Focus on Complement

December 1, 2016
Issue 44

International Complement Society

European Complement Network

Dear Readers,

Welcome to the December 2016 issue of 'Focus on Complement'. This 44th issue of FoC contains:

- **Season's Greetings** from the ICS and ECN Boards
- **Letter from the ICS President Andrea Tenner**
- **Flash News** will cover how intracellular C5 and inflammasome crosstalk regulates Th1 immunity and how complement contributed to synaptic loss and memory impairment after west nile virus infections
- **The Complement research teams around the world** series featuring several teams in Madrid, Spain, and the team of Dr. Wouters in Amsterdam, The Netherlands
- Part I of the meeting report on the **XXVIth International Complement Workshop** that took place in Kanazawa, Japan September 4 - 8th 2016
- **16th European meeting on Complement in human Disease** announcement
- **Work opportunities in complement research**

If you would like to contribute with an article to a future issue or have suggestions for a subject theme, please contact Claudia Kemper or Andrea Tenner; Claudia.kemper@kcl.ac.uk; atenner@uci.edu

Thank you for your continuous sponsorship:

On behalf of the Boards of the 'International Complement Society' and the 'European Complement Network', we wish you all a very joyful festive season.

We hope that the past year has been a happy and successful one and sincerely wish that the coming year 2017 will be prosperous, healthy and peaceful for you, your families, friends and collaborators.

Letter from the ICS President Andrea Tenner

Seasons Greetings to all of you who work to unveil the diversity of contributions of the complement system to health and creatively apply that understanding to enhance health and limit destructive inflammation.
For those that could make it to the 26th International Complement Workshop in Kanazawa, Japan, Professor Teizo Fujita and his Local Organizing Committee treated 327 participants to the best of science and wonderful cultural experiences. Fifty four talks were selected from over 282 submitted abstracts, and the remainder of the abstracts provided the basis of lively poster sessions. Four outstanding plenary lectures and two noontime talks provided many different fascinating views of the past, the present and the promise of exciting future discoveries in the realm of complement biology that can be applied to therapeutics and diagnostics for the benefit of many. The ICS Young Investigator Award was presented to **Mikkel-Ole Skjødt** of the Dept of Clinical Immunology/Rigshospitalet in Copenhagen, and the Lambris Complement Training Award was awarded to **Martin Kolev** of Kings College, London. Forty Travel Awards were presented to Trainees to facilitate their participation, with nearly 1 in 4 awards supported by a generous gift from Alexion Pharmaceuticals.

As this calendar year ends I hand the baton of the President to the capable hands of **Michael Holers** (Denver). In Kanazawa, we also elected a new Treasurer, **Dan Ricklin** (now heralding from Switzerland and thus befitting a Treasurer), a new Secretary, **Trent Woodruff** (who served as Councilor for 4 years, and gives the Executive Council its first representative from Australia), and a new President Elect, **Peter Garred** (just finishing a 6 year term on the ICS Council from Copenhagen). In addition, 4 new Councilors were elected to replace the 3 completing their terms and the spot vacated as Trent moved to Secretary. Those four dedicated new Councilors are **Viviana Ferreira** (US), **Claire Harris** (UK), **Joshua Thurman** (US), and **Nobutaka Wakamiya** (Japan). Thank you for your participation in guiding our Society for the next 6 years in conjunction with our continuing councilors **Michael Kirschfink** (Germany), **Bo Nilsson** (Sweden), **Denise Tambourgi** (Brazil), and **Peter F. Zipfel** (Germany) who will providing context, history as well as continued support to our Society. All complement researchers should feel welcome to contact them with suggestions or concerns that the Society may be able to address (or address more effectively).

Importantly, we owe our sincere gratitude to **Matthew Pickering** and **Santiago Rodriguez de Cordoba** for bringing their experience, judgment and service to our Council for 6 full years each. Special thanks also goes to **Claudia Kemper**, our amazing Secretary for 4 years (and Editor-in-Chief of Focus on Complement) who also had served as Councillor for 4 years prior to that, and Treasurer, **Wenchao Song**, who was the 2006 Beijing ICW Organizer followed by election to the Council in 2008 and then to Treasurer in 2012 for 10 continuous years of service to ICS. Lastly, **Zvi Fishelson**, was on the initial founding ICS Council in 2000-2002, was Secretary 2006-2010, during which he developed the framework for the ICS newsletter, “Focus on Complement”, and in 2010 was selected as President-Elect by the ICS members, serving as President 2012-2014 and completing his amazing formal service to ICW, as Past President the end of this year. We thank all of these individuals for their contributions to the then fledgling, now strong ICS organization.

