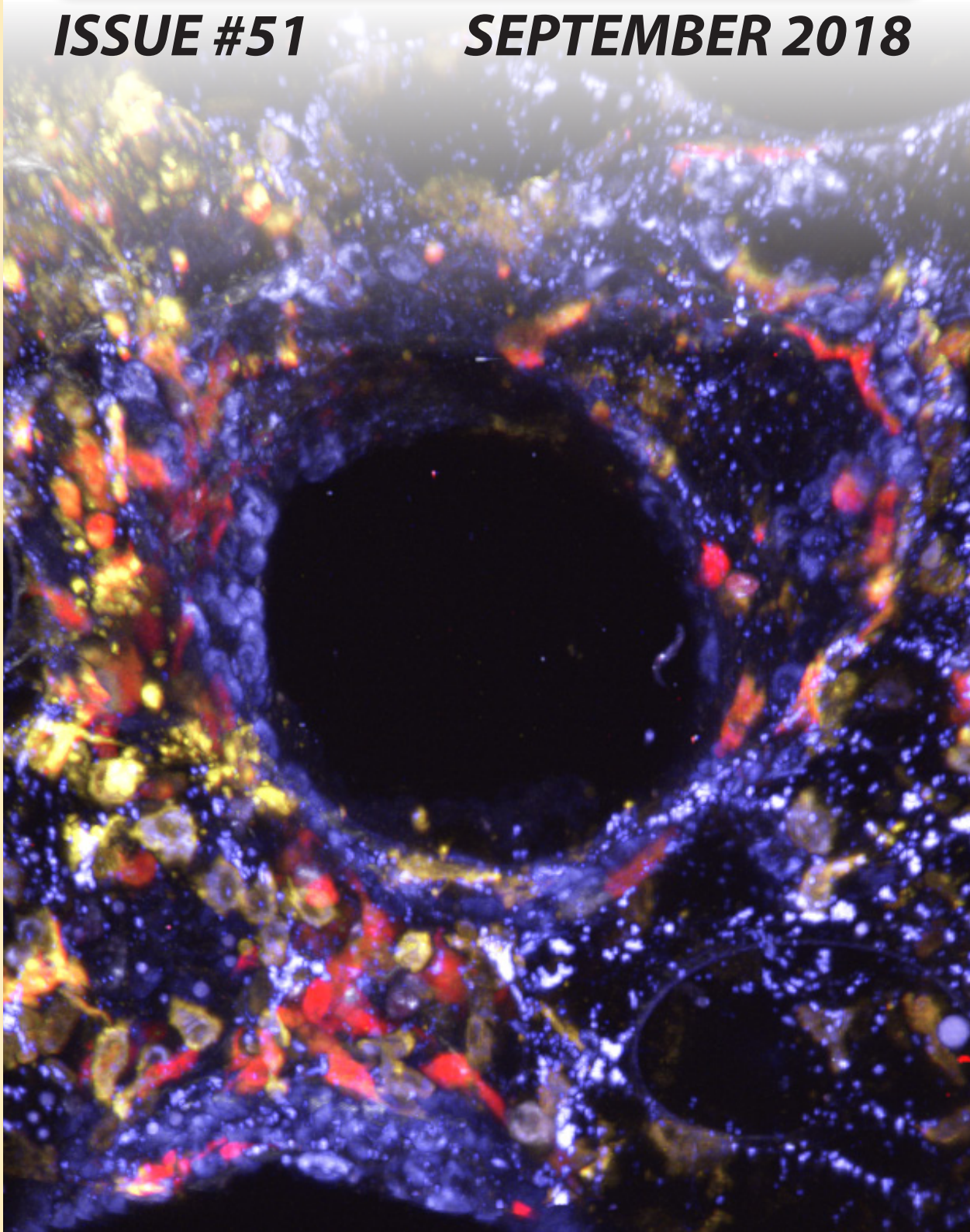
Focus on Complement

ISSUE #51 SEPTEMBER 2018



International Complement Society

9037 Ron Den Lane
Windermere
FL 34786-8328
T: 407-234-3274

ics@complement.org
www.complement.org

Focus on Complement

Issue #51

Editor
Trent Woodruff

Issue Contributor
Peter Zipfel

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In this 51st Issue of Focus on Complement:

From the Editor

Dear Readers,

Welcome to the 51st Issue of *Focus on Complement* – the official newsletter of the International Complement Society (ICS).

