Focus on Complement

December 1, 2013
Issue 32

International Complement Society

European Complement Network

Dear members of the complement and immunology community,

The December 2013 issue of 'Focus on Complement', the 32nd issue contains:

- **A seasonal** greeting.
- **Newsflash** that presents three publications of which two clearly connect the anaphylatoxins with retinal repair and one paper that shows how heterodimers between C5aR and C5L2 may transmit intracellular signaling events that are different from those of the single receptors.
- **Complement research teams around the world.** In this issue, we are introducing two research teams working on complement, one from The Netherlands – Dr. Leendert Trouw's team – and a research team from Germany, Dr. Guenther's laboratory.
- **Re-announcement** of the 2014 International Complement Workshop in Rio and the EMCHD 2015 in Uppsala.

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 Zvi Fishelson; Claudia.kemper@kcl.ac.uk, lifish@post.tau.ac.il

Seasonal greetings

On behalf of the editorial board of Focus on Complement, we would like to take this opportunity to 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 will be equally prosperous.

Newsflash I

Complement and Retinal Regeneration

The recent paper by Haynes et al. in Nature Communications 4:2312 (2013), is a reminder of the broad function of the complement system in both immune and in non-immune roles. C3, C3aR, and the alternative pathway components factor B and D were expressed in embryonic day E4 chick eyes. They then demonstrate a role for complement C3a, or a small peptide comprising the C-terminal 21 amino acids (referred to as C3a-p), in retinal regeneration in the chick embryo. Retinal regeneration can proceed through two main modes: 1) through transdifferentiation of retinal pigmented epithelium (RPE) cells; or 2) via proliferation of resident progenitors located in the ciliary margin. While C3a-p could induce some transdifferentiation of the RPE, it appeared that it primarily induced retinal regeneration via the latter mechanism. Interestingly, basic fibroblast growth factor (Fgf2) showed significantly more transdifferentiation of the RPE than C3a-p, suggesting that each may regenerate the retina through distinct mechanisms. This idea was supported further by the observation that inhibition of Fgf2 did not disrupt C3a-induced regeneration, and vice-versa with inhibition of C3aR. Importantly, the authors show that each layer of the retina can be restored in the regenerated retina, suggesting that C3a-p can regenerate a fully functional retina. Through a series of elegant inhibitor studies, Haynes et al. show that C3a acts via its receptor C3aR to induce phosphorylation of STAT3 via MAPK. This leads to transcriptional changes in inflammatory mediators (IL-6, IL-8 and TNFa), as well as genes involved in stem cell maintenance and progenitor cell development (Wnt2b, Six3 and Sox2).

This work builds on previous studies demonstrating a role for the complement system in regeneration of mouse liver, and newt lens and limb. This suggests a highly conserved role for complement in regeneration throughout evolution. A common theme in these studies is the upregulation of complement components post injury. This paper by Haynes et al. also builds on observations by Yu et al. (2012, IOVS 53:7684-92) that complement has an important role in retinal maintenance in postnatal development. These studies are, however, complicated by the plethora of data showing that SNPs in C3, FB and FH can lead to age-related macular degeneration. Clearly, the biology of C3a and C3aR are very complex and further knowledge on how these proteins act in both embryonic and postnatal development will hopefully teach us about the rules of engagement for these key players.

Tracy Haynes, Agustin Luz-Madrigal, Edimara S. Reis, Nancy P. Echeverri Ruiz, Erika Grajales-Esquivel, Apostolia Tzekou, Panagiotis A. Tsonis, John D. Lambris & Katia Del Rio-Tsonis (2013). Complement anaphylatoxin C3a is a potent inducer of embryonic chick retina regeneration. Nature Communications 4:2312.

Minzhong Yu, Weilin Zou, Neal S. Peachey, Thomas M. McIntyre, and Jinbo Liu. (2012) A Novel Role of Complement in Retinal Degeneration. Investigative Ophthalmology & Visual Science, 53(12):7684-7692.

