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FOCUS ON COMPLEMENT

A NEWSLETTER OF THE INTERNATIONAL COMPLEMENT SOCIETY

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ABOUT THIS ISSUE & MORE

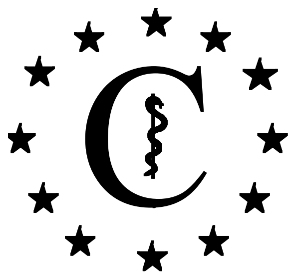
What's inside?

<1>Two flash news items, are presented by Dr. Anna Blom: **(a)** the role of complement factor H-related protein 5 (CFHR5) in glomerulonephritis, and **(b)** The regulation of T cell function by CD46.

<2> Dr. Blom also presents two complement teams: one from Copenhagen, Denmark and another from the US in Philadelphia. The Editorial Board of Focus on Complement (FoC) would like to extend sincere gratitude to Dr. Blom for her timely contributions.

<3> Under "ICS Projects" Dr Patricia Giclas presents a short report on behalf of the "Complement Standardization Committee", which has become a subcommittee within the International Union of Immunological Societies (IUIS).

<4> As promised earlier, the pictures from the XXIII ICW have been posted. To view them please visit the ICW 2010 website at: http://www.hsc.stonybrook.edu/ics2010/



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FLASH NEWS

CFHR5 and glomerulonephritis–*Reporter* : A. Blom

Identification of a mutation in complement factor H-related protein 5 in patients of Cypriot origin with glomerulonephritis.

Gale DP, de Jorge EG, Cook HT, Martinez-Barricarte R, Hadjisavvas A, McLean AG, Pusey CD, Pierides A, Kyriacou K, Athanasiou Y, Voskarides K, Deltas C, Palmer A, Frémeaux-Bacchi V, de Cordoba SR, Maxwell PH, Pickering MC.

Lancet 2010, vol 376, pp 794-801, email: p.maxwell@ucl.ac.uk

An internal duplication of exons 2 and 3 in complement factor H-related protein 5 (CFHR5) was found in 26 individuals from 11 unrelated families of Cypriot origin. All carriers of the mutation had renal disease characterized by persistent microscopic hematuria, recurrent macroscopic hematuria, C3 glomerulonephritis and progressive renal failure. Clinical PCR test was developed for screening of patients for CFHR5 nephropathy and the mutation was not detected in any of 36 sporadic cases of C3 glomerulonephritis from France and UK and therefore it may be specific for Cypriots and their descendants worldwide. The genetic alteration leads to expression of CFHR5 with duplicated complement control protein (CCP) domains 1 and 2. Such mutated CFHR5 binds less efficiently than the wild type to surfaces opsonized with complement but it does not differ in factor I cofactor activity. However, the physiological function of CFHR5 is still not fully established.

CD46 and T cell function –*Reporter* : A. Bom

Complement regulator CD46 temporally regulates cytokine production by conventional and unconventional T cells.

Cardone J, Le Friec G, Vantourout P, Roberts A, Fuchs A, Jackson I, Suddason T, Lord G, Atkinson JP, Cope A, Hayday A, Kemper C.

Nat Immunol. 2010, vol 11, pp 862-71, email: claudia.kemper@kcl.ac.uk

This study shows that engagement of CD46 on CD4+ T cells first promotes the effector potential of T helper type 1 cells but as IL-2 accumulates, the cells switch their phenotype to type 1 regulatory T cells (Treg). These cells attenuate IL-2 production via the transcriptional regulator ICER/CREM and upregulate IL-10 production after interaction of CD46 cytoplasmic tail with serine/threonine kinase SPAK. Most interestingly, CD4+ T cells from patients with rheumatoid arthritis failed to switch, consequently producing excessive interferon-g. Furthermore, γδ T cells expressed alternative CD46 isoform and were also unable to switch. Nonetheless, coengagement of T cell antigen receptor and CD46 suppressed effector cytokine production. Taken together, CD46 appears to use distinct mechanisms to regulate various T cell subsets during immune response and disturbances of these processes may be implicated in diseases such as rheumatoid arthritis.

