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Dear Readers,

Welcome to the March 2018 issue of '*Focus on Complement*'.
This 49th issue of FoC contains the following:

- Claire Harris reviews publications for the **News Flash**, which identify four distinct pathogenic groups of C3G/IC-MPGN patients, as well as identifying evidence for a differential role for natural IgM and the alternative pathway in kidney ischemia/reperfusion injury.
- **The Complement research teams around the world** series featuring Drs. Clark, Bishop and Day, in Manchester, UK, as well as Dr. Thielens, in Grenoble, France.
- 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. Claire Harris, UK)

News Flash 1:

[Cluster Analysis Identifies Distinct Pathogenetic Patterns in C3 Glomerulopathies/ Immune Complex-Mediated Membranoproliferative GN](#). Iatropoulos P, Daina E, Curreri M, Piras R, Valoti E, Mele C, Bresin E, Gamba S, Alberti M, Breno M, Perna A, Bettoni S1, Sabadini E, Murer L, Vivarelli M, Noris M, Remuzzi G. *J Am Soc Nephrol*. 2018; 29(1):283-294.

In 2010, the name C3 glomerulopathy (C3G) was proposed to describe a group of renal diseases with dominant glomerular C3 deposition and little or no Ig deposition (Pickering et al. C3 glomerulopathy: consensus report. *Kidney Int*. 2013 Dec;84(6):1079-89). C3G was separate from immune complex-associated membranoproliferative GN (IC-MPGN), which was characterised by significant glomerular Ig deposition. C3G was further divided by morphologic appearance into dense deposit disease (DDD), characterised by highly electron dense intramembranous deposits, and C3 GN (C3 glomerulonephritis). This classification was proposed to distinguish those pathologies caused by dysregulation of the alternative pathway of complement, whether that be from a genetic or acquired basis, from those where classical pathway was a driver of pathology. However, the distinction between these disease groups is not always straightforward as there is overlap between DDD and C3GN based on morphological changes, and there are genetic and acquired factors evident in some cases of IC-MPGN. In this paper, the authors use a mathematical approach to classify patients into disease sub-groups. A panel of 34 variables comprising clinical parameters, biopsy data, complement protein levels, genetics and the presence of autoantibodies was used for cluster analysis. This data-driven method was used to separate 173 patients with C3G/IC-MPGN into groups where disease could be characterised by specific pathophysiologic mechanisms.

Four groups were identified with relatively homogeneous phenotypes. Patients without alternative pathway abnormalities formed one group (cluster 4); the other three groups could be distinguished by underlying disease mechanism. Clusters 1 and 2 included patients with high prevalence of sub-endothelial deposits, high plasma SC5b-9, likely pathogenic variants (LPV) in complement genes, C3 nephritic factors, and low serum C3. Cluster 1 had a lower mean age of onset whereas cluster 2 included patients with higher levels of Ig and C1q staining compared to cluster 1, and more frequent occurrence of mesangial deposits. Patients in cluster 3 also had high prevalence of LPVs/C3 nephritic factor and low serum C3, but SC5b-9 was lower, indicating that C3 convertase activity predominated over C5 convertase activity. This cluster was characterised by higher prevalence of intramembranous dense deposits. Cluster 4 included patients with low prevalence of LPVs/C3 nephritic factor, higher serum C3 and later age of onset. When related to original disease classification, clusters 1 and 4 were composed prevalently of patients with C3GN and patients with IC-MPGN. Clusters 2 included mostly patients with IC-MPGN (94%) and cluster 3 included patients with DDD (64%). Interestingly, 21 of 25 patients with DDD and all three patients with IC-MPGN with intramembranous highly electron-dense deposits were included in cluster 3.

Critically, the four clusters demonstrate prognostic significance, with patients in cluster 4 having higher risk of end stage renal failure. Based on features available at onset, the authors propose a three-step algorithm to assign patients with C3G/IC-MPGN to specific clusters. These newly identified clusters may also be useful for better defining the molecular mechanisms underlying C3G/IC-MPGN and for predicting response to anti-complement therapies.

News Flash 2:

[Complement factor H protects mice from ischemic acute kidney injury but is not critical for controlling complement activation by glomerular IgM.](#)

Goetz L, Laskowski J, Renner B, Pickering MC, Kulik L, Klawitter J, Stites E, Christians U, van der Vlag J, Ravichandran K, Holers VM, Thurman JM. *Eur J Immunol.* 2018; Feb 1. doi: 10.1002/eji.201747240

Mutations in CFH are strongly associated with renal diseases such as aHUS and C3G, suggesting that factor H plays a critical role in protection of the kidney from complement-mediated damage. The glomerular basement membrane lacks endogenous regulators and depends on factor H to dampen the amplification loop. In mouse models of renal ischemia/reperfusion (I/R) injury, natural IgM can activate the classical pathway, however, factor H is sufficient to control downstream amplification. The investigators hypothesised that glomerular IgM would cause renal injury in animals post I/R if they were partially deficient in factor H. To investigate this hypothesis, the authors compared I/R injury in wild type (WT) mice to those with reduced factor H levels (*fH*^{+/-}) with or without soluble IgM (*sIgM*^{-/-}; mutation in the μ _s exon of the IgM gene).

