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Issue 13

Focus on Complement

EUROPEAN COMPLEMENT NETWORK

About this issue & More

<1> What's inside?

- Prof.Paul Morgan presents two Flash News: One involves N. meningitidis' ability to recruit factor H using protein mimicry of host carbohydrates; and the other describes structural and functional analysis of a C3b specific antibody that selectively inhibits the alternative pathway of complement
- Spotlighted in this issue are two complement groups: one from Madrid; and the other is from Cardiff.
- Part two of a report on the XXII International Complement Workshop held recently in Basel.

<2> Special thanks again to Zvi for his patience during the transition of the editorial office from Tel Aviv to Stony Brook and for helping in the distribution of the bulletin to the more than 3500 recipients.

<3> Special thanks also go to Paul Morgan, Piet Gros, Robert Rieben, Marten Trelendelberg and Jurg Schifferli for putting together their assignments on time and completeing the report.

<4>Information regarding the **12th European meeting on Complement** is at: <http://www.chd2009.com/>.

FLASH NEWS

meningitidis and fH: Reporter: Paul Morgan

Neisseria meningitidis recruits factor H using protein mimicry of host carbohydrates. Schneider et al. Nature 2009, Feb18 (Epub ahead of print).

There are now numerous examples of the pirating of human complement regulators by pathogens to escape complement killing in plasma; however, the details of how this clever survival strategy works are, for most interactions, unknown. The ability of organisms of the genus *Neisseria* to acquire complement resistance by binding fH was described over a decade ago by Peter Rice' group, and a specific fH-binding protein (fHbp) was subsequently identified in *N. meningitidis*. In this paper, Susan Lea and collaborators have solved at high resolution the 3D structure of the complex between fH (SCRs 6 and 7) and fHbp. They show intimate and extensive associations in the complex that explain the very high affinity of the interaction. The fHbp binding site in fH is predominantly in SCR6, overlapping the sugar-binding site described last year; however, the fHbp interaction is mediated by charged amino-acids, not sugars, a novel twist on molecular mimicry. The structure explains why *N. meningitidis* is an exclusively human pathogen (other species fH don't bind fHbp) and provoke the suggestion that binding of fH to *N. meningitidis* in systemic infections might precipitously reduce plasma fH levels, exacerbating systemic complement activation. The work also raises the possibility of structure-based vaccine design for this potentially devastating infection.

C3b-specific antibody: Reporter: Paul Morgan

Structural and functional analysis of a C3b-specific antibody that selectively inhibits the alternative pathway of complement. Katschke et al. J Biol Chem 2009, Feb 5 (Epub ahead of print).

Only recently has the key role of the AP in many and diverse diseases become widely accepted (see Holers; Immunol. Rev. 2008, 223, 300-316). This has provoked an increased interest in therapeutic agents specifically targeting AP activation. Indeed, there may be benefits in retaining CP activity, responsible for among other things, solubilising immune complexes. Potential targets include native, complexed or fragmented fB or C3, fD and properdin. C3 has key roles in all activation pathways and thus seems an unlikely target for AP-specific therapy. Here, a group from Genentech used state-of-the-art phage display technology to generate and optimise a fully humanised, high-affinity C3b-specific Fab (S77). Analysis of the crystal obtained from S77 in complex with C3b localised the binding site to a neoepitope, absent in native C3, formed by activation-induced re-orientation of the MG6 and MG7 domains. The reagent blocked binding of fB to C3b, thus preventing convertase formation. Of note, the recent structure for the C3bB complex (Torreira et al 2008; PNAS 106, 882-7) shows that the neoepitope is included in the C3b-fB interface, indicating that S77 directly blocks the interaction. In haemolysis assays, S77 inhibited AP but not CP activation; in itself, this is not surprising, given that the stage in the CP that is analogous to C3bBb formation is assembly of C4b2a. More surprising is that S77 inhibited the AP C5 convertase (C3bBbC3b) but not the CP C5 convertase (C4b2a3b). In these complexes the second C3b is considered the acceptor for C5; logic would have suggested that S77 binding the second C3b would inhibit both C5 convertases. The authors suggest that, in this context, C4b in the CP convertase may act as a C5 acceptor, permitting C5 activation. Whatever the precise mechanism, this reagent offers a new and much needed tool to selectively inhibit AP activation in disease.

