Focus on Complement

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Issue 37

International Complement Society

European Complement Network

Dear readers,

Welcome to the March 2015 issue of 'Focus on Complement'. This 37th issue of FoC contains:

- **News Flash** presenting two recent papers showing a critical protective role for the C3aR during *Listeria monocytogenes* infection and the generation of a new tool, a floxed-in GFP C5aR1 reporter mouse that will allow for detection of cell-specific deletion of the C5aR1 in mice.
- **The Complement research teams around the world series** featuring the teams of Drs. Viviana Ferreira in Ohio and George Hajishengallis in Philadelphia
- Part II of the **Meeting Report** on the 25th ICW held in September 2014 in Rio de Janeiro
- 15th European Meeting on Complement in Human Disease announcement
- Announcement if the ICS-sponsored Complement Symposium at the American Association of Immunologists meeting in New Orleans, May 10th 2015 http://immunology2015.org/program/index.html?loc=nav

If you would like to contribute with an article to a future issue or have suggestions for a subject theme, please contact Claudia Kemper or Andrea Tenner; Claudia.kemper@kcl.ac.uk, atenner@uci.edu

NEWS FLASH

NEWS FLASH 1: The receptor for the complement C3a anaphylatoxin (C3aR) provides host protection against *Listeria monocytogenes*-induced apoptosis. *Stacey L. Mueller-Ortiz, John E. Morales and Rick A. Wetsel. J Immunol. 2014 Aug 1;193(3):1278-89.*

The anaphylatoxins C3a and C5a are important effector molecules for many of the biological functions of activated complement. Both C3a and C5a bind to their cognate G protein-coupled receptors on the host cell surface. Compared with C5aR, which has been studied extensively in many complement-mediated immune and inflammatory responses and represents an attractive therapeutic target, our knowledge on the role of C3a/C3aR signaling in health and disease is still rather limited. In this paper, Mueller-Ortiz and colleagues demonstrated a striking phenotype of $C3aR^{-/-}$ mice in a model of *Listeria monocytognes* infection. The authors showed that C3aR plays a protective role such that in its absence mice had a higher mortality and increased bacterial burden, more severe liver damage, and elevated destruction of immune cells of the spleen important in *L. monocytogenes* clearance, including neutrophils, macrophages, dendritic cells, and T cells. The authors established that compromised immunity to *Listeria* infection is not due to defective macrophages or impairment in critical cytokine/chemokine production known to be critical for the initial clearance of the bacteria. Rather, they showed by TUNEL staining, together with Fas, active caspase-3 and Bcl-2 expression studies, that the increased susceptibility of $C3aR^{-/-}$ mice to *Listeria monocytognes* infection was largely caused by increased bacteria-induced apoptosis of myeloid and lymphoid cells. Although the exact mechanism by which C3aR deficiency caused this phenotype and the cell types on which C3aR confers the observed protection remain to be determined, this interesting paper provides a striking example of the important role of C3a/C3aR signaling *in vivo*, and one that could be used as a model to dissect novel immune regulating functions of the C3aR pathway.

Reported by Wenchao Song, University of Pennsylvania, USA

NEWS FLASH 2: Monitoring and Cell-Specific Deletion of C5aR1 Using a Novel Floxed GFP-C5aR1 Reporter Knock-in Mouse. *Karsten CM, Laumonnier Y, Eurich B, Ender F, Bröker K, Roy S, Czabanska A, Vollbrandt T, Figge J, Köhl J. J Immunol.194(4):1841-55. Epub 2015 Jan 14.*