Spotlight on teams - I

COMPLEMENT IN COPENHAGEN— DENMARK

Our group is particularly interested in the lectin pathway of complement. In the past we made in depth studies of the genetics of mannose-binding lectin (MBL) and the structural, functional and clinical consequences of MBL variants. We observed that the MBL gene (*MBL2*) harbours promoter polymorphisms that are associated with variation in the serum concentration of MBL. Additionally, we described how functional polymorphisms affected the oligomerization pattern, serum levels and function of MBL. We established the worldwide distribution of *MBL2* variants and found that these variants were very frequent in different parts of the world, particularly in sub-Saharan Africa and among indigenous people in South-America. We performed a range of genetic epidemiological disease association studies showing that alleles encoding low or dysfunctional MBL protein are to some degree associated with infections in small children, but not in healthy adults. However, MBL deficiency appeared to be a significant contributor to morbidity in patients with a concomitant immunodeficiency. A striking example was the influence of MBL deficiency on the disease course in patients with cystic fibrosis. We have been involved in studies showing that MBL may sequester dying host cells and provided evidence that MBL may function as an "eat me" signal not only towards pathogens, but also for altered self. Nevertheless, the notion that MBL may bind endogenous ligands could indicate that high MBL levels could be dangerous. In collaboration with colleagues we have, in series of studies, shown that genetically determined high levels of MBL are associated with increased cardiovascular morbidity and mortality in certain subgroups of patients. We and other groups have put forward the hypothesis that the high frequency of *MBL2* variant alleles in different populations is driven by selective advantages for heterozygous carriers. Although at first sight this might be an attractive hypothesis it has been difficult to prove by so-called molecular signature studies and remains an issue of debate. Nevertheless, we have performed studies in critically ill patients indicating that heterozygosity of structural *MBL2* variant alleles may favour survival.

In recent years we have changed our focus towards the ficolins and MBL/ficolin associated serine proteases (MASPs). Ficolins constitute a family of proteins whose biological role has been an enigma for many years. It has become evident that the ficolins function as recognition molecules in the lectin pathway of the complement system analogous to MBL. The three human ficolins, ficolin-1 (M-ficolin), ficolin-2 (L-ficolin) and ficolin-3 (H-ficolin or Hakata antigen) are encoded by the *FCN1* , *FCN2* and *FCN3* genes, respectively. In our early work we defined the inter-individual genetic variation in the three ficolin genes *FCN1*, *FCN2* and *FCN3* and could show that the *FCN2* harbour promoter variant that were associated with variation in serum concentration of ficolin-2, while variations in the fibrinogen-like domain of ficolin-2 affected binding to different ligands. We found the *FCN3* gene to be less polymorphic, but we did find a rare frame-shift variation in the *FCN3* gene. This variation leading to ficolin-3 deficiency and defective complement activation in homozygotes has been associated with life threatening infections. In order to have an easy functional tool to detect ficolin-3 deficiencies and down stream complement deficiencies we have recently developed a method based on the ELISA platform for evaluating ficolin-3 mediated complement activation and terminal complement complex deposition using acetylated compounds as binding matrix. This assay may be widely applicable both for research and clinical use. Currently we are trying to establish the relevance of ficolin variation in clinical settings. Another focus area has been the role of the ficolins to sequester host material. During these studies we discovered that ficolin-1, which is mainly synthesised by monocytes and neutrophils, is tethered to living cell surfaces through recognition of sialic acid by the fibrinogen-like domain. To elucidate the functional relevance of this finding is an ongoing project in our group.

