-1 to illustrate its potential physiological relevance on de-sialylated surfaces. **Dr. D.V. Pedersen** (Aarhus, Denmark) reported structural and functional studies with monomeric vertex structures generated from engineered recombinant human properdin. These structures form stable complexes with the AP C3 proconvertase and partially stabilize the AP C3 convertase. The crystal structure of these complexes confirms previous EM data and provides further insights into the interactions between properdin and the C345c domain of C3b. Finally, **Dr. C. Gaboriaud** (Grenoble, France) described the association of pathogenic mutations in C1R and C1S with Ehlers-Danlos syndrome, which may offer new areas to explore the functional role of the complement proteases.

Session: *Cardiovascular Diseases* **Chairs:** Bo Nilsson and Paul Morgan

This interesting and diverse session comprised six excellent presentations. **Katrine Pilely** described an examination of how cholesterol crystals bind C1q to initiate complement activation. She used flow cytometry and fluorescence microscopy to demonstrate that cholesterol crystals spontaneously bound pentraxins C-reactive protein (CRP) and long pentraxin 3 (PTX3); this in turn caused binding of C1q to the crystal-bound pentaraxins, leading to complement activation on the crystals. Opsonised crystals were more readily phagocytosed. In human atherosclerotic lesions, the pentraxins co-localised with cholesterol crystals and complement activation products. **Mai Abd el Hafez** reported a study of the effect of over-expression of human CD46 (membrane cofactor protein; MCP) in transgenic pigs. Pigs over-expressing both CD46 and the endothelial anticoagulant molecule thrombomodulin were tested in a myocardial ischaemia-reperfusion model. The double-transgenic pigs were protected from severe injury in the model as assessed by cardiac function, biochemistry and post-mortem analysis of infarct size. The data show that increasing complement and coagulation regulation on the endothelium protects from injury in this model of ischaemic heart disease. **Mikkel-Ole Skjoedt** used animal models to provide further evidence of the important roles of the alternative and lectin pathways as triggers for myocardial ischemia-reperfusion injury. They generated a hybrid inhibitor of both pathways comprising the lectin pathway inhibitor MAP-1 and the alternative pathway inhibitor factor H (the first 5 SCRs) and a humanised transgenic mouse in which human MBL replaced the mouse analogues. Myocardial ischaemia was induced in these mice, followed by treatment with the hybrid inhibitor, MAP-1 alone or no drug. Inhibition of both alternative and lectin pathways improved cardiac function and decreased infarct size in the model, even at low doses, suggesting that this approach might be a useful avenue of therapy. **Terje Espevik** presented interesting results concerning the functional relevance of cholesterol crystals (CC) which form precipitates in atherosclerotic plaques. Previous studies have shown that CC activate complement; here Espevik demonstrated that patients with stable angina and acute coronary syndrome had significantly elevated amounts of sC5b-9, C3bc, C4bc and C3bBbP in their plasma. There was pronounced accumulation of C1q and C5b-9 around CCs in carotid plaques of patients. mRNA profiling of carotid plaques demonstrated that C5a and TNF priming followed by CC stimulation significantly upregulated expression of NLRP3, ASC, CASP1 as well as IL-1β and IL-18, suggesting a NLRP3 inflammasome pathway involvement. **Sylwia Wasiak** described how the BET inhibitor of epigenetic regulators bromodomain and extraterminal proteins, apabetalone (RVX-208), down-regulates the complement cascade *in vitro* and *in vivo*. RVX-208 had previously been shown in phase 2 trials to reduce risk of adverse cardiac events. In cultured primary hepatic cells and in chimeric mice, RVX-208 reduced complement component synthesis. Proteomic analysis of plasma from patients treated with RV-208 showed a significant decrease in complement factors and regulators including factor B, properdin, collectin-11, SAP and C-reactive protein. Levels of complement activation products C5a, C3b and TCC were also reduced, implying a dampening of activation. **Hilde Orrem** presented data from non-ST segment elevation myocardial infarction (NSTEMI) patients treated with the IL-6R antagonist tocilizumab. She demonstrated that the expression of C5aR1 and C5aR2 was reduced by more than 50% at day 2 and 3 compared to placebo and healthy controls, but tocilizumab did not affect the degree of complement activation, quantified as sC5b-9. The data suggest that anti-inflammatory effects of tocilizumab administration may be explained by a pronounced reduction of C5aR1 expression.