In this paper, Karsten et al reports a new line of C5aR GFP knock-in mouse which simultaneously carries a floxed C5aR allele to allow tissue-specific deletion of C5aR. Many of complements' effector functions, from inflammation to regulation of adaptive immunity, are mediated by the anaphylatoxin receptor C5aR, a classic G protein-coupled receptor expressed highly on myeloid lineage cells. With the expanding role of C5aR in immune responses in mouse models of infection, autoimmunity, allergic response and organ transplantation, the mechanism by which how C5aR-mediated signaling contributes to inflammation and regulation of cellular immunity has become an important topic. In particular, while there is general consensus that C5aR is expressed on murine antigen-presenting cells such as macrophages, and perhaps certain sub-populations of dendritic cells in an organ- or disease-specific manner, there is conflicting data in the literature regarding whether C5aR is also expressed on murine T cells. Previously, a green fluorescent protein (GFP) knock-in mouse was generated in which GFP expression was under the control of the C5aR1 gene promoter and used as a surrogate of C5aR. Study of that mouse showed unexpectedly that naïve and activated mouse CD4 and CD8 T cells under the control did not express GFP. Considering that the GFP knock-in strategy in this study was different from that used in the previously generated GFP mouse (GFP knock-in at the second exon vs at 3'-UTR of the C5aR1 gene), this newly described mouse serves as a useful independent line to re-visit the issue of C5aR expression on various immune cells. Of note, authors of the current study likewise detected no GFP signal on naïve or activated T cells. This interesting paper thus describes an important research tool and draws new attention to an important issue regarding the mechanism of action of C5aR in murine models of T cell immunity and should be of interest to a broad group of complement investigators.

Reported by Wenchao Song, University of Pennsylvania, USA

COMPLEMENT TEAMS AROUND THE WORLD

Complement in Philadelphia, USA: George Hajishengallis' Team

Research in the Hajishengallis laboratory at Penn Dental Medicine of the University of Pennsylvania focuses on the interface of microbial pathogenesis and immunity and has illuminated novel mechanisms of dysbiosis and inflammation in periodontal disease. Our work has shown that complement is a major player in the pathogenesis of this prevalent disease, being involved in both the dysbiotic transformation of the periodontal microbiome and the ensuing destructive inflammation typified by loss of tooth-supporting bone. We combine basic scientific and translational research, leading to innovative therapeutic approaches to periodontal disease.

Our first "encounter" with complement occurred in the mid-2000s, when we discovered that certain periodontal bacteria activate Toll-like receptor 2 (TLR2) inside-out signaling to induce the high-affinity conformation of complement receptor 3 (CR3), which they exploit for safe entry into phagocytes. This excited our interest in further dissecting signaling crosstalk between TLRs and complement and started collaborating with complement experts, the team led by Dr. John Lambris at the Perelman School of Medicine of the University of Pennsylvania. We subsequently showed that "keystone pathogens" (*i.e.*, bacteria that exert community-wide effects while present in low abundance) modulate complement function to disrupt host-microbe homeostasis and promote dysbiosis. These pathogens express C5 convertase-like enzymes and generate high local concentrations of C5a for activation of the complement C5a receptor 1 (C5aR1) in tandem with TLR2. The resulting signaling crosstalk subverts the function of leukocytes, which now display impaired antimicrobial activity but enhanced inflammatory responses. This in turn ensures the persistence of the entire microbial community in a nutritionally favorable environment, as these bacteria rely on inflammatory tissue breakdown products for survival. We believe that the positive feedback loop between dysbiosis and inflammation drives the pathogenesis of periodontitis and recently implicated the central complement component C3 in the maintenance of this vicious cycle. Capitalizing on this discovery, we have moreover shown that C3 inhibition by the compstatin analog Cp40– a drug developed by John Lambris– blocks periodontal disease in non-human primates. Our findings pave the way to innovative and much-needed therapeutic strategies for the treatment of human periodontitis, which is our next and most challenging goal.

Besides John Lambris, we are fortunate to be among other leading experts in complement research including Wenchao Song (Perelman School of Medicine) and Hydar Ali (Penn Dental Medicine). We are glad to be based at the University of Pennsylvania, an international leader in the generation of new knowledge in both basic and translational biomedical sciences, and look forward to meeting with any of you from the international complement field visiting Philadelphia.