SPOTLIGHT ON TEAMS - I

Complement in Madrid

There are three teams in Madrid involved in complement research. Santiago Rodriguez de Cordoba (SRdC) heads the Molecular Pathology Group at the Centro de Investigaciones Biológicas (CSIC). They specialize in molecular genetics and among their contributions are the identification and characterization of various genes responsible for human diseases. The Group has a strong, long-standing interest in Complement genetics that was initiated back in the 1980s when SRdC described the "Regulators of Complement Activation" (RCA) gene cluster in chromosome 1q32 and characterized the genomic organization of this region of the human genome. Margarita Lopez Trascasa and Pilar Sánchez-Corral have affiliations with the Immunology Department and the Research Unit of the University Hospital La Paz, respectively. Dr Lopez-Trascasa's group is focused on inherited and acquired complement deficiencies and their association with human disease, specifically HAE, membranoproliferative glomerulonephritis, and other autoimmune renal diseases. Dr Sanchez-Corral's research efforts are aimed at delineating the role of factor H and the factor H related proteins in diseases, particularly atypical haemolytic uremic syndrome (aHUS).

During the last 10 years, work in these laboratories has contributed to understanding the pathogenesis of aHUS. The groups were the first to characterize functional abnormalities in the factor H mutant proteins associated with aHUS and to propose that the combination of an active complement system and a defective protection of cellular surfaces are critical to develop aHUS. Later, they reported that gain-of-function mutations in the complement factor B gene predispose to aHUS and showed that concurrence of different susceptibility factors (multiple-hit hypothesis) is critical in aHUS. Over the years they have also contributed to functional assays and the development of a murine model of aHUS that are facilitating diagnosis in patients and the development of therapies. Very recently they started to explore structural aspects of the AP C3 convertase. These structural data will be instrumental in understanding the association of complement mutations and polymorphism with disease and will aid the molecular design of therapeutic targets.

Through the years we have particularly enjoyed sharing our excitement for science by collaborating with friends and colleagues within and outside the complement community, and particularly the Cardiff group also featured in this issue. We are indebted to all of them for their partnership and support and to many students for their enthusiasm and excellence.

Madrid is the capital and largest city of Spain with more than 5 million people. We invite you to came and visit us. We will show you how we manage to combine passionate scientific discussions with intense cultural and artistic activities and a very lively nightlife.

Contact us at these addresses: srdecordoba@cib.csic.es
psanchez.hulp@salud.madrid.org
mlopeztrascasa.hulp@salud.madrid.org

SPOTLIGHT ON TEAMS – II

Complement in Cardiff

Complement research in Cardiff began in earnest in 1986 when Paul Morgan returned to a clinical lecturer post having undertaken post-doctoral training in the laboratories of Manfred Mayer and Alfred Esser in Baltimore and Gainesville respectively. Thanks to Fellowship support from the Wellcome Trust, he was able to establish a research group working on the terminal pathway and its regulation. The group has grown over the years and has "spun-off" several independent but collaborating groups working in diverse areas of complement research, all included in the umbrella term ***Cardiff Complement Research Group***.

