June 1, 2007
Issue 6

Focus on Complement

About this Issue and more

<1> This issue of "Focus on Complement" includes:
an article by Paul Barlow, Susan Lea and Steve Perkins on structural analyses of complement proteins, Flash news demonstrating involvement of complement CD46 in Tregs activation and Multiple Sclerosis and a second Flash news on functional lessons learned from analysis of the crystal structure of complement factor B, and last but not least, Spotlights on two complement teams in Sweden and Germany.

<2> 11th European Meeting on Complement in Human Disease.
will be held in 8-11 September 2007 in Cardiff, Wales. Paul Morgan and the organizing committee invite you to submit late breaking abstracts and attend this meeting. (http://www.complementcardiff.org.uk/index.html)

<3> Registry: Cancer in complement deficiency.
In an attempt to assess the involvement of the complement system in cancer (development, aggressiveness, metastasis etc.), we wish to initiate collection of information on all known cases of cancers that have been recorded in complement deficient patients. If you have such information or know of colleagues who may have it, please contact Zvi Fishelson (lifish@post.tau.ac.il).

EUROPEAN COMPLEMENT NETWORK

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Structural analysis of complement proteins

A brief comparison of available approaches*

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*compiled and edited by Paul Morgan

A battery of modern methods is available to study complement protein structures with each method having particular strengths in terms of the kinds of information generated. The major difference between the methods lies in the level of detail or resolution of the resultant structures, ranging from "atomic" level (provided by NMR spectroscopy and X-ray crystallography) to overall shape information obtained directly (e.g. by electron microscopy) or indirectly (e.g. using solution scattering or methods such as FRET that yield long-range distance restraints). None of the methods can generally be applied in totally physiological conditions. For example, they usually require proteins to be highly purified, and in many instances only portions of larger molecules can be studied. Nonetheless, used in **combination**, these methods have greatly increased our understanding of the biology of complement.

Crystallography:

For many years, X-ray crystallography has been the method of choice for determination of atomic level structural information. In principle, X-ray structures may be determined of proteins or protein-complexes ranging from a few kDa (e.g. C3a) to many megadaltons (e.g. viruses and ribosomes); however, large and complicated molecules present a challenge. Structural studies of C1 from the Arlaud group (Gaboriaud et al., 2004) were a significant achievement, while the recent crystal structure for C3 (Figure 1) represented a landmark for the complement field (Janssen et al, 2005). Other recent successes have included C2a (Milder et al, 2006; Krishnan et al, 2006), and C3b (Janssen et al, 2006; Wiesmann et al, 2006). The Lea group has focussed on structural analyses of the complement regulators and has successfully crystallised CD55, a complicated 4-module molecule (Lukacik et al, 2004). The strength of crystallography is in the level of detail provided; the weaknesses are first that it is only possible to determine the X-ray structure of proteins that crystallise and second that the structure of the protein packed in the crystal may not accurately represent the native protein where several domains or modules are present in an extended conformation (see the CR2 example discussed below). This problem is often cited as a major weakness of crystallography. However, there are now many examples of structures determined by

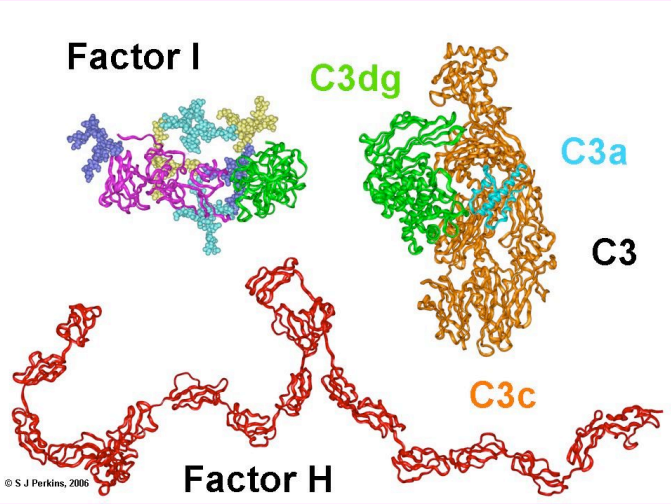

Figure 1. Models of Factor I and Factor H structures derived from solution scattering, and the crystal structure of C3.

multiple methodologies that are identical in domain structure and orientation, suggesting that crystal structures very often do approximate native structures. In some instances, crystallography can be very rapid with proteins going from initial trials to structures in a few weeks; however, crystallisation remains unpredictable and many key structures have taken months or years to yield atomic information.