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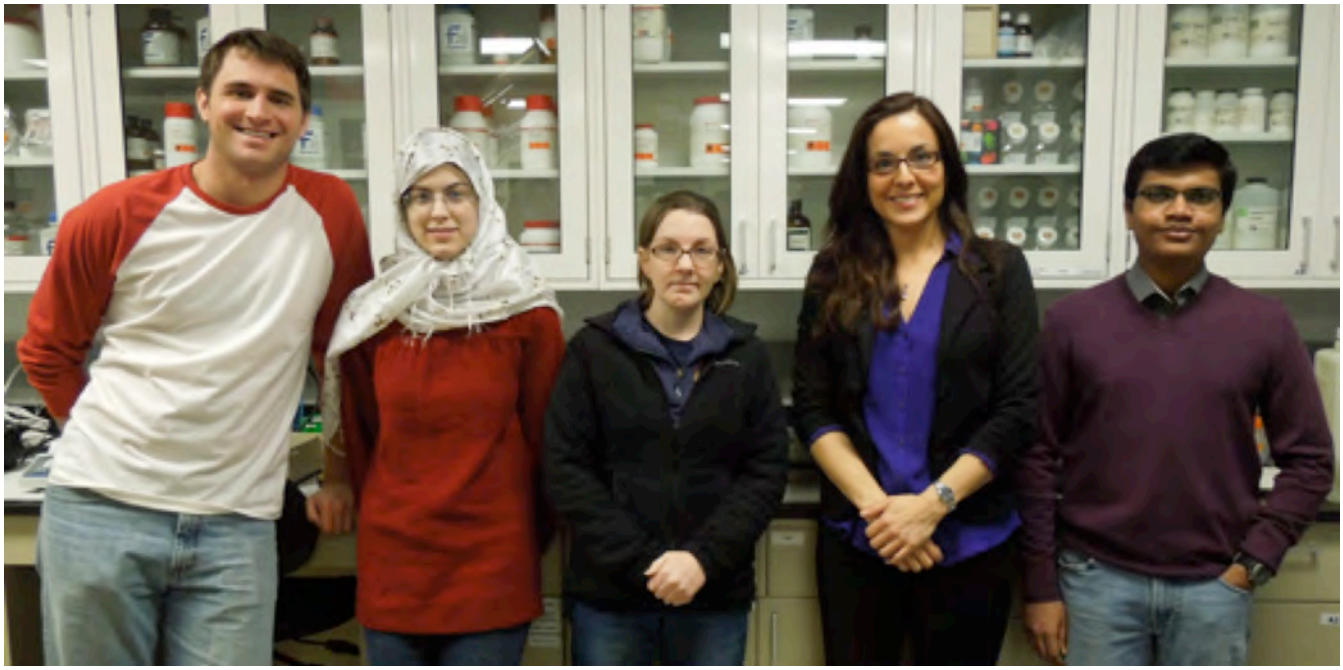
Complement in Ohio: Dr. Viviana Ferreira's Team

I received my D.V.M. and subsequently my Ph.D. from the University of Chile in Santiago, Chile. My Ph.D. thesis, co-directed by Drs. Arturo Ferreira (University of Chile) and Wilhelm Schwaeble (University of Leicester), defined the molecular basis of how *Trypanosoma cruzi*, the causal agent of Chagas' disease, uses its calreticulin to inactivate C1, as an immune evasion strategy. In 2004, I transitioned to a postdoctoral position in the laboratory of Dr. Michael Pangburn at the University of Texas Health Science Center at Tyler. Our research, which focused on human factor H, unveiled novel molecular mechanisms involved in the pathogenesis of atypical hemolytic uremic syndrome, paroxysmal nocturnal hemoglobinuria, and age-related macular degeneration. My amazing mentors were a continual source of encouragement during my training years and inspired me to begin my independent career in 2009 in the Department of Medical Microbiology and Immunology at the University of Toledo College of Medicine and Life Sciences. My research continues to focus on understanding the molecular mechanisms of activation and regulation of complement and has been made possible through the continuous support from the American Heart Association (since 2007) and recently from the National Institutes of Health.