Carmen van den Berg has established a group focussed on roles of complement in atherosclerosis and other vascular diseases. Her recent contributions include the characterisation of spider venom proteins that affect complement and coagulation (with Denise Tambourgi, Sao Paulo) and studies of C-reactive protein interactions with complement and its regulators. **Claire Harris** leads a group working on C3 convertase structure-function, complement regulation and dysregulation and complement therapeutics. In a very successful ongoing collaboration with Santiago Rodriguez de Cordoba, she has demonstrated the mechanisms by which mutations and polymorphisms in factor B alter function to cause disease. **Brad Spiller** and **Eamon McGreal**, working in the Paediatrics Department, have developed interests in dysregulation in complement and other immune parameters in neonates. Brad has continued to work on viral complement regulators and recent successes (in collaboration with Anna Blom) include characterisation of regulators in rhabdoviruses and identification of antibodies against the Kaposi virus encoded complement regulator in infected individuals. **Anwen Williams** explores roles of immune molecules, including complement and complement regulators in models of arthritis. In collaboration with Claire Harris and Paul Morgan, she has demonstrated that novel recombinant complement regulators can markedly inhibit arthritis disease in rodent models. **Rossen Donev** has taken a molecular biological approach to dissect the control of expression of complement regulators, particularly in tumor cells. He has identified mechanisms of regulation of CD59 in neuroblastoma and some other tumor types and devised a novel approach to tumor therapy – downregulating expression of CD59 to sensitise the tumor cells to antibody-directed complement killing. **Paul Morgan**, despite dalliances in other parts of the system, has stayed more-or-less faithful to the terminal pathway and its control. Cardiff, the capital city of Wales, is a compact, easy-going University city. It may not offer year-round sunshine (the weather is best described as changeable), or the dubious advantages of London. However, those who come to visit seem to like it and often settle down here. The Complement Biology Group provides ample evidence of this, a cosmopolitan collection of people, many of whom came for a short visit and stayed. If any of you, junior or not so junior, would like to see for your selves, we would be delighted to host you – just contact one of us at the addresses below.

morganbp@cardiff.ac.uk harriscl@cardiff.ac.uk
donevrm@cardiff.ac.ukspillerb@cardiff.ac.uk vandenbergcw@cardiff.ac.uk
williamsas@cardiff.ac.uk mcgrealep@cardiff.ac.uk

XXII International Complement Workshop
September 28 - October 2, 2008
Basel, Switzerland

Summary of oral sessions (part 2 of 2)

Robert Rieben, Piet Gros, Marten Trendelenburg, Jürg Schifferli

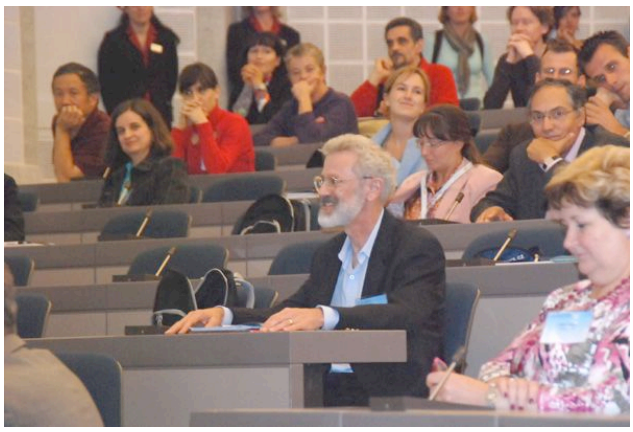
This is the 2nd part of the report on the XXII International Complement Workshop, which was held in Basel, Switzerland, from September 28 to October 2, 2008. The 1st part of the report appeared in the December 2008 issue of Focus on Complement and covered sessions 3-5. In the present, 2nd part we review sessions 1 and 2 (structure and function, summarized by Robert Rieben and Piet Gros) and sessions 6-9.

Session 1 (structure and function 1)

This session started with Suzan Rooijakkers, who showed how the staphylococcal convertase inhibitor SCIN stabilizes, dimerizes and inhibits the C3 convertase. Together with Jin Wu, she made and crystallized highly stable dimeric convertases, which resulted in the first crystal structure of a C3 convertase. Later in the session Piet Gros presented the crystal structures of the pro-convertase (CVF-B) and convertase (C3bBb-SCIN). Strikingly, the carboxy-terminus of CVF chelates the Mg^{2+} ion of factor B. Using the SCIN-convertase dimer he outlined the molecular mechanism of the convertase activity. David Isenman discussed mutational data on the binding of staphylococcal immune evasion molecules Sbi and CR2 to C3d. Sbi-IV and CR2 are competitive ligands of C3dg and bind to an acidic pocket on the concave surface of C3d. These data, however, conflict with the published C3d/CR2 (SCR1-2) crystal structure. James M. Kovacs used NMR to define CR2:C3d interactions in solution, which support the published crystal structure. The conflicting data on the C3d/CR2 interactions were heavily debated during the discussion sessions of these two presentations. Next Pietro Roversi presented the structure of the rat complement regulator Crry. Overlay of Crry domains 1-3 with DAF2-4 and fH1-3 shows an extended SCR arrangement that is likely critical for the regulator activity. In the next presentation Marie Phelan showed the first structure of a factor I-like module (FIM), that of C7, which is involved in C5b-C6-C7 interactions during MAC assembly. Gérard Arlaud presented their refined model of the structural arrangement of the very large C1 complex. The extensive structural and biochemical data point out an intricate set binding sites of the C1r-C1s-C1s-C1r tetramer with C1q; similar basic mechanisms probably apply to MBL-MASP and ficolin-MASP complexes. Finally, Umakhanth Girija presented details of the molecular interactions in the initiation complex of the lectin pathway of complement activation.

