About this issue & More

What’s inside?
- John Atkinson presents two Flash News on: (a) The Functional basis of protection against age-related macular degeneration conferred by a common polymorphism in complement factor; and (b) Expression of complement components coincides with early patterning and organogenesis in Xenopus laevis.
- Two complement teams: from Denver, Colorado and from Birmingham Alabama are presented in Spotlights on Teams.
- A report, by Paul Morgan, of a Preliminary meeting that was held in Budapest (May-1-2) to discuss the standardization of clinical complement assays is also included.

Many thanks to John Atkinson, for his promptness in sending in the team profiles, Claudia Kemper and Dennis Hourcade for the two interesting reports profiled in the Flash news, and Paul Morgan for the report on clinical tests standardization meeting held in Budapest.

The 12th European meeting on Complement in Human Disease to be held in Visegrád, Hungary, September 5-8, 2009, is open for registration. For details visit the meeting website at: <http://www.chd2009.com/>.
Expression of complement components coincides with early patterning and organogenesis in *Xenopus laevis* 

While complement has long been established as a first line of defense, reports of C3, C5 and their receptors playing roles in the regeneration of mouse liver and amphibian limbs (see Mastellos & Lambris, Trends Immunol 23:485) suggest that C proteins may also be involved in development. Here McLin et al. provide a systematic analysis of C gene expression during embryonic development of the African clawed frog, *Xenopus laevis*. The authors performed *in situ* hybridization analyses of embryos using *Xenopus* cDNA sequences (ESTs) that correspond to known mammalian C genes. Several remarkable expression patterns were observed during early development (gastrula/neurula stages). In the neuroectoderm, genes encoding properdin, C3, C9 and C1q are expressed in the neural plate and neural precursor tissue while the C1qR and C6 genes are expressed at the periphery of the neural plate and in the presumptive neural crest. Later in development, C genes are expressed during the formation of organs not usually associated with immune function: the pronephros (C1q and Factor I), the hindbrain and developing lens (properdin), and the developing vasculature (C1qR). These unexpected findings raise the possibility of C proteins functioning during amphibian development. Although most C immune activities are dependent on the C3 and C5 convertases, the expression of the genes that encode key convertase components either were not detected during early development (C4) or were not examined (FB, C2). Moreover, the C5/C5aR genes, mediators of potent downstream immune functions, were not found expressed during these developmental stages. Some C proteins are known to bind to the extracellular matrix and could, in principle, affect cell:cell contact and communication and cell movement. Given that C knockout mice appear to form normally, any developmental functions of the mammalian complement genes would likely be redundant.
Complement in Colorado

Research on the complement system in Colorado has traditionally focused at National Jewish Health, where Peter Henson and Patsy Giclas are long-time members, and other notable faculty such as Erwin Gelfand had worked in the area. Dr. Giclas continues to run an extraordinarily busy complement reference laboratory and serves as a major resource to all “newcomers” in the field. Research at the University of Colorado Denver School of Medicine (UCDSOM), formerly the University of Colorado Health Sciences Center, was catalyzed in 1993 by the recruitment of Michael Holers from Washington University to be the first Smyth Professor of Rheumatology. Dr. Holers, who was named the Rheumatology Division Head in 2000, brought his laboratory’s focus on translational immunology and the role of complement in disease pathogenesis and autoimmunity to the research community. His collaborative work with Dr. Gelfand on the role of complement in asthma has helped to rekindle translational research efforts in complement biology at National Jewish.

Over the subsequent 15 years since Dr. Holers’ arrival, several additional faculty members in Denver have also been brought into the complement field. One is Anne Lynch, whose longstanding research interests have focused on the immunobiology of pregnancy and its complications. Dr. Lynch has developed the Vanguard Study in pregnancy and recently shown that elevated complement alternative pathway activation fragments are found early in pregnancy in women who subsequently develop pre-eclampsia and other complications such as pre-term birth. Another faculty member is Susan Boackle, who has demonstrated that complement receptor type 2 (CR2/CD21) is a lupus susceptibility gene in humans. Dr. Boackle is currently evaluating a prospective cohort of patients with lupus for CR2-related changes as well as determining what are the functional consequences of the informative SNPs linked to this disease on receptor structure, function and expression. In addition to these efforts, two longstanding faculty members, Drs. Bill Arend and Nirmal Banda, have brought their expertise in arthritis to the study of complement pathogenesis.