In order to try to solve the complement activation potential of the ficolins we have started a

programme involving the MASPs. During this work we identified a splice variant of the *MASP1* gene and the resulting gene product is a novel serum protein of 45 kDa that is associated with MBL and the ficolins. This resulting protein was named MBL/ficolin-associated protein 1 (MAP-1). MAP-1 contains exons 1-8 and a novel exon encoding an in-frame stop codon. The corresponding protein of 45 kDa lacks the serine protease domains but contains most of the common heavy chain of MASP-1 and MASP-3, which both are derived from the *MASP1* gene. Additionally MAP-1 contains 17 unique C-terminal amino acids. By using quantitative PCR and MAP-1-specific immunohistochemistry, we found that MAP-1 is highly expressed in myocardial and skeletal muscle tissues as well as in liver hepatocytes and to some degree in nerve tissue. However, the expression profile is different from that observed for MASP-1 and MASP-3. MAP-1 co-precipitated from human serum with MBL, ficolin-2, and ficolin-3, and recombinant MAP-1 was able to inhibit complement C4 deposition via both the ficolin-3 and MBL pathway. Presently we are trying to better understand the biological role of MAP-1 in conjunction with the other MASP molecules.

We have also expanded our effort also to understand to biological role of different complement components outside the lectin pathway where we focus on the molecular and functional background of different complement defects. Our group consists of a mixture of students and experienced scientists with different backgrounds. We are situated at the main university hospital in Copenhagen giving us the possibility to translate basic science into daily clinical use.

Contact information, Professor Peter Garred: (garred@post5.tele.dk), Laboratory of Molecular Medicine, Department of Clinical Immunology sect. 7631, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark.

Picture legend: In the back row from left: Jakob T. Bay, Tina Hummelshøj, Vibeke Witved, Estrid Hein, Mette Brøns Jungersen, Lone Schejbel. In the front row from left: Mikkel-Ole Skjoedt, Lone Troelsen, Lea Munthe-Fog, Ying Jie Ma, Peter Garred, Hans O. Madsen.

SPOTLIGHT ON TEAMS - II

COMPLEMENT IN THE USA— PHILADELPHIA

Located only a stone's throw away from downtown Philadelphia at the first university ever founded in the United States (by Benjamin Franklin), the laboratory of John D. Lambris sets its full focus on complement and its involvement in health and disease. After a head start into complement research with positions in the laboratories of Gordon Ross and Hans Müller-Eberhard, John Lambris joined the University of Pennsylvania in 1990 and is now the Dr. Ralph and Sallie Weaver Professor of Research Medicine. His group of some 15 members covers almost all aspects of complement from deeply molecular studies on complement activation and regulation and the development of therapeutic inhibitors to disease-related *in vivo* studies using complement-deficient mice. Daniel Ricklin, who was appointed as Assistant Professor, joins forces with John in researching molecular aspects of immune evasion and complement inhibition. The close proximity to other key groups at Penn, like the ones of Wenchao Song, Youhai Chen, Hydar Ali or Oriol Sunyer, renders the university a stronghold of innate immunity and complement research.

Despite having boxes full of precious complement proteins, Lambris' group loves to think "outside the box". One mission of the lab is to establish complement not only as a "first line of defense" but as a key contributor to immune surveillance and cell homeostasis (Ricklin *et al.*, Nat. Immunol., 2010). Recently, the group provoked a paradigm shift concerning the role of complement in cancer development, and revealed important connections for complement in the development of thrombosis, in multi-organ damage during sepsis, and in tissue regeneration. Other energies in the

lab are directed toward the development of therapeutic complement inhibitors, with compstatin being the driving force in this respect. This is not surprising, as this peptidic drug, discovered by the

Lambris' group in 1996 was successfully tested in a clinical trial for age-related macular degeneration and recently licensed by Alcon. In close collaboration with the group of Piet Gros in the Netherlands, the Lambris lab published seminal papers that described key structures of the alternative pathway of complement activation and illustrated the molecular mechanisms behind its activation and regulation. Last, but not least, elucidating the fascinating strategies by which pathogens escape the deadly grip of complement has recently taken an important place in the group's research efforts.