In our ongoing efforts to raise awareness in the broader immunological community of the importance of the role of complement in basic and translational research, the International Complement Society is sponsoring its 4th ICS Guest Society Symposium at the American Association of Immunologists Annual meeting in Washington, DC, May 12-16, 2017. We hope to see many of you (and your colleagues) at this year's Symposium entitled "**21st Century Complement: Beyond the Textbooks"**. Our 4 distinguished speakers for this two hour symposium are:

Jörg Köhl, M.D., University of Lübeck, Germany and Cincinnati Children's Hospital:
Complement as a potential clinical driver and therapeutic target in Gaucher's Disease.

Baerbel (Barb) Rohrer, Ph.D., Medical University of South Carolina:
Complement and Age Related Macular Degeneration – anaphylatoxins and RPE signaling

Rick A. Wetsel, Ph.D, The Brown Institute of Molecular Medicine, University of Texas McGovern Medical School, Houston, Texas:
Complement Response to Listeria monocytogenes : Modulation of an Intracellular Beta-Interferon Response Pathway

Suzan H.M. Rooijakkers, Ph.D., Medical Microbiology University Medical Center Utrecht, Netherlands (an EMBO Young Investigator):
Complement Activation as a target for combating infections.

We would like to thank Comptech, Hycult and EMBO for their support of this Symposium.

Finally, mark you calendars now for the XXVII ICW to be held September 16-20, 2018 in the charming Santa Fe, New Mexico. More detailed information will be provided in the next Focus on Complement.

This is an exciting time for our field. Our challenge is to bring in new technology and new investigators while transferring to them the detailed knowledge derived from careful studies from past complement investigators. Complement is everywhere – we cannot escape it – but of course everyone loves complement!

With warm greetings,

ANDREA TENNER

NEWS FLASH (reported by Prof. Matthew Pickering, UK)

News Flash1:

T helper 1 immunity requires complement-driven NLRP3 inflammasome activity in CD4$^+$ T cells. *Arbore G, West EE, Spolski R, Robertson AA, Klos A, Rheinheiner C, Dutow P, Woodruff TM, Yu ZX, O'Neill LA, Coll RC, Sher A, Leonard WJ, Kohl J, Monk P, Cooper MA, Arno M, Afzali B, Lachmann HJ, Cope AP, Mayer-Barber KD, Kemper C.* ***Science 2016 352(6292).***

This is a landmark paper demonstrating that NLRP3 is an integral component of the Th1 response in CD4$^+$ T cells. Prior to this study NLRP3 inflammasome activity was thought to reside mainly within innate immune cells like monocytes and macrophages. However, the authors show that the NLRP3 inflammasome assembles in CD4$^+$ T cells upon T cell receptor (TCR) and CD46 co-stimulation and is able to initiate interleukin-1β secretion in a caspase-1-dependent manner. These events lead to interferon-gamma (IFN-γ) production and Th1 differentiation. Using a series of very elegant experiments that included data from patients with gain-of-function mutations in NLPR3 (cryopyrin-associates periodic syndromes, CAPS), the authors also showed that the assembly of NLRP3 required activation of complement C5 within the cell. This intracellular C5 activation leads to C5a generation, which interacts, with the C5a receptor 1 (CD88). C5aR2, also known as C5L2, present on the surface of the cells, in turn, negatively regulates this phenomenon. Altogether the data demonstrate for the first time that there is regulated cross-talk between intracellularly activated complement components (described by the authors using the novel collective noun: complosome) and the NLRP3 inflammasome and that this is essential for optimal IFN-γ expression and the physiological Th1 induction and regulation. Perhaps therapeutic agents can alter this complement-NLRP3 axis; if so, they would represent a novel means of modulating Th1 activity.

News Flash 2:

A complement–microglial axis drives synapse loss during virus-induced memory impairment.

Vasek MJ, Garber C, Dorsey D, Durrant DM, Bollman B, Soung A, Yu J, Perez-Torres C, Frouin A, Wilton DK, Funk K, DeMasters BK, Jiang X, Bowen JR, Mennerick S, Robinson JK, Garbow JR, Tyler KL, Suthar MS, Schmidt RE, Stevens B, Klein RS. ***Nature 2016 534(538).***

West Nile virus (WNV) targets hippocampal neurons and affected individuals frequently develop long lasting impaired memory and visual-spatial processing. The classical pathway is important in synaptic pruning by microglia. In this paper the mechanism of WNV-associated neurological abnormalities is revealed using a mouse model of WNV neuro-invasive disease. In these experiments viral infection of hippocampal neurons results in complement-mediated microglial elimination of presynaptic terminals. C1QA was up-regulated and localized to microglia, infected neurons and presynaptic terminals during infection. WNV-induced synaptic loss was dependent on C3 and C3a receptor. The authors propose that this novel model is a valid representation of the pathology that results in the neurocognitive impairment that can be seen in patients recovering from WNV neuro-invasive disease.