In this issue we highlight research groups from Pamplona, Spain (Dr Ruben Pio) and Paris, France (Dr Lubka Roumenina). Issue contributor Peter Zipfel reviews two recent research articles on C3 Glomerulopathy and Factor H binding proteins.

We also congratulate Katharina Quell, who is the winner of the inaugural FoC Young Investigator Cover Image Award. A description of Katharina's research and cover image description can be found on the next page.

I hope you all enjoy the this September issue of Focus on Complement!

Trent Woodruff, PhD.
Editor, FoC
Secretary, ICS
t.woodruff@uq.edu.au

Connect with the ICS

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).

Plus visit our website and follow us on Twitter to keep updated with the latest ICS and complement news.

www.complement.org @complementsoc

Katharina Quell, Winner of the Focus on Complement Young Investigator Cover Image Award

Katharina Quell is a third year PhD student in the research group of Yves Laumonnier at the Institute for Systemic Inflammation Research in Lübeck, Germany. Her project is part of the IRTG 1911 Program "Immunoregulation of Inflammation in Allergy and Infection" of the University of Lübeck, and is aimed at investigating the regulation and function of the C3a anaphylatoxin receptor (C3aR) in the establishment and severity of allergic asthma.

The cover image shows a section of lung from a non-allergic tdTomato-C3aRflx/flx reporter mouse, centered on an airway. In collaboration with the Institute of Anatomy at the University of Lübeck, the staining was performed on precison cut lung sections, stained with antibodies, and evaluated by confocal microscopy, using Z-stacks (40 µm with a resolution of 1 µm) allowing a three-dimensional presentation of lung structures. Colors correspond to autofluorescence of epithelial cells and collagen fibers (blue/white), eosinophils/alveolar macrophages surface marker Siglec F (blue), and antigen presenting cells presenting complex MHCII (yellow). The tdTomato-C3aR signal is visible in red. Here, the majority of the tdTomato signal is found around the airways but not in the alveolar compartment, and overlaps often with MHCII+ dendritic cells.

The ***Young Investigator Cover Image Award***. Each Issue the ICS board will select a scientific image to highlight on the front cover of FoC. The winning image will include a brief description of the image, and a profile of the winner within the newsletter.

Eligibility: graduate students, post-doctoral staff, and early career researchers (generally, but not exclusively under 40 years of age) are eligible to apply.

Interested applicants should email the FoC Editor (t.woodruff@uq.edu.au) at least 3 weeks prior to each issue release date (release dates: 1st March, 1st June, 1st September, 1st December), with one suggested image of their research. Images could include immunochemistry (tissues, cells etc), pathology, structures, or any other image of relevance to complement research. All images should not have any copyright that would be infringed if published in FoC (eg. work already published in a journal). Submissions should also include a brief profile of the researcher and a description of the image (~100 words each).

Winners of the Award will additionally receive a signed certificate from the ICS.

Complement Research in Pamplona, Spain

The group of Dr Ruben Pio

Complement research within the University of Navarra is led by Ruben Pio, the Director of the Program in Solid Tumors at the Center for Applied Medical Research (CIMA). We are located in Pamplona, a small city in northern Spain.

The objective of our Program is to develop new strategies for early cancer detection and new anticancer treatments. Our work primarily focuses on lung cancer, the leading cause of cancer death throughout the world. Our research building is directly opposite the university hospital, giving us the possibility to combine basic, translational and clinical research. In our team there is a mixture of scientists and students with different backgrounds and research activities.

Our interest for complement started during the postdoctoral fellowship of Ruben Pio at the National Cancer Institute (NIH). While searching for modulators of adrenomedullin, a peptide hormone involved in cancer progression, we came across complement factor H. In collaboration with Peter Zipfel, we characterized the regulatory role of factor H as an adrenomedullin binding protein.

