Focus on Complement

June 1, 2017
Issue 46

International Complement Society

European Complement Network

Dear Readers,

Welcome to the June 2017 issue of 'Focus on Complement'. This 46th issue of FoC contains:

- A **review of publications** describing evidence for a key role for complement activation in the developing mammalian brain, and the first identification of properdin in amphioxus. Contributed by Prof. Nobutaka Wakamiya.
- The **Complement research teams around the world** series featuring Prof Miki Nakao in Japan, as well as Dr Barbara Rolfe, in Australia
- Two 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).

NEWS FLASH (reported by Prof. Nobutaka Wakamiya, Japan)

News Flash 1:

Developmental activities of the complement pathway in migrating neurons. *Gorelik A, Sapir T, Haffner-Krausz R, Oleander T, Woodruff TM, Reiner O.* ***Nat Commun. 2017*** *May; 8:15096.* ***https://www.nature.com/articles/ncomms15096***

In recent years the notion that malfunctioning of the immune system may result in developmental brain diseases has emerged. It is reported that an activity of the classical complement pathway in the postnatal brain has been implicated in developmental pruning of synapse refinement of the mouse visual system. In addition, it has been shown that the complement fragment C3a and its receptor are important for collective cell migration of neural crest cells. However, the role of the complement system in the embryonic brain remains poorly understood. Neuronal migration is impaired in cases of intellectual disabilities, autism and schizophrenia, and the authors postulate that the complement pathway might be involved in regulation of migrating neurons. In this paper, the authors demonstrated that the complement cascade, particularly a lectin pathway, was functionally important in migrating neurons of the developing cortex in mice. Results showed that MASP1, MASP2 and C3, were expressed in the developing cortex in mice, and knockout or knockdown of *C3*, *Masp1* and *Masp2* genes caused the impairment of neuronal migration. Molecular mimics of C3 cleavage products rescued the migration defects that were seen following knockdown of *C3* or *Masp2*. Pharmacological activation of C3aR and C5aR rescued the phenotype of *Masp2* and *C3* knockdown, as well as *C3* knockout. The authors propose that complement activity is required for neuronal migrating progression, and thus a beneficial intervention may be possible.

News Flash 2:

Identification and characterization of properdin in amphioxus: Implications for a functional alternative complement pathway in the basal chordate. *Gao Z, Ma Z, Qu B, Jiao D, Zhang S.* ***Fish Shellfish Immunol. 2017*** *Jun; 65:1-8.* ***http://www.sciencedirect.com/science/article/pii/S1050464817301808***

Amphioxus, a cephalochordate, representative species of the most basal extant chordate lineage, is an important reference to the origin and evolution if immune system in vertebrates. Previously, the authors have shown that an alternative-like complement pathway operates and C3-like and fB-like proteins exist in amphioxus. However, properdin (factor P, fP), a positive regulator of the alternative pathway, remains elusive in amphioxus to date. In this study, the authors reported the identification and functional characterization of a properdin gene in the amphioxus *Branchiostoma japonicum*, BjfP, representing a typical structure of vertebrate properdins. The results from real-time PCR analysis showed that the BjfP was ubiquitously expressed and its expression was significantly up-regulated following the challenge with bacteria or LPS and LTA. Biochemical analysis indicated that recombinant BjfP (rBjfP) and its truncated proteins could interact with both Gram-negative and positive bacteria as well as LPS, and LTA and could also bind to C3b. The truncated rBjfP proteins could inhibit the binding of rBjfP to C3b, and could significantly suppress the bacteriolytic activity of amphioxus humoral fluid. These results suggest the involvement of BjfP in the alternative pathway in amphioxus. The authors propose that a properdin protein in the amphioxus *B. japonicum* might represent an ancient molecule from which vertebrate properdins evolved.

COMPLEMENT TEAMS AROUND THE WORLD

Comparative Complement Research in Fukuoka, Japan: The team of Prof. Miki Nakao

Complement system has been found in both vertebrates and invertebrates, suggesting its extremely ancient origin. Bony fish, an evolutionarily early vertebrate group, has a well-developed complement system comparable to that of mammals. Our lab studies diverse roles of complement in innate and adaptive immunity of bony fish using three model fish species that belong to Cyprinid family. Zebrafish (*Danio rerio*) is a popular vertebrate model in embryology and genetics. Because of very rich genomic, transcriptomic, and high-density genetic map data, as well as large panel of genetic mutants, zebrafish is effective for *in silico* cloning of immune-related genes and their functional assessment by reverse/forward genetics approaches. Carp (*Cyprinus carpio*) is a robust and edible fresh water fish, which are cultured in Asian countries. Due to its large size (~60-100 cm), carp is useful for cellular and biochemical works. Ginbuna crucian carp (*Carassius auratus langsdorfii*) can exist as triploid (3n=150) clonal lines reproduced by gynogenesis in nature. We have maintained two distinct triploid clones, OB1 and S3N, and its tetraploid hybrid clone, S4N. They are suitable for analyses of cellular adaptive immune responses, such as MHC-restricted T-cell cytotoxicity and the mixed lymphocyte reactions.