COMPLEMENT TEAMS AROUND THE WORLD

Complement in Madrid, Spain:

Complement research in Madrid has significantly grown over time as a consequence of the increasing interest in the field. Back in the 1990´s only a couple of labs, Prof. Santiago Rodríguez de Córdoba´s lab (SRdC) and Margarita López Trascasa´s lab (MLT), were dedicated to investigate complement in Madrid but, undoubtedly, they provided the ground for fostering the addition of new members into the complement community. Currently, six different groups are somehow involved in complement research: the above mentioned SRDC and MLT, Pilar Sánchez Corral (PSC), Óscar Llorca Blanco (OLB), Jose R. Regueiro (JRR) and, recently joined, Elena Goicoechea de Jorge (EGdJ). These labs are located across three different institutions in Madrid: the Biological Research Centre from the Spanish National Reseach Council (SRdC and OLB), the Immunology Department and the Research Unit of the University Hospital La Paz (MLT and PSC) and the Immunology Department of the School of Medicine at the Complutense University (JRR and EGdJ). In order to delineate common research objectives and to coordinate efforts between the different groups a Complement consortium was created in 2012. Through the years multiple publications have arisen demonstrating the fruitful collaborations between these labs.

The Complement consortium encompasses different areas of expertise which allows a multidisciplinary and translational approach to study the physiopathology of the complement system. SRdC´s lab has a strong track record on complement genetics and functional characterization of complement proteins. Throughout more than 30 years, SRdC´s lab has made very important contributions in the complement field. From the genomic organization of the Regulators of Complement Activation Gene cluster (RCA), to the functional characterization of common and rare gene variants associated with complement-mediated pathologies, the development of animal models of complement dysregulation, to structural studies of complement protein complexes which have been key for the understanding of the molecular mechanisms leading to disease. Importantly, throughout the years SRdC's lab has become the reference laboratory for the genetic diagnosis and molecular characterization of atypical haemolytic uremic (aHUS) and C3 glomerulopathy (C3G) samples. MLT´s lab, is a National Reference Centre for the diagnosis of primary complement immunodeficiencies and activation-consumption situations mediated by autoantibodies directed against specific complement components. Their research and diagnostic activities include biochemical and functional characterization of complement deficiencies, mutational screening in classical pathway complement proteins and characterization of autoimmune disorders, including anti-C1Inhibitor, anti-C1q, anti-CFH autoantibodies and nephritic factor. MLT' Lab is also referential in the study of classical and strogen-related forms of Hereditary Angioedema at the National and International levels. PSC´s lab mainly focuses on translational research aimed to improving the molecular diagnosis and treatment of renal diseases where there is a prominent role of the complement system, including aHUS and C3G, by using a proteomics-genomics approach. Its main contribution has been done in the understanding of the pathogenic mechanisms aHUS patients presenting abnormalities in the complement regulator factor H and the factor H-related proteins. JRR´s lab has a long-term interest in leukocyte physiopathology, with special emphasis in T lymphocytes from both sides of the immunological synapse in all areas of immunopathology (notably immunodeficiency/infection, but also in alloimmunity, autoimmunity and allergy).

Its main objective is the study of T cell development, activation and effector functions under inherited or acquired pathological conditions, in connection with both classical and non-classical MHC molecules. From this immunological perspective, JRR´s lab is a fundamental piece in the Complement consortium to tackle the new challenges that intracellular complement is posing. OLB´s lab is specialised in electron microscopy and image processing techniques. Their major contribution in the complement filed has been the structural characterization of the alternative pathway C3 convertase and several complement mutant proteins, which have contributed to understand how complement functions are altered in pathological conditions and identify targets in order to develop drugs. Last but not least, EGdJ is a young researcher recently established in Madrid. With a background on complement genetics, protein biochemistry and animal models, the main research interest of EGdJ´s lab is the understanding of the molecular mechanisms by which complement leads to aHUS and C3G. In particular, they focus on deciphering the biological role of the factor H family of proteins. All together, we believe complement research in Madrid in going through a thriving period.