Following kidney I/R, tubular damage was evident in wild type (WT) mice and to a more severe level in mice with reduced levels of factor H, however, glomerulus morphology appeared normal in both cases. IHC illustrated that C3 was deposited throughout the tubulointerstitium in both mouse strains, but not the glomeruli, and that membrane attack complex was more prominent in the tubulointerstitium of *fH*^{+/-} mice. IgM was detected in the glomeruli where it colocalised with C4, but not C3, indicating control of the amplification loop at this site. These data indicated that following I/R, even low levels of factor H were sufficient to protect the glomeruli from C3 deposition. Little difference was seen in post-ischemic renal injury in *fH*^{+/-} mice, with or without soluble IgM, and also when antibody C2, a monoclonal IgM which binds multiple glomerular cells *in vivo*, was injected. Glomerular C3 was absent in all cases.

The authors conclude that activation of the alternative pathway is an important cause of kidney injury after I/R, particularly with regards to complement-targeting of the tubular epithelial cells, but IgM deposited in the glomeruli does not play a major role in tissue damage, even though it activates the classical pathway. This is in contrast to the role of natural IgM in tissue damage after ischemia of other organs and in chronic models of glomerular disease. The authors emphasise the importance of understanding endogenous mechanisms for controlling complement activation on host cells, and the need to identify molecular events leading to complement dysregulation in many different glomerular diseases. Greater understanding of these processes is essential to validate indications for the array of anti-complement therapies progressing through the clinic, and to develop new therapies capable of blocking or reversing these pathogenic processes.

COMPLEMENT TEAMS AROUND THE WORLD

Complement Research in Manchester, UK: Drs. Clark, Bishop and Day's Research Groups

Dr. Simon Clark, Prof. Paul Bishop and Prof. Tony Day are based in the Faculty of Biology, Medicine and Health at the University of Manchester, UK, and are studying the regulation of complement within the extracellular matrix at various sites around the body. Over the last 12 years our work has focused on complement regulation within the eye and its association with age-related macular degeneration (AMD), the most common form of blindness in the developed world. Together with the lab of Dr. Richard Unwin, we form the AMD Research Group, which is investigating the molecular and cellular mechanisms underlying AMD and developing novel therapeutics to treat 'dry' AMD. A key resource for our research is the Manchester Eye Tissue Repository, a biobank we set up in 2014 (in conjunction with the Manchester Royal Eye Hospital) that contains eye tissue from over 1,000 donors, generously donated for use in scientific research. We genotype and phenotype the tissues and use them to determine how complement drives AMD pathogenesis. For example, in previous studies we have identified Factor H-like Protein-1 (FHL-1) as the main complement regulator protecting the Bruch's membrane and choriocapillaris in the eye (where AMD develops) and demonstrated that the outer blood-retinal barrier separates complement regulation into two distinct compartments.

In order to apply a multi-disciplinary approach to understanding complement-mediated disease, especially AMD, the sixteen-strong team covers a broad range of disciplines, allowing a variety of techniques to be applied including genomics, proteomics, transcriptomics, cellular models, protein and sugar biochemistry, bioimaging and translational medicine. The genomics team, along with our collaborator Prof. Graeme Black, has identified novel complement mutations that drive early-onset AMD and has established an early-onset macular degeneration clinic at Manchester Royal Eye Hospital to care for these patients. Our proteomics team is developing a targeted mass-spectrometry assay capable of measuring all soluble complement regulators simultaneously in human samples and has recently undertaken a proteomic study of macular tissue from donors with distinct genotypes to elucidate differences in protein levels associated with particular risk modifying genetic variants. The cell model team has identified novel interactions between the retinal pigment epithelial cells and the underlying Bruch's membrane that may alter the cells' reaction to complement-mediated inflammatory stimuli. Finally, the translational team is developing novel therapeutic biopharmaceuticals for the treatment of AMD and is aiming to progress these into clinical trials.



We have been privileged to have collaborated with numerous labs around the world and wish to extend a welcoming environment for any visiting students and researchers, who would like opportunities to learn more about complement biology in the eye.