One of the most striking features of bony fish complement system is that many of the complement components are present as diversified isotypes encoded by multiple genes. In particular, C3, C4, factor B and C7 genes are multiplied regardless the chromosome polyploidy. Structural and functional diversification among these isotypes is a key to understand evolutionary significance of the multiplication of the complement component genes. In the case of C3, bony fish C3 isotypes are grouped into two major types, His-type and non-His-type defined by the presence and absence of a histidine residue at about 100 residues C-terminal from the thioester site, where the His catalyzes cleavage and binding reaction of the thioester towards hydroxyl-group. His-type C3 is a universal type, whereas non-His-type is found as an optional isotype in limited animal groups including fish. Interestingly, our functional study using carp C3 has suggested that non-His-type C3 plays a versatile role in bony fish, based on its wider binding spectrum against microbial targets. C4 is also duplicated to generate His- and non-His-types in bony and cartilaginous fish species, suggesting their ancient functional differentiation.

Negative regulation of complement activation by endogenous regulatory factor(s) is essential to avoid excess and autoimmune complement activation, which may cause inflammatory damage to the host. Hence the regulatory mechanism must be one of the phylogenetically conserved features of the complement system. Our interest has been focused on a CD46-like homologue, designated Tecrem (Teleost Complement Regulatory Membrane protein), which was originally identified from zebrafish genome databases, and then from carp and S3N ginbuna clone. Functional assay of carp and ginbuna Tecrem demonstrated its regulatory role in C3 and C4 deposition and immunomodulatory role on T-cell proliferation. Homeostatic function of CD46 may also have an ancient origin, thus we are now looking at roles of Tecrem expressed on epithelial cell lines established from carp and ginbuna fin.

Our team has also worked on bony fish thrombocytes, especially focusing on their functions as phagocytes with MHC class II. This would propose a novel framework of innate and adaptive immunity in the evolutionarily early vertebrates. Complement analysis should also provide a novel insight to our deeper understanding on this regard.

The comparative immunology team is the defending champion of the softball tournament of our department. (Prof. Miki Nakao, 4th, and Assoc. Prof. Tomonori Somamoto, 5th from the left of 1st row)

Complement in Cancer Research in Brisbane, Australia: The group of Dr. Barbara Rolfe

Barbara Rolfe's team from The University of Queensland, Australia is studying how the complement system is involved in the immune response to tumours.

With former PhD student Jamileh Nabizedeh (now a Post-Doctoral Fellow at Leibniz Institute for Plasma Science and Technology, INP Greifswald) and Post-Doctoral Fellow Dr. Helga Manthey, Dr. Rolfe has used murine tumour models to investigate the role of complement components C3a and C5a in tumour development and growth. They demonstrated for the first time that C3aR deficiency or antagonism inhibits the growth of B16 melanoma, and that the anti-tumour effects of the C3aR inhibition could be reversed by neutrophil depletion, thus indicating the importance of neutrophils in this response. C3aR deficiency or antagonism was also protective in other murine tumour models, suggesting the potential of C3aR as a therapeutic target for a range of cancers (Nabizedeh et al., 2016, *J Immunol*, 196:4783-92).

In addition to their work on C3a, the group have used the same murine melanoma model to confirm previous reports of tumour inhibitory effects of the C5aR1 antagonist, PMX53. In accord with previous studies in other tumour types, tumour infiltrating leukocyte populations (MDSC, macrophage and T cell subsets) were altered in the absence of C5aR1 signaling. Interestingly they found no additive effects of combination C3aR/C5aR1 inhibition, implying that the inhibition of either receptor alone is sufficient to impair tumour growth. However, the mechanisms by which these receptors exert their effects appear to be subtly different, as indicated by the differing tumour infiltrating leukocyte profiles in the absence of C5aR1 compared with C3aR. The group is also examining roles for the alternative C5a receptor, C5aR2 in tumorigenesis. They are now focused on gaining a better understanding of the role of these three complement receptors in tumour growth, and in particular how neutrophil function is altered in the absence of C3aR signaling.

Dr Rolfe's laboratory also has ongoing collaborations with Assoc Prof Kris Thurecht (Centre for Advanced imaging) investigating the complement response to polymeric nanomaterials, and the use of these nanomaterials for targeted drug delivery to tumours.

The research is funded by grants from the Queensland Cancer Council, and the National Health and Medical Research Council of Australia.

(Left to right) Research assistant Nadya Panagides, Dr Barbara Rolfe, and Dr Helga Manthey in their laboratory at the Australian Institute for Bioengineering and Nanotechnology, The University of Queensland.

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ANNOUNCEMENTS

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.

Deadline for late breaking abstracts is June 30th, 2017.

Deadline for early bird registration is July 1st, 2017.

For the preliminary program, accommodation and travel information, please see ***http://emchd2017.dk***

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

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.

COMPLEMENT REAGENT RESOURCES