During these years we have also enjoyed immensely the collaboration and friendship with colleagues around the world. We always look forward to initiating additional exciting collaborations within and outside the complement community.

MLT´s and PSC´s research groups from the Immunology Department and the Research Unit of the University Hospital La Paz.

SRdC´s, OLL´s, JRR´s and EGdJ´s research groups from the Biological Research Centre (CSIC) and the Immunology Department of the School of Medicine at the Complutense University.

CONTACT INFORMATION:
Santiago Rodríguez de Córdoba: srdecordoba@cib.csic.es
Margarita López Trascasa: mltrascasa@salud.madrid.org
Pilar Sánchez Corral: pilar.sanchezcorral@salud.madrid.org
José Ramón Regueiro: regueiro@med.ucm.es
Oscar Llorca: ollorca@cib.csic.es
Elena Goicoechea de Jorge: egoicoec@ucm.es

Complement in Amsterdam, The Netherlands: The team of Dr. Diana Wouters

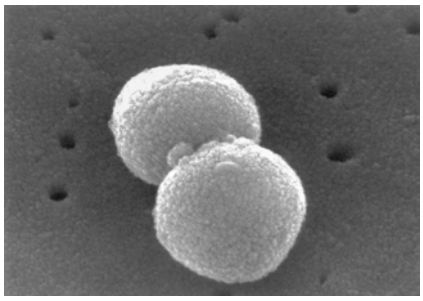
For over 30 years the complement system has been studied at the department of Immunopathology at Sanquin Research. Sanquin is the national blood supply foundation in the Netherlands which next to the blood bank also houses a diagnostics department and a research facility for fundamental and translational research relating to blood transfusion medicine and immunology. Dr. Diana Wouters was trained as a PhD student by Prof Erik Hack at this institute and is currently leading the Complement research group.

Our research group investigates the balance between complement activation and regulation on different surfaces both under conditions of normal homeostasis and pathology. We are especially interested in how complement activation can be influenced in order to tip the balance to decrease unwanted complement activation and prevent damage on host cells. For proper therapeutic intervention, an appropriate balance should be found between suppressing unwanted complement mediated pathology whilst allowing sufficient complement mediated protection against infection.

To investigate the contribution of the different membrane expressed regulators to the protection of human cell surfaces against unwanted complement activation, we have successfully established knockouts for membrane complement regulators (CD46, CD55, CD59 and double/triple combinations) on a human cell line using CRISPR/Cas9 technology.

These model cells can be used to investigate the individual contribution of each complement regulator against spontaneous alternative pathway activation, bystander activation by infection and classical pathway activation by (auto)-antibodies. In addition, we study factor H (FH) as important regulator of the alternative pathway on human host cells. Next to investigating the therapeutic applicability of plasma purified FH, we have identified a unique monoclonal antibody that potentiates the function of FH. We are currently investigating the mechanisms by which this monoclonal antibody affects FH function and we are exploring whether this antibody may be used therapeutically in diseases with excessive alternative pathway activation.

Next to protecting host cells, complement regulator FH is exploited by numerous pathogens such as *N. meningitidis, S. pneumonia,* and many others to protect themselves against complement activation and thereby increase their survival in blood. Due to the high homology between certain domains of FH and the FH-related proteins (FHRs), some microbes also bind certain FHRs, but the significance of this phenomenon for immune evasion is not known yet. If FHRs indeed counteract the function of FH, as suggested before, high plasma levels of (certain) FHR proteins may render pathogens more vulnerable for complement attack. Variation in FHR proteins may therefore (partially) explain differences in vulnerability for certain infections.



Streptococcus pneumonia
Source:
CDC, Public Health Image Library
ID 263

As part of a European consortium, Euclids, we are investigating plasma levels of FH and all FHRs in children suffering from various bacterial infections to investigate their role in susceptibility and severity in life-threatening bacterial infections in childhood. To this end, we have generated large panels of monoclonal antibodies against all members of the FH-protein family and set up specific assays to quantify these proteins.

This work is done together with Prof. T.W. Kuijpers, head of the department of Pediatric Hematology, Immunology and Infectious Disease of the Emma Children's Hospital at the Academic Medical Center in Amsterdam.

The complement research group of Dr. Diana Wouters has a strong link with Sanquin Diagnostics services (department Immunochemistry under supervision of Dr. Kyra Gelderman), where all complement patient diagnostics is being performed for hospitals in the Netherlands.