Session: *Genetics and neurological diseases*
Chairs: *Veronique Fremeaux-Bacchi and Ninette Genster*

This session opened with **Simon Clark** presenting research showing that complement proteins synthesized locally on either side of Bruch's membrane, or on the choroidal side derived from the circulation, predominantly remain on their side of origin. In particular FHL-1, FD, and the anaphylatoxin C5a can diffuse freely, while the proteins including FI, FH, FB, and C3a cannot. BrM creates two semi-independent compartments which should be kept in mind when developing AMD treatments. The second talk by **Inkeri Lokki** presented a genetic association study suggesting that complement C3 receptor modifications caused by missense variants in CR3 and CR4 genes might influence the interaction between C3 and phagocytes and possibly also platelet function, thereby affecting the susceptibility to preeclampsia. **Rosella Piras** from the Mario Negri institute described the CFH and CFH-Related rearrangements identified in three large cohorts of patients with aHUS, C3G and IC-MPGN. The homozygous *CFHR3-CFHR1* deletion associated with anti CFH associated aHUS was not associated with C3G and IC-MPGN and, in contrast, that hybrid genes or duplications involving *CFHR4* was found in all three diseases. Next **Maria-Grazia De Simoni** presented interesting data from a mouse model of cerebral ischemia showing that the vascular damage and platelet IL-1a release was driven by MBL. The session ended with a nice talk by **Trent Woodruff** who presented in vivo and in vitro models demonstrating a role for the anaphylatoxin receptors in the normal development of the brain. In contrast to C3aR, C5aR1 promoted proliferation of neural progenitor cells and blockade of C5aR1 in mice during development resulted in behavioral and structural changes in the adult mice.

Session: *Late Breaking* **Chair:** Peter F. Zipfel

Dror Mevorac from the Hadassah-Hebrew Medical Center in Jerusalem, Israel, reported on patients with genetic complement deficiency and the clinical management of these patients with complement inhibition. Mutations in the CD59 gene are associated with the neuronal disease Guillain Barré syndrome a severe neuronal disease with sensory motor demyelination neuropathy and secondary axon damage often associated with stroke and neurological insults. The lack of CD59 results in uncontrolled complement activation on the cell surface. Dror Mevorac has identified five patients with this severe neurological disorder. He reported on the successful clinical experience on terminal complement inhibition, using Eculizumab, of four patients over a period of 3 years. In addition, he presented very recent findings in a large Muslim Arab family with protein losing enteropathy. He and his team identified a novel frame shift mutation in the CD55 gene. The patients are homozygous for this mutation in congenital CD55 deficiency and show abnormal complement regulator expression and abnormal complement activation with enhanced TCC deposition on granulocytes and erythrocytes. Also, this group of patients responded to terminal complement inhibition by Eculizumab. Thereby, Dror Mevorac was able to demonstrate two examples where genetic findings were successfully translated into the clinic.

Session: *Infectious Diseases* **Chairs:** Reinhard Würzner and Claire Harris

Antonio Inforzato from Milan reported an investigation into the role of the pentraxin PTX3 in clearance of the fungal pathogen, *Aspergillus fumigatus*. PTX3 works in concert with factor H to promote effective opsonisation of the pathogen with C3 fragments, thus promoting phagocytosis and killing. The group in Italy are unravelling the molecular mechanisms underlying the synergistic role in clearance. Attention then turned to immune evasion mechanisms of the pathogen, *Bordetella pertussis*, in a talk given by **Ilse Jongerius** who is based in Utrecht. Ilse's group has been exploring the role of Vag8 in triggering consumption of classical pathway components in the vicinity of the bacterium, thus hindering effective targeting of the pathogen. Although a surface-associated inhibitory activity cannot be totally ruled out, it seems that the primary role of Vag8 is to diffuse from the pathogen surface and bind to C1inh, resulting in the production of activated C1s and C1r which cleave and consume C4 and C2 locally. The implications for vaccine development were discussed. **Thiago Ferreira de Araujo Rosa** presented data from a collaboration with Baltimore and Jena on a complement evasion mechanism executed by plasmodium falciparum sporozoites during their short (approx. 20 mins) transition from the skin to the liver. As for many other pathogens, factor H is also here the target molecule which is hijacked to downregulate complement activation, thus favoring pathogen survival in the human host. **B.W. Bardoel** from Utrecht detailed a mechanism how antibiotics, effective against gram-positive bacteria, can also penetrate the outer membrane of gram-negative bacteria. This is facilitated by the membrane attack complex (MAC) formed out of the added serum which then allows the antibiotic nisin to reach its target sites. **Dani Heesterbeek** and colleagues from the same institute investigated the role of C5 convertases in the MAC dependent killing of gram-negative bacteria. They could show that purified C5b-9 on its own does not kill bacteria, killing is only effective when C5 convertases are attached, as these are essential for a stable insertion of the MAC into the bacterial membrane.

Session: *Infectious and Kidney Diseases*
Chairs: Daniel Ricklin and Zotlán Prohászka