Session 2 (structure and function 2)

Jin Wu presented the crystal structure of C3b in complex with factor H domains 1-4. C3bfH has a discontinuous, extensive but weak interface that involves many domains of C3b and fH. Using the structures of C3bfH and the convertase, she discussed the implications for the mechanism of decay acceleration and cofactor activity. This presentation was followed by a very lively discussion in which Jin Wu elegantly addressed the questions with a series of prepared slides. Next, Claire Harris presented functional data on common factor B polymorphic variants. Markedly, some fB polymorphisms reside in the Ba domain and are not part of the active enzyme, apparently affecting the rate of convertase formation. Lubka Roumenina showed that gain-of-function fB or C3 mutant proteins, yielding "super C3 convertases", are associated with atypical hemolytic uremic syndrome with a poor outcome. Mutations in the MIDAS of fB yield gain of function of C3 convertase due to increased affinity of C3 binding. The next four talks focused on fH. Using NMR Christoph Schmidt delineated fH binding sites for C3b/c/d and polyanions. Viviana Ferreira showed data from a collaborative study of the group of Michael Pangburn and the Edinburgh University Biomolecular NMR group of David Kavanagh, indicating that the binding of fH to a complex of physiological polyanions and C3b on the cell surface is disrupted in aHUS. Next, a clinical study of Veronique Frémeaux-Bacchi's group was presented by Marie-Agnes Dragon-Durey. They had asked the question whether CFHR1 deletion is a susceptibility factor for aHUS by itself and tested the deletion for association in aHUS cases and controls. The frequency of homozygous CFHR1 deletion was highest in the population which had also anti-fH autoantibodies. The role of anti-fH antibodies was then discussed by Stefanie Strobel, Jena, and she showed that autoantibodies against fH from patients prevent fH binding to sheep red blood cells and make them susceptible to lysis. The antibodies mostly bind to the C-terminal part of fH and reduction of autoantibody levels by immunosuppressive therapy is beneficial for the patients. As the last speaker in the session Elena Goicoechea de Jorge (Matthew Pickering group, London) discussed the involvement of C5 activation for the development aHUS in CfH-/- fH_16-20 mice.



Session 6 (anaphylatoxins)

The first speaker of this session was Ke Li, who showed that T cell responses are reduced when dendritic cells (DC) from C3aR -/- mice are used. The C3a-C3aR interaction upregulates DC function in antigen presentation in vitro and enhances their capacity to stimulate antigen-specific T cell responses in vivo. Cyclic AMP plays a critical role in C3a-receptor mediated regulation of DC in antigen uptake and T cell stimulation. Edimara Reis from Jörg Köhl's group then presented evidence for a regulatory role of the C5a anaphylatoxin in Th17 cell differentiation. Interaction of C5a with C5aR, but not with C5L2, on DC seems to favor a cytokine pattern which induces the generation of Treg and also Th-17 cells. The presentation of studies on C5a was continued by Rick Wetsel, who showed that in double ko mice deficient in CPN and the anaphylatoxin receptors lethality due to acute complement activation is caused by C5a but not C3a. These data were generated in a CVF induced mouse lung injury model. Andreas Klos, Hannover, presented data on the role of C5aR in acute and chronic dextran sulphate induced models of inflammatory bowel disease.