Above: *Dr. Diana Wouters' (second row, 1st from the right) group at Sanquin*

CONTACT INFORMATION:
Dr. Diana Wouters
Sanquin Research, dept. of Immunopathology
Amsterdam, the Netherlands
d.wouters@sanquin.nl
http://www.sanquin.nl/en/research/who-is-who/diana-wouters/

ICW 2016 Meeting Report

Below is PART I of the summaries of the Scientific Sessions and Lunchtime Seminars of the 26th ICW in Kanazawa, Japan (September 4th to 8th 2016), each composed by the respective chair persons.

SCIENTIFIC SESSIONS

SESSION I: Intra-cellular Complement
Chairs: Peter F. Zipfel (Jena, Germany) and Claudia Kemper (London, UK)

This session focused on the recently newly discovered location for complement activity: The unexpected observation that complement can be activated intracellularly and the capacities of complement receptors to signal within the cell and direct from there effector functions. The first presentation was given by Nathalie Niyonzima (abstract 108) from Terje Espevik's and Claudia Kemper's laboratories. In line with previous findings that intracellular C5aR1 activation in human $CD4^+$ T cells is critical to normal Th1 induction during infection, this current work demonstrated for the first time that intracellular C5aR1 activity in human monocytes is also key to the induction of sterile inflammatory responses towards cholesterol crystals with subsequent NLRP3-depedent IL-1β secretion.
The next talk by Hrish Kulkarni (abstract 41) from John Atkinson's laboratory then further supported a likely broad role for intracellular complement activity in a range of cells by demonstrating that an intracellular C3-C3aR system is also present in human lung epithelial cells (LECs). Although part of intracellular C3 expressed by LECs is secreted, another portion of intracellular C3 is constitutively cleaved into C3a and C3b and it was noted that intracellular C3a co-localized with intracellular C3aR to intracellular membrane fractions. The exact identity of these subcompartments as well as the biological importance of this system in LECs is now focus of further studies in the Atkinson lab. Hide Yamamoto (abstract 156) from the Kemper laboratory then presented data that surprisingly suggested that the loss of intracellular C3 production and tonic C3a generation in human prostate epithelial cells correlates with induction of prostate cancer. Importantly, re-instatement of intracellular C3a levels via adenovirus-mediated delivery normalized hyperproliferation of malignantly transformed cells, suggesting that the intracellular C3 system regulates normal cell proliferation in the prostate and that dysregulation of intracellular C3 expression contributes to cancer. Finally, Michelle Elvington (abstract 145) from John Atkinson's laboratory then shared her data on a novel C3(H_2O) recycling and degradation pathway of the intracellular complement system in freshly isolated human peripheral blood cells. This group excitingly observed that C3(H_2O) can be taken up by blood-circulating cells and is then shunted into either a pathway that generates C3a as well as other biologically active C3 fragments intracellularly or a pathway that 'returns' the C3(H_2O) via secretion to the extracellular space. The group suggest that alterations in these pathways could contribute to immune diseases and may be a novel therapeutic target.
In all, this session showed that intracellular complement emerges as critical factor in directing cell functions but that we also are just at the beginning of understanding how this 'system' functions in health and disease.

SESSION III: Collectins
Chairs: Viviana P. Ferreira (Toledo, USA) and Peter Garred (Copenhagen, Denmark)

This session focused on the recognition molecules of the lectin pathway collectin-10 (CL-10 or CL-L1) and collectin-11 (CL-11 or CLK1) and hybrids of the two molecules, named CL-10/11 or CL-LK. Dr. Yasuyuki Matsuda et al. (abstract 187) elucidated the interaction properties between CL-10 and CL-11 and with the MASPs purified from human plasma. They showed that CL-11 covalently interacts with CL-10 by interchain disulfide bridges. In the circulation they showed that CL-10/CL-11 heteromeric complexes and also CL-10-free CL-11 and CL-11-free CL-10 exist. In comparison with MBL, CL-11 was associated with a relatively high amount of MASP-3, whereas MASP-2 binding was low, but detectable. These results indicate that a complex pattern of different oligomeric forms of CL-10 and CL-11 is present in the circulation and that MASP-3 is the preferential lectin pathway enzyme binding to these complexes. A series of presentations addressed that locally produced CL-11 released from stressed cells may initiate complement activation. Dr. Giorgia Fanelli et al. (abstract 106) showed that under hypoxic conditions CL-11 is present on the surface of retinal pigment epithelium (RPE) cells and may mediate deposition of complement, suggesting a role for CL-11 in the pathophysiology of diseases like age-related macular degeneration. Dr. Xia Dong *et al.* (abstract 72) showed that CL-11 could enhance phagocytosis of RPE cells and modulate cytokine responses by phagocytosis, providing evidence that CL-11 may also have a homeostatic role in the retina. In an *in vivo* rodent model of tubulointerstitial fibrosis Dr. Weiju Wu et al. (abstract 61) showed that CL-11 plays a critical role in the pathophysiology of renal fibrosis by enhancing leukocyte infiltration, tissue inflammation and extracellular matrix production. Moreover, they showed *in vitro* that CL-11 by itself had a potent effect in stimulating neutrophil and mono/macrophage migration. Finally in this session, Dr. Conrad Farrar et al. (abstract 144) provided evidence that the known CL-11 ligand L-fucose, which is exposed on stressed cells, may be a therapeutic option in renal ischemia reperfusion injury. In mice subjected to renal ischemia-reperfusion injury, administration of L–fucose and control sugars into the peritoneal cavity prior to the induction of ischemia, showed that L-fucose significantly protected the mice against injury. All together these results suggested that targeting of CL-11 may be a treatment possibility in ischemic induced inflammation.