This session provided a smooth transition between infectious and kidney diseases and offered exciting insight about complement regulation and dysregulation at the interface of defense and disease. The first talk of the session, given by **David Emert** from Lund University, introduced a novel immune evasion mechanism employed by Group A streptococci (GAS). Based on the observation that immunoglobulin therapy exacerbated GAS infection in transgenic mice expressing human C4BP, the study revealed that the bacteria's virulence factor Protein H simultaneously binds C4BP and the Fc part of IgG. This "crosslinking" near the bacterial surface increases the avidity of C4BP and confers increased protection for the pathogen. **Anna van Beek** from the University of Amsterdam then talked about novel insight into the composition and plasma levels of Factor H-related proteins (FHR), particularly in the context of infection. By utilizing improved methods for detecting FHRs, they could show that FHR1, 2 and 5 solely exist in hetero- and/or homo-dimeric form but that they rapidly exchange monomeric units. Whereas pediatric patients with *Streptococcus pneumoniae* infections did not show marked differences in the levels of FH or FHR1 and 2 dimers when compared to healthy donors, the levels of FHR5/5 were significantly elevated during the acute phase of infection, thereby highlighting FHR5 as an interesting protein for functional and/or diagnostic studies. In the third presentation, **Weiju Wu** from King's College in London revealed an important connection between the anaphylatoxin C5a and bacterial adhesion in urinary tract infection. The study showed that human renal tubular epithelial cells express C5aR1 and that its stimulation by C5a increases the exposure of mannose moieties on the cell surface in a TNFα-dependent manner, thereby facilitating the adhesion of uropathogenic *E. coli* via their fimbriae. Next, **Lubka Roumenina** focused on the question why the kidney is particularly susceptible in atypical HUS. Resting and heme-activated endothelial cells of glomerular, dermal, and umbilical vein origin were analyzed for expression of complement regulators and C3b deposition. While in the resting state the various cell types behaved in the same manner, heme-activated glomerular endothelial cells (in contrast to other cell types) failed to adapt to the exposure and responded with increased C3b deposition. Inefficient Heme Oxygenase 1 expression may be a reason for the lack of adaptation to hemolysis explaining the greater renal susceptibility of endothelium injury in aHUS. **Talat H. Malik** from the Pickering Lab followed up next by examining the interaction of mouse FHRB and renal-bound C3 in vivo. Using the CRISPR/Cas9 genome editing technology various FH and/or FHR deleted mice strains were generated. Similar to FH deficiency, there was spontaneous plasma C3 depletion and glomerular C3 deposition in FH and FHR deleted mice. In vivo and in vitro evidence was presented that mCFHR2/B is binding to glomerular capillary wall fixed C3. The novel mice strains may provide unique tools to study the role of CFHRs in the pathogenesis of C3 glomerulopathies. In the final presentation, **Kate Smith-Jackson** from the Newcastle Complement Therapeutics Research Group presented the first study on the treatment of aHUS with BB5.1, a monoclonal anti-mC5 antibody. Importantly, the gain-of-function aHUS mice, having C3 D1115N conditional knock-in, showed all important hematologic and histology features of thrombotic microangiopathy. The disease could be treated effectively by the anti-C5 antibody treatment leading to decreased deposition of C3 and C9 in kidney. These data confirm that the mechanism of TMA in this strain is highly similar to aHUS and validate the model as an appropriate test bed for complement therapeutics.

Session: *Kidney Diseases*
Chairs: Mohamed Daha and Matthew Pickering

In the session on kidney diseases, **Sophie Chauvet** (Paris, France) further investigated the relation between C3G and monoclonal gammopathy. She showed that a small percentage of patients contained antibodies reactive with complement factors, including FH and CR1. A larger proportion show the capacity to augment C3 convertase activity. Patients with C3G and MIg showed a worse prognosis. **Paraskevas Iatropoulos** (Bergamo, Italy) provided data to discriminate different forms of C3G and IC-MPGN. Input of clinical, pathological and laboratory results, combined with hierarchical clustering, identified 4 subgroups with specific characteristics. Patients in group 1-3 show fluid phase complement activation, whereas patients in group 4 showed solid phase complement activation, with normal C3 levels in circulation, late onset of disease and worse prognosis. **Yuzhou Zhang** (Iowa, USA) showed data of a FH / FD double KO, in which, although C3 levels in circulation were restored compared to the FH-KO, renal biopsies still showed glomerular C3 deposition. In vitro experiments with serum of these mice showed some AP haemolytic activity, which was explained by a slow kallikrein-mediated activation of C3bB. Finally, **Felix Poppelaars** (Groningen, the Netherlands) studied the process of brain-death in an experimental mouse model using multiple complement-deficient mice. Mice deficient in C4 showed significantly diminished renal injury and inflammation.

MEETING ANNOUNCEMENTS

ICS Guest Society Symposium

New Discoveries in Complement: Impact on Health and Disease

Sunday, May 06, 2018 12:30 PM - 2:30 PM

Chairs:
Viviana P. Ferreira, University of Toledo College of Medicine

Rick Wetsel, University of Texas

Speakers:

- **Betty Diamond**, Feinstein Ins. For Med. Res., *C1q as an immune modulator of pro-inflammatory pathways*
- **Claudia Kemper**, NIH, *Intracellular complement is required for basic physiological processes in immune cells*
- **Ronald Taylor**, Univ. of Virginia, *Cancer and complement therapeutics: molecular structure to treatment regimens*
- **Michael Carroll**, Harvard Med. Sch., *CD21 blockade of neurological symptoms of lupus*

Link: http://www.immunology2018.org/scientific-program/

MEETING ANNOUNCEMENTS

DATE CLAIMER: JUNE 23-28, 2018
11th International Conference on Complement Therapeutics

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