After moving to Spain, we contributed to the understanding of the strategies used by cancer cells to control complement activation. We demonstrated that factor H is expressed by non-small cell lung cancer cells, protecting them from the deleterious effect of complement. We also showed that factor H partially inhibits the antitumor activity of cetuximab, an anti-EGFR monoclonal antibody with clinical benefit in cancer patients. In collaboration with Anna Blom and Marcin Okroj we also evaluated the role played by other soluble and membrane-bound complement regulators in lung cancer.

Ten years ago, work by John Lambris and his group brought about a significant shift in our research focus. They demonstrated a tumor-promoting role of complement in an immunocompetent model of cervical cancer. Several studies have validated and extended this initial observation, supporting that complement assists the escape of tumor cells from immunosurveillance, induces angiogenesis and promotes cancer metastasis. In collaboration with John Lambris, we found that the activation of complement on lung tumor cells leads to the release of complement components such as C5a, which contributes to tumor progression by the generation of an immunosuppressive microenvironment where myeloid-derived suppressor cells are involved. We also dissected the mechanisms by which complement is activated in lung cancer cells, finding that lung tumors activate the classical complement pathway and generate C4d. Moreover, our results suggested that the deposition of C4d on primary tumors, as well as its release to biological fluids, is associated with poor prognosis and that the determination of C4d may be of value for the diagnosis of lung cancer. We have recently shown that C5aR1 expression is associated with poor prognosis and the metastatic potential of lung cancer cells.

Based on these later studies, and others from seperate groups, we believe that C5a/C5aR1 represent a novel therapeutic target for cancer. More specifically, we propose that C5a/C5aR1 blockade may enhance the antitumor activity of immunotherapies based on PD-1/PDL-1 inhibition. In fact, in pre-clinical *in vivo* models we have demonstrated that the

simultaneous blockade of C5a/C5aR1 and PD-1/PD-L1 markedly reduces tumor growth and metastasis, and leads to prolonged survival. This effect is accompanied by a negative association between the frequency of CD8 T cells and myeloid-derived suppressor cells within the tumors, which may result in a more complete reversal of CD8 T-cell exhaustion. These studies provide support for the clinical evaluation of anti-PD-1/PD-L1 and anti-C5a/C5aR drugs as a novel combination therapeutic strategy for lung cancer.

During all these years we have been privileged to collaborate with many leading experts in complement (those already mentioned and others such as Santiago Rodriguez de Cordoba, Barbara Rolfe or Trent Woodruff). We are indebted to all of them for their partnership and support. At present, complement has become one of the major research fields in our laboratory, and we wish to keep it that way. So, we look forward to initiating additional collaborations within the complement community.

Contact information:
Ruben Pio
Program in Solid Tumors
Center for Applied Medical Research, University of Navarra, Pamplona, Spain
Email: rpio@unav.es
Webpage: http://cima.unav.edu/en/investigacion/programas-verticales/tumores-solidos-biomarcadores

Complement Research in Paris, at the Cordeliers Research Center

The group of Dr Lubka Roumenina

The group of Dr. Roumenina emerged from the Complement and diseases team of V. Fremeaux-Bacchi. We continue to work in close collaboration with her group and with Dr. MA Dragon-Durey on two prototypical diseases, associated with alternative pathway over-activation – the atypical hemolytic uremic syndrome (aHUS) and C3 glomerulopathy (C3G). We aim to understand complement-mediated diseases, using protein structure-function relationships.

Dr. Roumenina's current research has two main focuses – one on the role of complement in hemolytic diseases, such as aHUS and sickle cell disease (SCD), and another dedicated on studies of complement in context of malignant disease. We develop *in vitro* and *in vivo* models and, thanks to collaborations, we have access to patient cohorts. The work is funded by the French National Research Agency, by different foundations (CARPEM, ARC, SFNDT) and we receive support from industry.