SESSION IV: Complement and Diseases I
Chairs: Claire Harris (Newcastle, UK) and Kristina Ekdahl (Uppsala, Sweden)

The first of three *Complement and Diseases* sessions started with a captivating talk by Jörg Köhl (Lübeck, Germany, abstract 181) who presented convincing evidence of another disease driven by complement, Gaucher disease (GD). Individuals with GD lack the lysosomal enzyme b-glucocerebrosidase (GCase) and suffer continuous inflammation triggered by excessive glycosylceramide (GC) accumulation in immune cells. The investigators showed that GCase-deficient mice exhibit high levels of complement-activating anti-GC antibodies driving C5a production, both paralleled in the human disease. In experimental GD, C5aR deficiency and both C5 and C5aR1 blockade rescued the disease phenotype opening the door to new treatment regimes in this devastating disease. The session continued with a presentation by Delu Song (Pennsylvania, USA, abstract 130). Previous data from this group demonstrate that mice lacking properdin (P) and carrying low levels of a factor H mutant have severe C3 glomerulopathy. The investigators have extended their study by examining the eyes in these mice and they observe pathology very similar to the retinal manifestations in AMD; onset of retinopathy was rapid, 2-3 months. This mouse model provides opportunities for testing therapies targeted at AMD. Feng Lin (Cleveland, USA, abstract 6) presented data on complement activation in an experimental autoimmune uveitis model utilizing C4 and C1q KO and WT mice. Progression of disease was followed by ophthalmoscopic techniques and at the end of the study the mice were subjected to quantification of antigen-specific Th1 and Th17 T-cells. C4 KO mice developed significantly milder disease compared to WT, with no difference between C1q KO and WT mice. The authors conclude that activation of the LP but not the CP is operative in EAU and propose the LP as a novel therapeutic target in autoimmune uveitis. The talk triggered fascinating discussion around potential immune-modulating properties of C4a. Finally, Natsumi Sakamoto (Fukushima, Japan, abstract 81) reported data on complement activation in a model of lupus-like glomerulonephritis MRL//pr mice, which were KO regarding MASP-1/3. In contrast to WT, MASP-1/3 KO mice maintained their C3 when they aged, had higher levels of plasma IgG, similar amounts of immune complexes and did not develop proteinuria. The results demonstrate that the LP has an essential role in the development of glomerulonephritis in this disease model.

SESSION V: Lectin pathway and MASP
Chairs: Wilhelm Schwaeble (Leicester, UK) and Nicole Thielens (Grenoble, France)

This session summarized the latest key findings related to the three effector enzyme components of the lectin pathway (LP) and the truncated enzymatically inactive alternative splice product of the *MASP1* gene first described as MAp44 or MAP-1.
The first Young Invesitgator Award talk by Mikkel-Ole Skjoedt of Peter Garred's group (abstract 170) reported an antithrombotic effect of recombinant MAP-1 in mouse models of $FeCL_3$-induced thrombus formation, work performed with Greg Stahl (Boston, USA). In human plasma and whole blood *in vitro* coagulation models, MAP-1 reduces activation of all MASPs, coagulation factor XII, thrombin and kallirein and delays clot formation *in vitro*. In the discussion, Jens Jensenius (Aarhus, Denmark) mentioned that preparations of recombinant MAp44 failed to regulate LP functional activity *in vitro*. Future work needs to assess the therapeutic utility of MAP-1.