Nuclear Magnetic Resonance (NMR):

While NMR-derived 3D structures are of slightly lower resolution (i.e. are less accurate) than ones solved by X-ray crystallography, NMR can offer several advantages over its rival. With NMR, the structure is studied free in solution (although the protein concentration is not generally in the physiological range) rather than packed into a crystal; the 3D structure may be studied by NMR at physiological pH and ionic strength; and any flexibility within the protein is preserved and can be measured. As with crystallography, pure protein is required; however, because there is no need to grow crystals, one of the major constraints of crystallography is eliminated and NMR can often deliver results rapidly. NMR is especially powerful for mapping binding sites on proteins. A major limitation of NMR is that it is only capable of solving the structures of smaller proteins (i.e. less than 30-40 kDa). For complement proteins, most of which are built up from multiple small domains, innovative approaches are possible. Some groups have developed a "dissect-and-rebuild" approach in which individual domains of complement proteins are expressed and studied, in isolation, by NMR (Jenkins et al, 2006). By also solving structures of pairs of neighbouring domains in order to reveal their relative arrangements, it is possible to “rebuild” the structure of the intact molecule.

In addition, the Barlow group in Edinburgh has recently started to employ fluorescence resonance energy transfer (FRET) as a means of studying the architecture of intact proteins such as factor H and complement receptor type 1. By attaching fluorescent probes at strategic positions they hope to be able to measure distance of up to 80 Å with reasonable accuracy (Figure 2). This will allow the very detailed NMR-derived structures at the single and double CCP module level to be strung together to give reliable models of intact proteins. Furthermore, because FRET data can be collected very rapidly, the method affords the opportunity to observe these proteins as they interact with their targets in the complement cascade.

Solution scattering:

Scattering methods offer the possibility of studying intact, large molecules in near-physiological conditions. They are applicable to proteins of any molecular weight above about 15 kDa, meaning that small complement proteins and protein fragments can also be studied. The “rebuilt” NMR structures mentioned above can be validated by scattering. The first global views of many of the complement protein structures have been obtained by X-ray and neutron scattering or by ultracentrifugation (at a “low” structural resolution) followed by constrained modelling, a complex process requiring considerable computing input. In the modelling process, thousands of possible domain

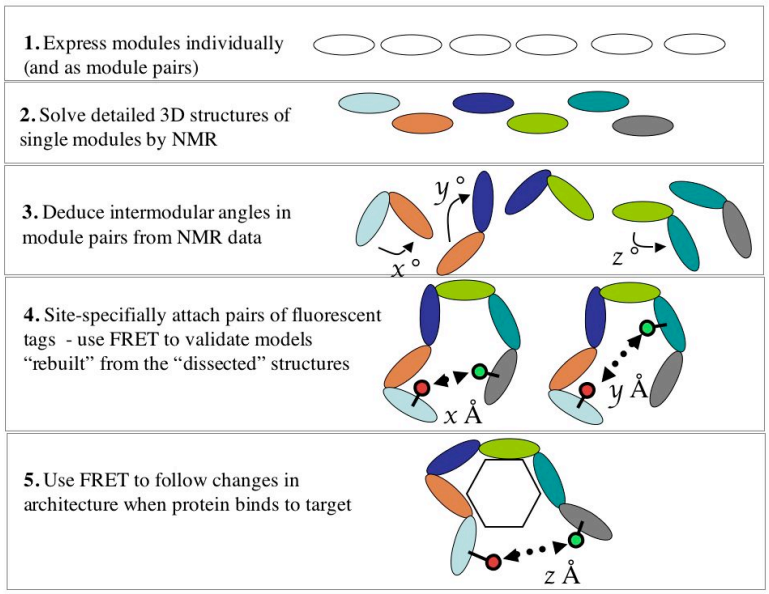

Figure 2. Rebuilding and FRET to define complex structures.