Mechanisms of complement-induced inflammation and endothelial cell damage in hemolytic diseases: In some diseases, complement over-activation coincides with severe hemolysis, as in aHUS or in SCD. Nevertheless, the interaction between complement and hemolysis-derived products, as well as their interplay in the pathophysiology of these diseases is still not well understood. Our objective is to investigate the crosstalk between complement and hemolysis in the induction of inflammation and cell damage. With Dr. M. Frimat, we demonstrated that heme renders endothelial cells susceptible to complement attack. With Dr. N. Merle, we found that this process occurs *in vivo*, using a mouse model of intravascular hemolysis or after administration of exogenous heme. Heme and heme-loaded erythrocyte microvesicles were the hemolysis-derived products triggering complement. With Dr. O. May, we found that the glomerular endothelium was highly susceptible to complement deposits. This is in part due to inefficient binding of Factor H to heme-exposed glomerular endothelial cells and by their inability to up-regulate the heme-degrading enzyme HO-1. Further, Dr. Merle found that complement contributes to hemolysis-mediated renal injury. These discoveries helped us to better understand the pathophysiology and organ injury in aHUS and SCD. The ensemble of these results allowed us to propose a novel concept to explain the variable penetrance and the fact that not every encounter of a triggering event induces an aHUS manifestation. In our model, hemolysis-derived heme represents a secondary hit, amplifying endothelial damage and thrombosis in aHUS.

Analyzing plasma samples and kidney biopsies from SCD patients and model mice with SCD, we found a significant sC5b-9 increase in the circulation and tissue deposits of complement C3 and C5b-9. These results confirmed the previous notions for complement over-activation in this hemolytic disease. The fact that intravascular hemolysis triggers complement activation *in vivo*, and its association with SCD nephropathy, places complement as an underestimated pathogenic factor in SCD.

Complement in tumor progression: The Cordeliers research center is one of the leading institutions in Paris, working on tumor

immunology. Therefore, it offers an excellent environment for research in the emerging field of complement and cancer. To understand how complement is initiated in cancer and how it modulates the tumor microenvironment, we used tumor models in complement-deficient mice as well as human clear cell renal cell carcinoma and non-small cell lung cancer cell lines and primary tumors from different patient cohorts, in close collaboration with Prof. WH Fridman and Prof. I Cremer from our institute. Marie Daugan and Remi Noe found that in situ orchestrated production of C1q by tumor-associated macrophages and C1r, C1s, C4 and C3 by tumor cells, associated with IgG deposits, led to C1 complex assembly and complement activation. Our data identify the classical complement pathway as a novel inflammatory mechanism activated by cooperation between tumor cells and tumor-associated macrophages, highlighting novel therapeutic targets to restore an efficient immune reaction.

These collaborations stimulated our decision to establish the Inflammation, Complement and Cancer Team of the Cordeliers Research Center, joining the efforts of the groups working on complement and diseases and the ones, studying cancer, immune control and escape. We will keep our strong interest to renal diseases and will develop topics on complement and cancer, nourished by this novel environment. We have numerous fruitful partnerships in France, Europe and worldwide and we are always open for new collaborations and visiting scientists.

Contact information:
Dr. Lubka T. Roumenina
Complement in hemolytic diseases and cancer group, PI
Inflammation, complement and cancer team, deputy director
Cordeliers Research Center
15 rue de l'Ecole de Médecine, 75006 Paris, France
lubka.roumenina@crc.jussieu.fr

Prevention of Fatal C3 Glomerulopathy by Recombinant Complement Receptor of the Ig Superfamily

Wang X, Van Lookeren Campagne M, Katschke KJ Jr, Gullipalli D, Miwa T, Ueda Y, Wang Y, Palmer M, Xing G, Song WC

J Am Soc Nephrol. 2018 Aug; 29(8):2053-2059.

C3 glomerulopathy (C3G) describes a rare human kidney disease which presents in multiple forms and with many symptoms. This disease is associated with defective complement regulation in the fluid phase and on glomerular surfaces. Several genetic causes in the form of Factor H, C3 and CFHRs gene mutations have been described. In addition, acquired autoimmunity forms, with autoantibodies to C3 convertase, the C4- and C5 convertase, as well as to Factor H, that have been linked with this group of kidney diseases. As no specific therapy is currently available, there is an urgent need to understand disease pathology further and to develop specific inhibitors.