The second presentation (abstract 31) by Lorenz Jenny of Verena Schroeder's group (Bern, Switzerland) reported the effect of a recombinant catalytic fragment of MASP-1 (rMASP-1cf) on clot formation using a microvasculature-on-chip model. Addition of rMASP-1cf reduced the clotting time of recalcified whole blood, indicating that the thrombin-like activity of rMASP-1cf accelerates clot formation and that MASP-1 may link LP driven inflammatory conditions with thrombotic complications.

The subsequent talk given by Wilhelm Schwaeble (Leicester, UK) showed that MASP-1 or MASP-3 deficiency provides no protection from the severity of cerebral ischaemia reperfusion injury (IRI). He collaborated with Maria-Grazia De Simoni (Milano, Italy). When assessing MASP-2, or MASP-1/3 or C4 deficient mice, cerebral IRI was strictly MASP-2 dependent, but independent of C4, MASP-1 and MASP-3. The administration of an antibody-based inhibitor of mouse MASP-2 achieved significant therapeutic protection in WT mice. These data indicate that the hypothesis that MASP-1 is an essential activator of MASP-2 functional activity needs to be revised (abstract 84).

Nirmal Banda from Michael Holers' group (Denver, USA) reported that small interfering RNAs for MASP-1/3 or Df can attenuate pathology in a mouse model of collagen antibody-induced arthritis (CAIA) (abstract 2). Further, proDf expressed in the adipose tissue of MASP-1/3 deficient mice was effectively converted into active Df by MASP-1 and MASP-3 expressed in the allo-transplanted liver tissue of Df deficient mice and that this conversion occurs in circulation. In context with their previous study showing that MASP-1/3 deficient mice are protected from CAIA, this identified targeting MASP-1/3 as a promising therapy for the treatment of rheumatoid arthritis.

Gabor Oroszlan and colleagues (Budapest, Hungary) (abstract 166) reported the isolation of an inhibitory peptide that blocks MASP-3 functional activity. Using an *in vitro* model of proDf cleavage in "resting blood" (where neither coagulation nor complement activation occurs) they showed that inhibition of MASP-3 blocks the activation of proDf. This supports the role of MASP-3 as an essential converting enzyme of proDf. The pioneering work of Minoru Takahashi and Teizo Fujita (Fukushima, Japan) has now led to the general acceptance of the concept that the LP plays a critical role in the maintenance of AP functional activity – with these presentations providing support for the central physiological role of MASP-3 as a critical regulatory component of alternative pathway (AP) functional activity.

SESSION VI: Complement and the Nervous System Receptors
Chairs: Trent Woodruff (Brisbane, Australia) and Jörg Köhl (Lübeck, Germany)

This session opened with Andrea Tenner presenting research investigating brain C1q expression (abstract 42). Using transgenic C1q floxed mice crossed to cre mice to provide inducible and cell-specific ablation of C1q, it was clearly demonstrated that microglia are the sole source of C1q in healthy aged mice, and in mouse models of Alzheimer's disease. The second presentation by Michael Hernandez from the Tenner laboratory (abstract 125), looked further into Alzheimer's disease, investigating mechanisms for C5aR1-mediated microglial dysfunction in the 'Arctic' mouse model of AD. Arctic mice deficient in C5aR1 show remarkable protection from cognitive decline, and transcriptome analysis of sorted microglia surprising found major alterations in lysosomal, assembly, and protein degradation pathways in these immune cells. Next, Trent Woodruff presented research highlighting a major role for C5aR1 in Parkinson's disease (abstract 131). C5a and C5aR1 were shown to activate the NLRP3 inflammasome in microglia and to be increased in mouse models and human patient brains, and blockade of C5aR1 resulted in protection from loss of dopaminergic neurons, and improvements in motor dysfunction in mouse models. Rui Li from the Woodruff lab, followed up next by examining the second C5a receptor, C5aR2 in neurodegeneration (abstract 171). Mice deficient in C5aR2 showed remarkable protection from cognitive and motor deficits in mouse models of Parkinson's and Huntington's disease. In the final presentation, Maria-Grazia De Simoni (abstract 82), demonstrated in a cohort of 28 patients with severe brain trauma, local deposition of MBL, Ficolins-1-3 and MASP2 in and around brain vessels. Importantly, MASP2 levels correlated with disease severity. Overall, this session provided further evidence for a key role of complement in driving pathological sequela in neurodegenerative disease, representing a further disease class that may benefit from complement-targeted therapeutics.