C5aR is highly expressed in acute disease, C5aR-deficiency is beneficial in acute but harmful in chronic disease. Therefore, C5aR antagonist treatment against inflammatory bowel disease may only make sense during the acute phase. Rahasson Ager, Andrea Tenner's group, showed results from a study in a mouse model of Alzheimer's disease. In this model, the use of the C5aR antagonist PMX205, a cyclic peptide based on the C-terminus of C5a, resulted in reduced amyloid β plaques, inflammatory microglia and reactive astrocytes. The role of C5a and C5aR in neutrophil activation and glomerulonephritis induced by anti-neutrophil cytoplasmic antibodies was then highlighted by Adrian Schreiber, Berlin. Complement was not suspected in ANCA disease so far, but the presented animal studies suggest its importance. A role for C5a and C5aR was also shown in the development of airway hyperresponsiveness (AHR) in allergic asthma. Meenal Sinha from Rick Wetsel's group showed mouse data which suggest that C5aR protects against AHR by inhibiting the cysteinyl leukotriene pathway. Finally, Jörg Köhl talked about pathogen-driven CCR5/C5aR heterodimerization, which initiates a JNK2/JIP1-dependent signaling pathway that protects from Toxoplasma gondii infection. T. gondii-derived cyclophilin-C18 induced IL-12 production depends on the presence of both C5aR and CCR5, which then form a heterodimer able to sense danger and trigger an adaptive immune response.

Podcast of the closing session!
John Atkinson summarized the highlights of the meeting in a memorable closing session, which was scientifically to the point and entertaining at the same time. We recorded this speech and merged it with the slides to a podcast which can be downloaded here in three different sizes:
https://www.wuala.com/rrieben/ICW2008_Basel?key=ICW2008

Use iTunes or a similar program to see the slides and listen to the presentation. Duration: 25:23 minutes.

Session 7 (animal models)

Ischemia/reperfusion injury was a major theme in this session. Ludmila Kulik, from Michael Holer's group, started with a talk on intestinal I/R injury in a mouse model. Cr2-/- mice are known to be more resistant to intestinal I/R injury than wildtype, and this was traced back to reduced levels of natural antibodies against annexin IV in Cr2-/- mice. The theme was continued by Thusitha Gajanayake (Robert Rieben's group), who showed that low molecular weight dextran sulfate reduces I/R injury by modulating the activation of complement and the MAPK pathway. These data were generated in a rat aortic clamping model. Wen-Chao Song then presented a whole array of triple ko mice. DAF1 and CD59a double ko mice were crossed with several specific C-component and Ig ko to produce triple ko mice, and the effect on renal I/R injury was assessed. Only fB and properdin triple ko mice were protected from renal I/R injury, suggesting that the AP is mostly responsible for the injury in this model. Renal I/R injury in mice was also the theme of the talk by Joshua Thurman. He presented data on the expression of annexin A2 in the kidney after I/R injury, which seems to be a ligand for binding of fH and thus lead to inhibition of the AP and limitation of I/R injury. In her second talk in this session, Ludmila Kulik presented data on a highly inhibitory monoclonal antibody against the C3d binding site on human CR2/CD21. In hCR2 transgenic mice this antibody suppresses the generation of an antibody response against SRBC. Yuko Kimura (Wen-Chao Song's group) showed that gene targeting of properdin ameliorates the disease development in the K/BxN mouse serum transfer model of arthritis. fP-/- mice had a lower disease score due to reduced AP activity and significantly lower levels of IL-1β. However, in the discussion it was made clear that AP activation is possible without properdin (Michael Pangburn). A novel alternative to complement depletion by CVF was then presented by Carl Vogel. They made a human C3/CVF hybrid protein for

therapeutic complement depletion and tested its in vivo activity as well as toxicity in primates. The construct seems to work well, C was depleted, lots of C3a but almost no C5a were generated, and it was well tolerated. Also the final talk of this session, by Hidechika Okada, was on a non-human primate model. AcPepA, a complementary peptide to hC5a with acetylation of its N-terminal alanine, was shown to suppress a lethal cytokine storm in monkeys injected with LPS.