The group of Wenchao Song from Philadelphia and collaborators have developed a new approach to inhibit complement in a mouse model of C3G. In this recent paper the authors describe the design and the generation of a fusion protein which uses the Fc component to link the complement receptor of the Ig superfamily (CRIg-Fc). This inhibitor was evaluated in a previously described mouse model. Mice with mutations in Properdin and Factor H genes show alternative pathway activation with C3 consumption and these animals develop C3G at an early stage. The new inhibitor CRIg-Fc inhibited the alternative pathway in these animals and reduced the consumption of C3 as well as Factor B and C5. Treatment with this novel inhibitor blocked alternative pathway activation and the survival of the animals was increased. In addition C3 fragment and C9 deposition was reduced in the glomeruli. It will be of interest to develop this novel inhibitor further and to determine if CRIg-Fc is similarly effective in human C3 glomerulopathy patients. Also the long term outcomes are of relevance and to see if the novel inhibitor CRIg-Fc can be used to treat all patients who have all spectral forms of C3G.

Factor H binding proteins protect division septa on encapsulated S. pneumoniae against complement C3b deposition and amplification

Pathak A, Bergstrand J, Sender V, Spelmink L, Aschtgen MS, Muschiol S, Widengren J, Henriques-Normark B

Nat Commun. 2018 Aug; 9(1): 3398.

Many pathogenic microbes aim to survive in a complement competent host, and to this end these microbial pathogens have developed means to evade host complement recognition and attack. Several complement inhibitors have been identified and characterized from a broad panel of pathogens, including fungi, Gram-positive and Gram-negative bacteria as well as multicellular parasites. Most pathogens express multiple complement and immune evasion proteins and the distribution on the surface and the co-ordinated action of the factors is not understood in detail. Streptococcus pneumoniae is a respiratory commensal and pathogen that uses multiple strategies to interfere with, and to block host complement in order to avoid activation of the alternative complement pathway and C3b deposition. The pneumococcal polysaccharide capsule forms a mechanical shield and prevents C3b from covalent binding. In addition, pneumococci use evasion proteins like the choline binding protein PspC to recruit numerous complement and immune regulators such as Factor H, vitronectin, poly IgA-receptor and sIgA. PspC is a highly polymorphic pneumococcal immune evasion protein and the reasons for this polymorphism are not completely understood. In this paper, Birgitta Henriques-Normark and colleagues fuse super resolution microscopy and mutant pneumococcal strains to study PspC localization and function. The authors used an S. pneumonia strain, CC138, that expresses two variants of PspC, which they term PspC1 and PspC2. With their experiments, the authors identify a coordinated spacial effect of the capsule and the two PspC variants for immune evasion. Using precision imaging the authors show that the capsule is differently distributed on the pneumococcal surface and is less abundant at the cell wall septum. In this case, i.e. reduced protection by the pneumococcal capsule, PspC steps in and assists in complement inhibition. At these specific sites, at the division septa, PspC1 is localized and by binding Factor H and vitronectin PspC controls complement activation, amplification and C3b deposition. Thus by combining super resolution imaging with mutant strains and functional analyses the authors show that pneumococcal virulence factors co-ordinate local complement attack. The co-ordinated action by the capsule and the two PspC variants is essential for control of host complement attack. The local co-operation of multiple factors and immune evasion proteins in complement control can explain why S. pneumoniae, as well as the other microbial pathogens, express multiple complement and immune evasion proteins. This aspect is relevant for selection of candidate proteins for vaccine development.

1st Complement Society of the Youth (CoSY) Meeting Report

The first meeting of the "**Co**mplement **S**ociety of the **Y**outh" (CoSY) was held from 25th – 27th May 2018 at the University Hospital in Regensburg, Germany. The workshop was an interactive forum for young scientists working in different areas of complement research. Here, we review the proceedings at this meeting and highlight the issues raised during discussions on the future of complement research in health and disease.

Held in the historic, Bavarian surroundings of Regensburg, this meeting provided a unique opportunity for a small group of early-career scientists from five different European countries to communicate their work and discuss the future direction of various projects in complement research. The meeting commenced on Friday evening with dinner in a cosy Bavarian surrounding at the "Regensburger Dult" supported by Merck/ Millipore (picture).

The following day featured a total of nine talks in the seminar room of the Institute for Experimental Ophthalmology (Regensburg, Germany). Mariana Gaya da Costa (University of Groningen, Netherlands) began on Saturday morning with "The relation between the complement system and cardiovascular disease in hemodialysis patients", discussing an increased risk for cardiovascular events as a result of dialysis in renal diseases. Ex vivo blood analysis and experimental data from an in vitro model revealed an association of C3d/C3-ratio, properdin and MBL levels with long-term complications of dialysis and suggested complement inhibition as an improvement for dialysis protocols.