Several young colleagues in Denver have also recently joined the complement research community in Denver. One is Josh Thurman, a nephrologist at UCDSOM whose laboratory is studying the role of the alternative pathway and other components of the complement system in renal injury and HUS. In addition, Philip Stahel, a trauma surgeon whose research focus is on the role of complement and other inflammatory mediators in traumatic brain injury, has been recruited to Denver Health Medical Center, where Michael Flierl has recently joined his research group following a successful fellowship with Peter Ward at the University of Michigan. An upcoming addition to the group is Tem Morrison, who is joining the Microbiology program and will continue his study of complement and infection. Finally, Denver is the research base for Taligen, a complement-focused therapeutics company whose co-founder and first CEO was Woody Emlen, a rheumatologist whose prior academic research career included a fellowship in Cambridge where he worked with the outstanding complement research group there.

Overall, the complement basic and translational research effort in Denver remains a thriving enterprise, built upon a strong foundation and with many highly energetic young investigators who will undoubtedly carry the area forward scientifically in the years to come. Denver lies at the foot of the Colorado Rockies, and is a vibrant and growing city. The mountains are filled with exciting and challenging activities. Your colleagues in Denver are always interested to have visitors, so please stop by.
Spotlight on teams - II

Complement in Birmingham, Alabama

In Birmingham at the University of Alabama at Birmingham, Scott Barnum leads a group of investigators examining the role of complement in the human demyelinating disease multiple sclerosis (MS) using experimental autoimmune encephalomyelitis (EAE), the animal model of MS. These studies started many years ago when the lab, along with other groups discovered the production of complement proteins and receptors by all the major cell types in the central nervous system (CNS). Following these studies, the lab developed and characterized a transgenic mouse that produced a soluble complement inhibitor (sCrry) only in the CNS. These mice had significant protection from EAE and the results of these studies added to the growing literature indicating a significant role for complement in this disease and, importantly a potential role in therapeutics as well. Since that time, the lab and has “walked” the complement system, in collaboration with Jane Hu and Drs. Alex Szalai and Dan Bullard at UAB, in an effort to determine which complement proteins and/or pathways may be the most important with respect to disease development and progression. In the course of these studies, Drs. Jillian Wohler and Sherry Smith examined the role of the complement receptors CR3 and CR4 in EAE and discovered that these receptors were not only critical to disease development but that they were differentially expressed on multiple T cell subsets including ab, gd and Tregs (CD4+CD25+FoxP3+).

Most recently we have extended the studies of the complement phagocytic receptors on T cells to include other members of the b2-integrin family of adhesion molecules. We have now shown that expression of these receptors on gd T cells is not essential to their role in disease development, but are critical to the development of demyelinating disease. They traffic rapidly to the CNS (both brain and spinal cord) based on studies using luciferase expressing gd T cells. We have also seen that these receptors are differentially expressed on Tregs and appear to have unique functions on these cells as well. Exacerbated disease in transferred EAE experiments using LFA-1-deficient mice performed by Dr. Kari Dugger suggested an important role for this receptor on Tregs. We subsequently showed that these mice have a significant reduction in the number and function of Tregs compared to wild type mice. These studies have allowed us to more carefully address possible therapeutic routes using complement inhibitors for demyelinating disease.

Birmingham is a quiet Southern city set in the foothills of the Appalachian mountains. The weather is pleasant year round, although it can get a bit humid and hot in the summer. If it gets too hot, the sugar white sands of the Gulf coast are not too far away. We’d love to show you some Southern hospitality and the energetic research efforts at UAB – contact at us at the e-mail address below.
sbarnum@uab.edu
Report of a Preliminary Meeting to discuss the standardisation of clinical complement tests held in Budapest, Hungary, 1st and 2nd May 2009.

A group of like-minded complementologists from across Europe and the US (see participant list at end) met in the attractive surroundings of central Budapest to discuss whether standardization of clinical complement tests was necessary and, if so, how it could be achieved. The meeting was convened by George Füst, expertly organised by him and his local collaborators, Lilian Varga and Zoltan Prohaszka, and generously sponsored by Instand e.v., a Dussledorf-based non-profit organisation specialising in standardisation and quality control for laboratory medicine.

The stated aim of the meeting was to explore the value and feasibility of introducing an international standard or standards for measurement of complement analytes, including components, regulators, activation products and autoantibodies in clinical samples.