Reporters: Dr Angela Jeanes and Dr Trent Woodruff

Newsflash II

C5aR and C5L2 heterodimers

New evidence is shedding light into putative regulatory roles for C5L2 through heteromer formation with C5aR. In this study Crocker et al., (Immunol Cell Biol. 2013 doi: 10.1038/icb.2013.48) use C5aR-Rluc8 and C5L2-Venus constructs and bioluminescent resonance energy transfer (BRET) techniques, to demonstrate that C5aR and C5L2 form constitutive heteromers in transfected HEK 293cells. Administration of C5a dose-dependently induced further heteromer formation, but only at doses higher than normally required to induce C5aR signalling (i.e. >100nM). Interestingly, at similar concentrations to C5a, C5a des Arg did not induce heteromers. Heteromer formation was also inhibited in the presence of a selective C5aR antagonist (PMX53), demonstrating the absolute requirement for C5a-C5aR engagement in order to associate with C5L2. In further studies, native human monocyte-derived macrophages bearing both receptors challenged with either C5a or C5a des Arg, induced internalization and colocalization of C5aR with C5L2 in the cytoplasm. Finally, using these same cells, the authors demonstrated that both C5a and C5a des Arg dose-dependently potentiated the release of anti-inflammatory cytokine IL-10. However, despite showing equal IL-10 generation at low (1nM) doses, at doses shown to cause heteromer formation (>100nM), significantly greater amounts of IL-10 were generated by C5a, compared to C5a des Arg. The authors speculate that this difference in cytokine generation at high C5a concentrations may be due to heteromer formation by C5a, which is absent with C5a des Arg. These studies open up new insights into the interplay between C5a and C5aR/C5L2 downstream functions, although the relevance of this work to whole animal physiology is yet to be determined.

Croker DE, Halai R, Fairlie DP, Cooper MA. (2013). C5a, but not C5a-des Arg, induces upregulation of heteromer formation between complement C5a receptors C5aR and C5L2. Immunol Cell Biol. doi: 10.1038/icb.2013.48.

Reporters: Dr Mike Wu and Dr Trent Woodruff

Focus on Complement Research Teams

Complement in The Netherlands: Dr. Leendert Trouw's Team

My small research team in the Department of Rheumatology at the Leiden University Medical Center in Leiden, The Netherlands, focuses on complement in autoimmunity and on autoantibodies.

My interest for complement was initiated during my PhD training with Prof. Moh Daha at the Department of Nephrology also in Leiden. My work on anti-C1q autoantibodies paved the way for a career on both complement and autoantibodies. Next my post-doc training in Malmo Sweden with Prof. Anna Blom on complement regulators on apoptotic and necrotic cells was a decisive moment to work on complement in autoimmunity. These two great mentors provided massive inspiration to pursue a scientific career in complement and autoimmunity. Upon moving back to the Netherlands I joined the Department of Rheumatology (Prof. Huizinga and Prof. Toes) where I was given the opportunity to combine my expertise and drive for complement research in the context of the autoimmune diseases Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE). Initially working on this topic myself together with technician Nivine Levarht and PhD student Nina Daha we pursued research lines of candidate gene studies on the risk to develop RA. The high hopes of C1q being a major risk factor for RA were somewhat downscaled by the release of GWAS data. In addition the expected effect of the famous Factor H SNPs (shown to have major impact on other AP driven diseases) on RA, as an AP driven disease, turned out to be completely absent. Later studies on the complement activating potential of anti-citrullinated protein antibodies (ACPA), the strongest serological marker for diagnosis/prognosis in RA, were much more fruitful and revealed that these human autoantibodies activate both the CP and the AP. Next a new PhD student was recruited to the team, Parawee Suwannalai from Thailand who worked on the avidity of this ACPA response. She observed that the ACPA avidity is in general low and that the limited avidity maturation all takes place before onset of symptoms. Interestingly the severity of joint destruction in RA was associated with the lowest avidity and these low-avidity antibodies were shown to have the highest capacity to activate complement. The molecular make-up, epitope specificity and quantity of these ACPA have next been studied in detail by PhD student Annemiek Willemze.