The second stand-out talk was delivered by Caroline Hennig (University of Lübeck, Germany) presenting "C5aR2-regulated NK cell function". She showed that MAP kinase p38 phosphorylation and activation is involved in C5aR2 signalling in murine natural killer cells, which exclusively express C5aR2 without the presence of C5aR1. Caroline Hennig was honored for her excellent presentation by all participants with the CoSY-presentation award, sponsored by Hycult.

Later on, we heard Felix Poppelaars (University of Groningen, Netherlands) with "The complement system in kidney transplantation, the donor's perspective". We learnt about increased complement activity in donor grafts of brain dead animals. Complement

activation in these donor organs depended on C3, C4, properdin and C5aR1 activity. He suggested that kidney function could be improved using C1-Inhibitor.

Before lunch, Lorenz Jenny (University of Bern, Switzerland) presented his studies on "The complement system enhances clot formation in a microvascular whole blood flow model". Dr. Jenny described a microvascular flow model as a tool to examine the role of the complement system in diabetic vascular complications. The close connection between the complement pathway and the clotting system was promoted in his talk.

During the following lunch seminar, Loek Willems from Hycult (Uden, Netherlands) shed light on to various aspects of collaborations between academia and industry. Especially for young scientists, this perspective opened up new future opportunities for complement research.

Agustín Tortajada (University of Madrid, Spain) and Nicole Schäfer (University of Regensburg, Germany) started the afternoon session, discussing the role of the Factor H-related protein family and associated mutations in the development of different diseases. Dr. Tortajada highlighted in his talk "Factor H-related proteins: lessons from naturally occurring mutations", the importance of FH and FHR competition on complement activity. We learnt from genotype-phenotype correlations, that the local self-surface regulation of FHR/FH-ratio is different in multiple tissues. Therefore, e.g. FHR-3 is disease promoting in age-related macular degeneration, but not in hemolytic-uremic syndrome. Quite in line with this, Nicole Schäfer presented "FHR-3 - the evil agent in the human retina", a novel function of FHR-3 on retinal pigmented epithelium (RPE) cells, which maintain the blood-retinal barrier in the eye. She showed a pro-inflammatory effect of FHR-3 on RPE cells, including a cell-associated cleavage of autocrine C3 in C3a and C3b.

Next, Xavier Dervillez (University Luxembourg, Luxembourg) introduced "A novel immunotherapeutic approach using complement-mediated destructive tumor-cell targeting", an innovative therapeutic strategy for tumor cells and bacteria. Immunoconjugates consisting of small antibody fragments, a multimerisation domain and FHR-4 (CoMiX) prevent FH from binding and promote complement-dependent cytotoxicity on target cells.

The COSY meeting was completed by a presentation from Diana Pauly (University Hospital Regensburg, Germany) introducing "The eye - a perfect model for complement research". She presented the eye as an immune-privileged tissue, which produces, regulates and activates the complement system locally in stress situations and during aging. This independence from the systemic complement system, the easy accessibility and two controls in one individual makes the eye a unique organ for complement modulation and research.

In a subsequent dinner at a Mexican restaurant - in preparation for the ICW2018 in Santa Fe - we discussed the results from this productive scientific day and debated about possible (critical!) funding opportunities for collaboration projects.

The first meeting of the "Complement Society of the Youth" (CoSY) was completed on Sunday morning with a boat trip on the river Danube to the Abbey Weltenburg, which was supported by Hycult. During the boat tour and the farewell lunch we summarized the meeting.

With the 1st CoSY meeting, we got together the young and future generation of complementologists. We shared new ideas, discussed research and exchanged lab protocols. In this cosy atmosphere, we set up new collaborations, which will promote complement research in different areas.

All participants voted unanimously for a 2nd CoSY meeting in 2019. We are looking forward to it! We thank the participants and our sponsors for this fantastic meeting.