Lunchtime Seminar I: Taroh Kinoshita (Osaka University, Japan)
Molecular pathogenesis of paroxysmal nocturnal hemoglobinuria
Chair: Carl-Wilhelm Vogel (University of Hawaii, USA)

The lunchtime seminar was presented by **Dr. Taroh Kinoshita** from Osaka University, Japan. Dr. Kinoshita's presentation was entitled **"Molecular pathogenesis of paroxysmal nocturnal hemoglobinuria"**. During the first part of his presentation, Dr. Kinoshita described the role of complement in the pathogenesis of paroxysmal nocturnal hemoglobinuria (PNH). The disease is a clonal blood cell disorder characterized by a deficiency in the membrane attack complex inhibition factor (CD59) and decay accelerating factor (DAF, CD55). These two membrane components on normal red blood cells inhibit complement activation at the stage of the C3 convertases, both in the classical and alternative pathways, as well as the formation of the membrane attack complex (MAC). As these two membrane-bound regulatory components are missing on PNH cells, complement activation can proceed, leading to intravascular hemolysis. Dr. Kinoshita subsequently described that CD59 and DAF are glycosylphosphatidylinositol (GPI)-anchored proteins, and that their absence in PNH is caused by a somatic mutation in the phosphatidylinositol glycan class A (PIGA) gene involved in the first step of GPI biosynthesis. Dr. Kinoshita elaborated that beyond DAF and CD59 there are approximately 30 more GPI-anchored proteins in hematopoietic stem cells, and that over 150 human GPI-anchored proteins are known. Whereas mutations in many genes of the GPI synthesis pathway can disrupt GPI biosynthesis, the PIGA gene is the most important as it is located on the X-chromosome (with the second copy on the other X-chromosome in females being inactive) therefore requiring only a single somatic mutation. Dr. Kinoshita then described the mechanism by which eculizumab, a humanized anti-C5 monoclonal antibody, prevents lysis of PNH cells in PNH patients: eculizumab binding to C5 prevents its activation and the subsequent generation of the MAC. He further described the issues associated with eculizumab therapy which include the risk of gram negative meningitis, as well as the occurrence of C5 variants resistant to eculizumab. Significantly, inhibition of C5 activation does not inhibit the underlying C3 activation on PNH cells, leading to accumulation of C3 activation product on PNH cells and in some cases extravascular hemolysis. The development of next generation of PNH therapy must address these issues. Subsequently, Dr. Kinoshita described that PNH, in addition to hemolysis, can also manifest bone marrow failure and venous thrombosis. The mutant hematopoietic stem cells can escape from immunological attack that kills normal hematopoietic stem cells and causes bone marrow failure, and that intrinsic mechanisms in the PNH cells may also be involved in the clonal expansion. In the last part of his presentation, Dr. Kinoshita presented some rare forms of PNH with abnormalities in chromosome 12 (2 patients) and two other patients with a combination of a germline mutation and a somatic mutation of the phosphatidylinositol glycan class T (PIGT) gene on chromosome 20.

ANNOUNCEMENTS

COPENHAGEN 2017
16th European Meeting on
COMPLEMENT
IN HUMAN DISEASE

On behalf of the organizing committee, Professor Peter Garred invites members of the complement community and beyond to the 17th European Meeting on Complement in Human Disease. The meeting will take place in Copenhagen, Denmark from September 8th to 12th 2017.

For opening dates for abstract submission, the preliminary program, accommodation and travel information, please see ***http://emchd2017.dk***

ALEXION PHARMACEUTICALS

ALEXION

Title: **Research Scientist III, Protein Sciences**
Location: **Cheshire, CT, USA**

Position Summary:
Provides leadership in identifying and prosecuting discovery research programs, specifically in the field of complement biology, and also in other disease pathways as needed; participates in proposing, identifying, evaluating new targets/programs for the research portfolio; provides leadership in designing screening cascades in aid of lead identification, in developing cellular and PK/PD assays in support of the discovery projects; participates in performing diligence activities in support of Business Development initiatives and in performing competitive intelligence analyses; establishes and manages external collaborations as needed.

Qualifications:

- Ph.D. in biochemistry/cell biology /molecular biology /pharmacology/structural-biology with 5-6 years of relevant industrial/academic research experience
- Extensive knowledge in complement biology, structure-function relationships, disease areas related to complement dysregulation
- A sound understanding of the theory governing macromolecular behavior
- Experience in research programs towards identifying therapeutic lead molecules is a plus
- Experience in collaborating/managing/directing within a matrix research organization desirable
- Ability to effectively allocate efforts amongst multiple projects and drive to aggressive timelines
- Good oral and written communications skills

COMPLEMENT REAGENT RESOURCES