Current research activities are focussed around autoantibodies as biomarkers and on their properties to activate complement and on the role of C1q in autoimmunity. Regarding the biomarkers for RA, we made an interesting discovery that in RA there is, in addition, to an autoantibody response against citrullinated proteins, also an autoantibody response against carbamylated proteins. PhD student Jing Shi characterized these antibodies and showed their prognostic value in several patient cohorts. PhD student Marije Verheul is now taking over this research line and is studying the molecular details of this antibody response as well as its effector mechanisms such as complement activation. Regarding the role of C1q in autoimmunity PhD student Rosanne van Schaarenburg is studying C1q deficiency. We have characterized two new cases of C1q deficiency in Leiden and she recently conducted a survey on life-expectancy and quality of life in C1q deficient individuals.

The opportunity to co-organize the European complement meeting in Leiden, EMCHD2011, was a great way of interacting with even more scientist active in complement research. Now serving as a board member of the ECN I hope to further contribute to the development of complement research and to stimulate (inter)national collaboration.

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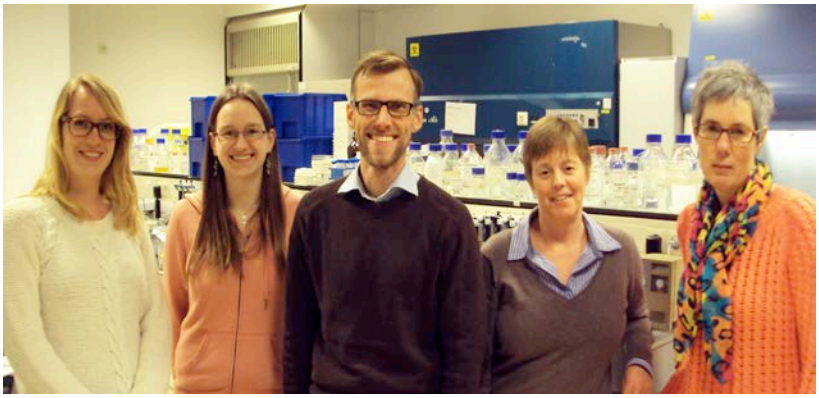
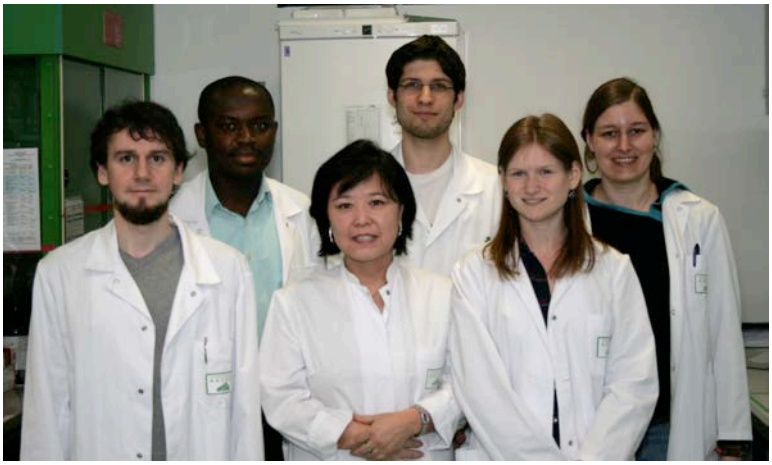