Currently, most complement assays offered for clinical use are inadequately standardised, meaning that values obtained in one laboratory cannot readily be compared with those in another. A straw poll of delegates confirmed this and showed that in most clinical laboratories only C3, C4, and sometimes C1-INH, measurements were standardised through National or Trans-national External Quality Assurance (EQA) schemes (for example, the NEQAS scheme in the UK). Other complement assays either lack standardisation, or rely upon ad-hoc standards generated in the laboratory or, for commercial assays, provided by the manufacturer. Lack of universal standards and quality control (QC) creates a number of problems:

• For assays that lack proper QC it is impossible to compare results between laboratories regardless of assay methodology;
• In most countries, assays that are not externally validated through a QC scheme are subject to variable restrictions with regard to use in patient management and often need to be reported with disclaimers;
• Without EQA there is no way of assessing the performance of a particular assay methodology or testing laboratory in comparison with others;
• Without EQA there is no way of comparing results obtained for a particular analyte measured using different methods.

The participants unanimously agreed early in the meeting that there was a current and urgent need for improved QC and QA in complement assays. Several immediate targets were identified:

• Create an International Standard (or standards) for calibrating assays measuring complement components, regulators, activation products and autoantibodies in different laboratories across the world;
• Identify a “preferred method” for testing each of the complement analytes;
• Select “Reference Laboratories” in the participating countries that would participate in an international QC/QA scheme;
• Develop a QC/QA scheme in which participating laboratories receive standard samples at regular intervals for assay and report results back to “centre” for analysis.

Discussions then centred on the practicalities of starting and maintaining such a scheme.

Top of the list was cost. Preparation, storage and shipping of standards will be expensive. Although once established, it may be possible to fund from payments made by participating
laboratories (as currently happens with many other QC/QA schemes), there was agreement that “pump-priming” funds would be needed to establish the scheme and prove its value.

Second, there was debate around the nature of the “gold standard” used to calibrate the standards distributed for the QC scheme. It was broadly agreed that the ideal standard would be the pure protein, assigned an absolute concentration value by well-validated biophysical methods and doped into a serum or plasma matrix lacking that component. The “gold standard” would then be used to assign absolute values to serum/plasma pools used as secondary standards. This may be possible for some component proteins but not for all, and certainly not for activation products.

Third, there was discussion about which analytes should be included. There was general agreement that the first targets should be those already widely measured in clinical labs – C3, C4, C1q, C1-INH. The next priority would be assays for activation products including terminal complement complex (TCC; SC5b-9) and fragments of C3, C4 and factor B. There was some discussion about whether functional hemolytic assays could be standardised, a challenge for a later date.

A number of practical steps were agreed:

- One or two laboratories to explore ways of efficiently collecting a large plasma pool (6 litres!), rapidly aliquoting (0.5ml or 1ml) and freezing for storage at –80°C – this would comprise the primary standard for component assays;
- Several labs will collaborate to explore different methods of generating an activated serum pool for use as standard in assays of complement activation products;
- Selected labs will explore the possibility of purifying a particular complement protein to “gold standard” quality and in sufficient quantity to be distributed to all lead laboratories – C1q, C3, C4 and C1-INH as priorities;
- Participating labs to collect where possible anonymised samples from patients with pathological complement activation, abnormal levels of individual components or autoantibodies against components/regulators, stored in small aliquots for sharing with participating labs;
- A provisional timetable for implementing the first stage of the QC scheme in spring 2010 was agreed.

Participants:
Moh Daha (Leiden), George Füst (Budapest), Patricia Giclas (Denver), Sakari Jokiranta, (Helsinki), Michael Kirschfink (Heidelberg), Tom Mollnes (Oslo), Paul Morgan (Cardiff), Bo Nillson (Uppsala), Michael Pangburn (Tyler), Zoltán Prohászka (Budapest), Hans Reinauer (Instand e.V, Düsseldorf), János Szebeni (Budapest), Franco Tedesco (Trieste), Lilian Varga (Budapest), Peter Zipfel (Jena).

The participants agreed to meet again in September 2009 during the European Complement Workshop. Before that time we would welcome constructive criticism! Mail your thoughts and ideas to me, Professor Pangburn or Professor Füst. If you measure clinical samples in your lab and want to get involved, again please mail one of us and we will include you in future communications.

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