The organizers
Nicole Schäfer and Diana Pauly (University Hospital Regensburg, Germany)

Participants of the first meeting of the "Complement Society of the Youth" (CoSY) 2018
From left: Maria Reichenthaler, Caroline Hennig, Loek Willems, Nicole Schäfer, Agustín Tortajada, Mariana Gaya da Costa, Lorenz Jenny, Xavier Dervillez, Felix Poppelaars, Diana Pauly, Dominik Rogalski.

You are invited to attend the 27th International Complement Society meeting being held in historic Santa Fe, New Mexico…UNESCO World Heritage City of Crafts. Santa Fe is a magical city with legendary history and culture that will fascinate and inspire you. The art galleries and diverse visual arts span ancient traditional art to the most contemporary, making Santa Fe one of the largest and most important art markets in the country.

The meeting will be held at the Santa Fe Convention Center, located in the historic center and one block from the hotels and the cities best galleries, museums, and handicraft stores. Fly into either Santa Fe Airport (SAN) or for more flight choices Albuquerque Airport (ABQ), and take a scenic 50-minute shuttle to Santa Fe.

The Program includes an exciting mix of formal presentations and panel discussions on diverse topics of scientific and educational interest to the Complement community. We look forward to seeing in historic Santa Fe!

Local Organizing Committee:

Andrea Tenner, PhD (Chair)
John Atkinson, MD
Nirmal Banda, PhD
Ashley Frazer-Abel, PhD
Joshua Thurman, MD
Rick Wetsel, PhD

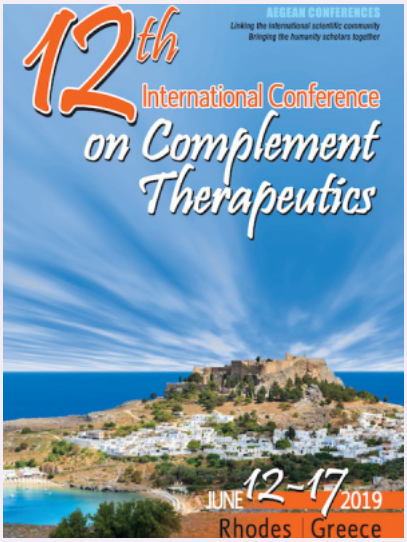
Plenary Speakers:

Michael Diamond, MD, PhD
Washington Univ School of Medicine, St Louis, MO

Eric Huang, MD, PhD
Univ of California School of Medicine, San Francisco, CA

Marina Noris, PhD
Mario Negril Institute for Pharmacological Research, Bergamo, Italy

12th International Conference on Complement Therapeutics



The field of complement-targeted drug discovery has experienced an profound transformation during the past decade. With the first complement-specific drugs on the market, clinical experience is gained and novel indications are being explored. At the same time, efforts in both academic and pharmaceutical research have produced new innovative therapeutic concept that interfere at different levels of the complement cascade; many of these candidates are currently undergoing clinical evaluation. Finally, genetic and molecular studies continue to reveal contributions of complement in both orphan and highly prevalent diseases. Apart from offering new hope for patients suffering from such diseases, the study of complement pathways, mutations, and deficiencies also teaches us important lessons about the role of complement in health and disease and allows us to refine our models and tools for applied and basic research. This conference aims to bring together academic and industry scientists and clinical development experts who are focused on contemporary and emerging aspects of complement-mediated disease pathogenesis and the development of therapeutics that modulate this system in a beneficial manner.

Topics discussed during the conference include: Molecular mechanisms and targets in complement-related diseases; Novel inhibitors & pipeline compounds; Hematological disorders; Organ & cell transplantation, I/R injury and chronic rejection; Kidney diseases; Neurological & ocular diseases; Acute and chronic inflammatory disorders; Infectious diseases & sepsis; Cancer; Informative complement biomarkers in therapeutic development; Novel and unexpected indications.

Organizing Committee:
John Lambris, PhD
Dimitrios Mastellos, PhD
Edimara Reis, PhD
Daniel Ricklin, PhD
Antonio Risitano, MD
Richard J.H. Smith, MD

12th - 17th June 2019
Sheraton Rhodes Hotel

If you would like to promote your Complement-focused meeting in FoC, please contact Trent Woodruff (t.woodruff@uq.edu.au) or Michael Holers (Michael.Holers@ucdenver.edu).

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