Complement in Germany: Dr. Erdenechimeg Guenther's Team

The Guenther's "Complement research group" is one of the groups in the Institute of Molecular Cardiovascular Research (IMCAR) located at the RWTH Aachen University Hospital in Germany. After targeting complement anaphylatoxin C5a receptor (C5aR) in neointima formation in arterial injury in atherosclerosis-prone mice, a contribution that was published in Circulation (Shagdarsuren et al., 2010, 122:1026-36; note: Shagdarsuren is Guenther's publishing name), the Guenther group has since then been pursuing the complement system in cardiovascular diseases like atherosclerosis, vascular injury and myocardial infarction. We have recently identified the presence of C5a receptor like-2 (C5L2) in different stages of human atherosclerotic lesions and this second receptor of the complement anaphylatoxin C5a seems to be overexpressed upon atheroprogression both at protein and mRNA levels. To address the question as to whether C5a receptors are ''drivers'' or mere ''passengers'' in atherogenesis, we are currently analyzing atherosclerotic plaques and C5aR/C5L2-mediated mechanisms affecting atherosclerotic lesion formation and restenosis in C5aR and C5L2 deficient hyperlipidemic mice. We are also using myocardial infarction models to address the functional role of these two receptors in this disease. Whereas C5aR deficient mice show reduced myocardial infarct size and significantly improved cardiac parameters, C5L2 deficiency does not affect infarct size, an outcome that reflects the expression levels of these two receptors in the mouse heart. Cooperation between the complement and FcγR effector pathways has been described where C5aR modulates FcγR in inflammatory diseases. As atherosclerosis is a known chronic inflammatory disease, we are pursing this cooperation mechanistically *in vitro* under proatherogenic conditions and also in atherosclerosis models *in vivo* employing FcγRIII deficient hypercholesterolemic mice. Being situated in a University Hospital means we have the luxury of collaborating with other clinical departments. In collaboration with the Nephrology and Pathology departments, the cardio-renal interaction during myocardial infarction, with or without unilateral nephrectomy, in spontaneous and diet-induced atherogenic mouse models, is being pursued.



Front row (L to R): Jaco Selle, MSc; Erdenechimeg Guenther (MD), Ass. Prof.; Tanja Vajen, BSc
Back row (L to R): Yaw Asare, PhD; Nico Joeres, Cand. Med; Janine Kohncke, MSc
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And Other Business

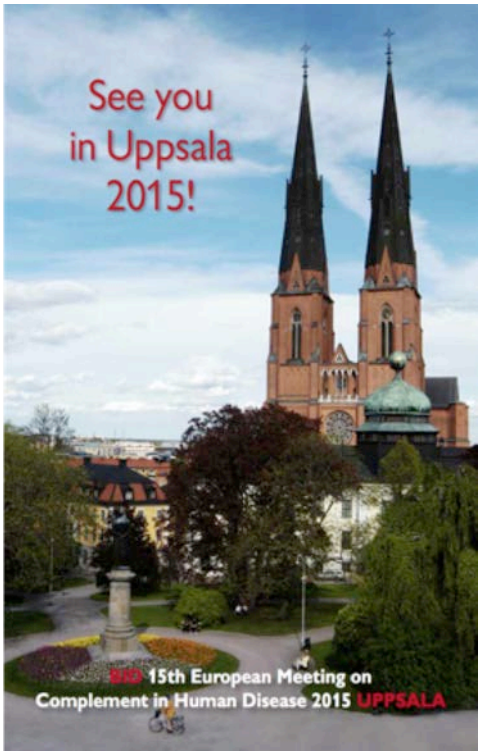
We are happy to announce that the website of the XXV International Complement Workshop (ICW) scheduled for September 14th to 18th 2014 in Rio de Janeiro 'is live' (www.ICWRIO2014.com**).**

Please be sure to plan this meeting early – particularly since there are visa application requirements for several countries for travels to Brazil!

XXV INTERNATIONAL COMPLEMENT WORKSHOP

SEPTEMBER 14 - 18
WINDSOR ATLÂNTICA HOTEL
RIO DE JANEIRO - BRAZIL

We are equally pleased to announce that the venue of the EMCHD 2015 will be in Uppsala, Sweden (June/July 2015).



Complement Reagent Resources