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Complement Research in Grenoble, France: The team of Dr. Nicole Thielens

Complement research in Grenoble was initiated in the mid-1970s by Maurice Colomb and Gerard Arlaud who were interested in the molecular mechanisms underlying the activation of the classical complement pathway. Following a PhD thesis focused on the classical C3 convertase under M. Colomb's supervision and a post-doc on C9 in A. Esser's Laboratory (Gainesville, Florida), Nicole Thielens joined the team of Gerard Arlaud in 1988 and they moved to the newly opened Institut de Biologie Structurale (IBS) in 1992. The team including Monique Lacroix, Isabelle Bally and a PhD student, Veronique Rossi, pioneered a dissection strategy of the C1 complex aiming at deciphering its mechanism of activation, through structural analyses of fragments involved in the sensing and proteolytic properties of the complex and in its assembly. This was made possible thanks to a tight collaboration with Christine Gaboriaud from a crystallography laboratory of the IBS. Similar approaches were later applied to the initiating complexes of the lectin complement pathway, with the participation of Evelyne Gout and in collaboration with the Danish team of Jens Jensenius and Steffen Thiel in Aarhus, and the Japanese team of Teizo Fujita in Fukushima. A novel cellular approach focused on the role of C1q and calreticulin in apoptotic cells recognition and phagocytosis was initiated by Philippe Frachet and Pascale Tacnet in 2003.

The IBS group currently headed by Nicole Thielens and entitled "Immune Response to Pathogens and Altered Self (IRPAS)" was created in 2011 and, in addition to master and PhD students, gathers 10 permanent members with expertise in biochemistry (I. Bally, A. Chouquet, V. Rossi, N. Thielens, C. Wicker-Planquart), structural biology (C. Gaboriaud, J.-B. Reiser) and cell biology (P. Frachet, J.-P. Kleman, P. Tacnet). The IRPAS group is located, since 2013, in the European Photon Neutron (EPN) science campus of Grenoble. Besides the IBS, the EPN campus hosts the European Synchrotron Radiation Facility (ESRF), the Institut Laue Langevin (ILL), and the EMBL (European Molecular Biology Laboratory), which provide access to state of the art equipment to analyze biological systems at different scales of resolution. Members of the IRPAS group operate the surface plasmon resonance (SPR) and cell imaging IBS platforms.

The first research axis of the IRPAS group aims at deciphering the role of C1q in apoptotic cell elimination (efferocytosis) by analyzing the interactions between apoptotic cells opsonized by C1q and receptors on different types of phagocytes and investigating the consequences of the capture of these cells on immune tolerance. We use a multidisciplinary integrated approach combining (i) protein engineering, biochemistry and structural biology, (ii) functional imaging of the C1q-receptor complexes at the efferocytic synapse using super resolution fluorescence microscopy, and (iii) cell biology techniques.

The fact that several of our proteins of interest are implicated in different pathologies opens the way to collaborative research, aimed at deciphering the molecular mechanisms of pathogenesis. One example is the study of the role of anti-ficolin autoantibodies in the pathogenesis of lupus nephritis in collaboration with Chantal Dumestre-Perard (Grenoble Alpes University Hospital), aiming at understanding the possible interference of these autoantibodies with the function of ficolins. A second example is linked to the recent discovery of an association of a rare disorder, the periodontal Ehlers-Danlos syndrome (pEDS) with heterozygous mutations in the *C1R* and *C1S* genes. In collaboration with the Austrian Medical University of Innsbruck (Johannes Zschocke, Ines Kapferer-Seebacher, Heribert Stoiber), our objective is to analyze the structural and functional impact of these mutations in the C1r and C1s proteins, with a view to obtain new clues about the molecular mechanisms responsible for pEDS and relate them to the clinical consequences of the disease.

The Institut de Biologie Structurale (IBS) is a joint research center of the CEA, the CNRS and the University Grenoble Alpes. The research of the IRPAS group is mainly funded by grants from the French National Research Agency (ANR).



IRPAS group

Left row, top-down:

Jean-Philippe Kleman
Christine Gaboriaud
Guillaume Fout
Nicole Thielens
Anne Chouquet
Isabelle Bally

Right row, top-down:

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MEETING ANNOUNCEMENTS



Please plan to attend the
[International Complement Society Guest Symposium](#)

New Discoveries in Complement: Impact on Health and Disease

Sunday, May 06, 2018

12:30 PM - 2:30 PM

American Association of Immunologists 2018 meeting
Austin Convention Center. Austin, TX.

Chairs:

Viviana P. Ferreira, University of Toledo College of Medicine

Rick A. Wetsel, University of Texas Health Science Center at Houston

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*

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

MEETING ANNOUNCEMENTS

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

The field of complement-targeted drug discovery has experienced a profound transformation during the past decade. 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.

Organizing Committee:

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 Michael Holers, MD
 Daniel Ricklin, Ph.D.
 Antonio Risitano, MD
 Richard J.H. Smith, MD



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DATE CLAIMER: SEPT 16-20, 2018; 27th INTERNATIONAL COMPLEMENT WORKSHOP




WEBSITE: <https://www.regonline.com/builder/site/Default.aspx?EventID=1858584>

Organizing Committee:

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


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