Session 8 (pathogens and regulatory proteins)

This session started with three consecutive talks by members of John Atkinson's group. The first was by Elizabeth Moulton, who presented data on the interaction between host complement and the poxviral Ectromelia inhibitor of complement enzymes (EMICE). She showed that in mice the virulence of Ectromelia virus, a model for smallpox, is dependent on the host complement system and expression of EMICE by the virus. Kathryn Liszewski then showed that the poxviral regulators of complement activation also provide attachment of the virus on host cells, most probably via chondroitin sulfate E and/or heparin. Finally, Panisadee Avirutnan reported data on a newly discovered immune evasion strategy of dengue virus, namely the inhibition of human C4 by dengue virus nonstructural protein NS1, which prevents deposition of C4b on target cells. The next two talks were on Staphylococcus aureus, which later was declared 'bug of the meeting' by John Atkinson. A novel way of acquisition of fH by S. aureus was presented by Michael Reuter (Peter Zipfel's group): S. aureus binds fH and FHR-1 via Sbi (S. aureus binder of IgG) in combination with C3b and C3d. Daniel Ricklin (John Lambris' group), then followed with novel insights into target specificities and molecular mechanisms of extracellular fibrinogen-binding protein Efb of S. aureus. He presented the key residues and thermodynamics of C3d:Efb-complement binding. Reinhard Würzner, presented data on the protective role of fH in entero-hemorrhagic Escherichia coli (EHEC) induced HUS by ameliorating shiga toxin action. The latter seems to directly activate the AP and lead to destructive complement activation, which can be slowed down by fH on the cell surface. The final talk of this session was one of the few presentations at this meeting which looked at evolutionary aspects of complement. Ayuko Kimura showed nice data on the endodermal expression of C3, Bf and MASP genes of the cnidarian sea anemone Nematostella vectensis. Complement-related genes were cloned, characterized, and their anatomical location determined by in situ hybridization. Analogs of C3, fB, and MASP, but not of C6 or factor I, were found in N. vectensis.

Session 9 (complement in health and disease)

The multitude of diseases in which the complement system plays an important role became once again clear during this session, as the number of talks almost equaled the number of diseases covered. The session started with a talk by Svetlana Hakobyan (Paul Morgan's group) on the measurement of fH and the Y402H polymorphism in Alzheimer's disease. Elevated levels of fH and a differential expression of Y402 and H402 fH variants was found in AD patients. Nadine Lauer, from Peter Zipfel's group, then presented new findings on how the V62I and Y402H variants of fH, which are associated with age-related macular degeneration (AMD), affect protein function and complement control. A reduced binding to pentameric and monomeric forms of CRP was found for the I62 and H402 forms of FHL-1

and fH, which could explain the impaired complement control of these AMD-related alleles. Rossen Donev, Cardiff, put the focus back on Alzheimer's disease. He presented a new strategy for protection of neurons from complement-mediated degeneration triggered by beta-amyloid plaques. Treatment of neurons with a peptide derived from neural-restrictive silencer factor (REST) modulates the expression of CD59 and by this protects the neurons from complement-mediated damage. Anne Lynch, from Michael Holers' group, then presented data on Bb, angiogenic factors and preeclampsia. In a prospective study with >650 women, elevated Bb was found to be an independent predictor of preeclampsia. Fleur Bossi (Francesco Tedesco's group) showed rather unexpected data on C1q, which displays strong pro-angiogenic properties. At least in vitro the pro-angiogenic effect of C1q was just as strong as the one of VEGF. Guido Moll, Uppsala, had looked into complement activation triggered by human mesenchymal stem cells intended for clinical use, and he could show that MSC tested at early passages and grown in AB serum instead of FCS, displayed reduced complement activation. Cornelia Bigler, from Marten Trendelenburg's lab in Basel, then showed that autoantibodies against C1q specifically target C1q on early apoptotic cells, but don't recognize C1q on immune complexes. Finally, results of a phase III study with human recombinant C1-inhibitor for HAE treatment were presented by Jan Nuijens from Pharming Technologies, Leiden / NL. The rhC1inh "Rhucin" is isolated from the milk of transgenic rabbits. 28 double-blinded treatments of patients suffering from acute angioedema attacks were made with Rhucin 100 U/kg or placebo. Rhucin worked well, but one anaphylactic reaction in a healthy volunteer with pre-existing rabbit allergy was observed.

Complement Reagents Corner