arrangements are fitted to the scattering data to identify a small family of best-fit structures, yielding a model at a "medium" structural resolution. This is exemplified by the solution structural models for Factor I and Factor H shown in Figure 1. The resolution is sufficient to identify the most probable domain arrangements, both proteins here showing domains folded-back on each other (Chamberlain et al, 1999; Aslam & Perkins, 2001). The structural precision of this approach can be sufficient to identify functional roles – for example, it enabled the first analyses of structural effects of mutations in Factor H leading to atypical haemolytic uraemic syndrome, now confirmed by recent NMR and crystal structures (reviewed in Saunders et al, 2007). See http://www.fh-hus.org/

Even when "high" resolution crystal or NMR structures (in which atomic detail is visible) do become available, it may be important to verify the observed domain arrangement in solution to precisely answer specific points of biology. Some complement regulatory protein crystal structures have had to be revised in the light of scattering modelling, as the inter-domain linker flexibility influences the way in which crystals form. For example, crystal structures of the terminal domain pair of CR2 suggested folded-back domain structures, but the two domains were found to adopt a more opened up conformation in solution (Gilbert et al, 2005).

Summary:

This brief review illustrates the strengths and weaknesses of structural methods applied to complement proteins. No one technique is alone adequate and best results are achieved by combining information from high-resolution and low-resolution methods. The recent explosion of new structures has had a major impact on the complement field and emerging data from the growing number of structural complement groups will further strengthen and extend our understanding of this fascinating biological system.

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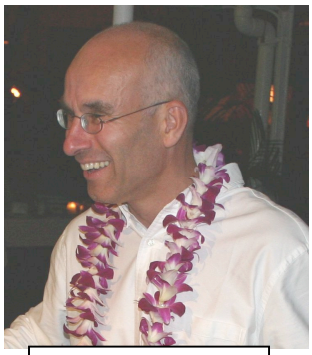


Paul Barlow

Susan Lea

FLASH NEWS

Complement, CD46 and Tregs: Reporter: Claudia Kemper

Alterations in CD46-mediated Tr1 regulatory T cells in patients with multiple sclerosis.

Anne L. Astier, Gregory Meiffren, Samuel Freeman, and David A. Hafler.
J. Clin. Invest. 116:3252-3257 2006.

In this exciting study, Anne Astier and colleagues provide evidence that a defect in the induction and function of CD46-induced IL-10-secreting regulatory T cells may be connected with the development of multiple sclerosis (MS) in humans. Concurrent activation of CD46 and the T cell receptor on $CD4^+$ T cells leads to the generation of a distinct immunomodulatory T cell population (Tregs) that are characterized by their production of high amounts of the immunosuppressive cytokine IL-10 and of granzyme B. Through these two agents, CD46-induced Tregs suppress the activation of bystander effector T cells. Here, the authors show that $CD4^+$ T cells purified from blood of MS patients demonstrate a defect in IL-10 production if the cells are activated with antibodies to CD3 and CD46. The loss of CD46-mediated IL-10 production was then traced to an altered regulation of the cytoplasmic domain 2 (CYT-2) of CD46, which is known to transduce intracellular signals. This is the first report of an involvement of CD46-induced Tregs in a human autoimmune disease. Thus, studies like this will become key to understanding the role of complement-modulated T cells in human disease.

Factor B structure: Reporter: Dennis Hourcade

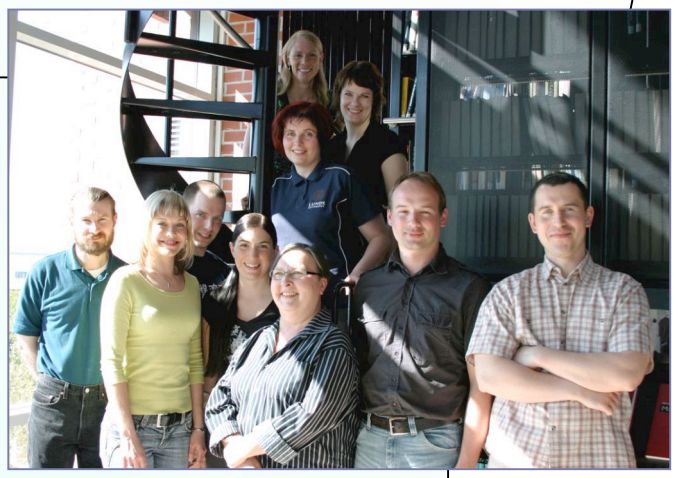
Factor B structure provides insights into activation of the central protease of the complement system.