Yet scientific exchange is at least as important for John Lambris as research itself, and he therefore maintains an impressive network of more than 30 national and international collaborators. The Aegean Conferences, an ever growing series of focused scientific meetings (ranging from innate immunity to systems medicine) on beautiful Greek islands are organized by a non-profit educational organization founded by John Lambris; they not only have proven to be an invaluable forum for discussing cutting-edge science but also for establishing fruitful collaborations across disciplines. Given his longtime involvement in the complement community, John Lambris is more than delighted to host the next Complement Workshop in Greece in 2012 (for more information see www.complement2012.org), as he is convinced that only collaboration and discussion drives the field forward. His philosophy, aptly, can be summed up by the words of Benjamin Franklin: "Tell me and I forget. Teach me and I remember. Involve me and I learn."

For more information visit www.complement.us or www.lambris.com

ICS PROJECTS

THE COMMITTEE FOR THE STANDARDIZATION AND QUALITY ASSESSMENT OF COMPLEMENT MEASUREMENTS

SUBMITTED BY PATRICIA C. GICLAS

During the clinical diagnostic process, it is not uncommon for a patient to have the same tests performed in different laboratories. In order to provide valid diagnostic results that can be compared in spite of diverse methods, it is necessary to standardize the testing by using reliable preparation of the analyte. Complement analysis varies widely between labs because—except for a very few proteins such as C3 and C4— there are no such standard preparations available on a wide scale. This is especially true for the inter-laboratory variation of CH50 or AH50 (APH50), or lectin pathway function. Most physicians look at the results in a qualitative manner: either the patient's result is within the normal range for the test, or it is outside that range, but this may not be sufficient for diagnosis. The same dilemma faces the research scientist whose publications must meet similar criteria.

At the 2008 International Complement Workshop in Basel, Switzerland, a group of interested ICS members met to discuss the formation of a standardization committee. The group (see picture) first met in Budapest in May 2009. At this meeting, which was organized by Gorgy Füst and his colleagues, the need for standards was discussed and a plan was evolved to create several large pools of plasma and serum that would be evaluated in labs across Europe and the United States for defined

proteins of the complement system. At this meeting, Hans Reinhauer of Instand e.V, Dusseldorf, offered the assistance of his organization in storage and distribution of the final product.

This standards committee is now a subcommittee within the International Union of Immunological Societies (IUIS). Michael Kirschfink (Heidelberg) is Chairman, and Bo Nillson (Uppsala) and Patricia Giclas (Denver) serve as Co-Chairs. The mission of the subcommittee is to provide the scientific and clinical communities with a well-characterized standard preparation.

The first pool of 6 liter of serum was prepared in Sweden under the direction of Bo Nillson. Six thousand aliquots of 1.0 mL each were prepared, frozen and stored. One thousand of these will be retained for the purpose of quality control, and assurance of continuity when a new lot must be prepared. Several samples were distributed to each of 10 laboratories for analysis of C3 and C4 protein levels, C1-INH level and function, and classical, alternative and lectin pathway functions. The data from this first analysis was discussed at the ICW in NY in 2010, and it was decided to repeat the analysis one more time. A second standard for activation markers is being prepared in the laboratory of Tom Eirik Mollnes (Oslo). It will provide a fully activated serum sample and will be analyzed for the complement split products. Plans include additional proteins to be added to the "normal" sample so that, ultimately, all of the pathways will be covered. It is hoped that in the long run, there will be a consensus regarding the use of these products around the globe. Each lab requesting the standard for its use will be asked to use it to create an internal secondary standard. This requirement for preparation of a secondary standard is in use by the WHO and other sources of calibrated standards today and helps preserve the valuable resources.

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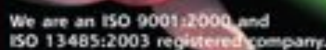

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