Milder FJ, Gomes L, Schouten A, Janssen BJC, Huizinga EG, Romijn RA, Hemrika W, Roos A, Daha MR, Gros P.
Nature Structural & Molecular Biology. 2007 March 1; 14 (3):224-8.

The alternative pathway C3 convertase is the central protease of the feedback loop. As such it is a workhorse of complement activation. Its catalytic site, which lies in the factor B zymogen, is activated by cleavage of factor B by factor D. This can occur when factor B is associated with C3b. Previously, a number of investigators have focused on the structure/function of specific portions of factor B. This article presents an x-ray crystallographic analysis of the full factor B structure. It demonstrates a novel mechanism that "locks" the protein in its quiescent form until cleaved by factor D. It shows interactions between the CCP domains and the VWA domain that bear on factor B:C3b association and it lends clues to the activation of the catalytic site. This study provides an important key to our understanding of convertase assembly and regulation.

SPOTLIGHT ON TEAMS

Anna Blom
Lund University, Sweden



Our Medical Protein Chemistry research group is located at the Wallenberg Laboratory within University Hospital in Malmö, southern Sweden. Our current main areas of interest are complement inhibitors and their role in several biological systems. Our ambition is to make high quality basic science based on clinically relevant questions and yielding novel diagnostic and clinical interventions.
Excessive or misguided activation of complement contributes to pathogenesis of most chronic and acute inflammatory diseases. So far there is no approved pharmaceutical complement inhibitor that can be used clinically and detailed knowledge of how the system works on the molecular level, to which we contribute, is required for development of such compounds. We want to identify factors contributing to detrimental complement activation in joint disease such as rheumatoid arthritis. Therefore, we study interactions between complement factors and components of extracellular matrix. Also we want to gain insight into complicated processes of regulation of cell death and clearance of dying cells by complement and their implications to autoimmune diseases. The genetic and molecular basis for deficiencies and defects of human complement inhibitors are investigated in order to understand mechanisms underlying diseases caused by these deficiencies and to better understand functions of these proteins in relation to their structure. Furthermore, we study mechanisms used by major human pathogens to circumvent complement attack such as secretion of proteases and acquisition of human complement inhibitors or the production of own homologues such as inhibitor produced by Kaposi´s sarcoma associated herpes virus (KCP).

Our group has formed extensive network of collaborations and we always enjoy and welcome visitors in our laboratory!

Contact info: Lund University, Dept. of Laboratory Medicine, Section of Medical protein Chemistry, The Wallenberg Laboratory floor 4, Malmö University Hospital entrance 46, S-205 02 Malmö, Sweden

SPOTLIGHT ON TEAMS

Peter Zipfel — Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany

Complement work in Germany is pursued in Jena at the Leibniz Institute for Natural Product Research and Infection Biology. Our interest is to understand the role of complement and in particular complement regulation in health and disease. A major focus is the complement evasion of pathogens particularly human pathogenic fungi such as *Candida albicans* and *Aspergillus fumigatus*. Additional relevant topics include the role of complement in autoimmune diseases. The severe kidney disease Hemolytic Uremic Syndrome is caused by defective alternative pathway control, induced either by gene mutations in multiple complement components or autoantibodies that bind to the immune inhibitors Factor H and FHR-1. In addition, the role of Factor H gene mutations is analyzed in membranoproliferative glomerulonepritis Type II and in Age Related Macular Degeneration of the eye. These aspects are covered in close collaboration between the Department of Infection Biology (Peter F. Zipfel) and the Junior group for Cellular Immunbiology (Mihály Józsi).

We are interacting and collaborating with complementologists and other groups including clinicians and basic research teams in Germany, Europe and in America. We are open for further cooperation and fruitful collaborations with guest researches.

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