We have previously demonstrated that the C-terminus of human factor H is essential for its ability to bind to the combination of C3 fragments and polyanions on cell surfaces, allowing the N-terminus to carry out its regulatory functions. These studies used a recombinant protein composed of the C-terminus of factor H (rH19-20) that competitively inhibited the interaction of factor H with cell surfaces, but did not affect factor H regulation in the fluid phase of blood *in vitro*. Collaborators are using our murine version of rH19-20 in various *in vivo* disease models, including renal disease, asthma, and arthritis, to elucidate the role of factor H in preventing disease pathogenesis.

Motivated by the work of Drs. Kemper, Hourcade and Atkinson that rediscovered the ability of properdin to initiate complement activation as originally described by Pillemer, we and others have contributed to defining properdin as a highly selective recognition molecule of the alternative pathway. Recently, we determined properdin binds human activated platelets and promotes alternative pathway activation, suggesting a role for properdin in cardiovascular disease. Platelets are the first blood cells that rapidly adhere to tissue, to each other, and to leukocytes in response to vascular injury. An increased number of activated platelet/leukocyte aggregates (PLA) are found in the blood of patients with inflammatory cardiovascular diseases and play a role in the initiation and progression of disease. We are investigating the molecular basis of how properdin and factor H regulate this cellular interaction, promoting the formation of PLA and thrombi in human blood. Understanding these mechanisms may lead to new treatments for numerous cardiovascular diseases. The long-term goal of my lab is to contribute to the basic knowledge that will allow the design of drugs to control complement, either by inhibiting its consequences in inflammatory diseases, or by enhancing its activity on unwanted cells (i.e. microbes or cancer cells). We look forward to initiating additional exciting and challenging collaborations with the extraordinary and highly collegial ICS network and beyond!



Research group (left to right): *Adam Blatt (M.D./Ph.D. student); Sabina Pathan (2nd yr. medical school student); Heather Emch, Phys. Assist. (Research assistant); Viviana Ferreira, D.V.M., Ph.D.; Koustubh Kulkarni, M.S. (Research assistant).*

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The **15th European Meeting on Complement in Human Disease (EMCHD)** will take place in Uppsala, Sweden, the 27th to 30th of June 2015. Abstract submission is now closed and the early bird registration dead line is May 12th 2015.

For further information, please go to the meeting web page:

https://akkonferens.slu.se/emchd2015/

The ICS 'has sponsored a 'Guest Society symposium' on Complement during the upcoming annual meeting of the American Association for Immunologists (AAI) held in New Orleans, LA, USA, May 8-12 2015.

For further information, please go to the meeting web page:

http://immunology2015.org/program/index.html

MEETING REPORT – ICW 2014 IN RIO DE JANEIRO

The 25th International Complement Workshop took place September 14-18, 2014 in beautiful Rio de Janeiro, Brazil, at the Copacabana and was hosted by Dr. Denise Tambourgi (Instituto Butantan, San Paulo) and colleagues. The remote venue imposed relatively higher travel expenses; nevertheless, 155 senior ICS members and 90 postdocs and students participated in this excellently organized conference and made it highly productive and rich in novel findings and concepts. The meeting was organized in 10 Scientific Sessions plus 3 Plenary Sessions in which selected invited speakers presented an overview of their work. The respective session chairs summarized the highlights of each session and the summaries on the Plenary Sessions I to III and on the Scientific Sessions I to III were published in the last issue 36 of Focus on Complement. The summaries for the Scientific Sessions IV to X are below. We thank all session chairs for their contribution during the meeting and for summarizing their sessions for us all to read and remember.

Session IV: C3a, C5a and their receptors in health and disease I
(Chairs: Mohamed Daha, Leiden, The Netherlands and Jörg Köhl, Lübeck, Germany)

In the first presentation (Abstract 77), Bosmann *et al*. showed that intra-tracheal administration of C5a results in acute lung injury (ALI) with disruption of the alveolear/epithelial barrier and infiltrating neutrophils. C5a drove phosphorylation of Akt, MEK and ERK1/2, the inhibition of which markedly reduced C5a-induced ALI. Also, C5a liberated CCR5 ligands CCL4 and 5 from neutrophils suggesting involvement of CCR5. In line with this, C5a-mediated ALI was attenuated in CCR5-deficient mice. Karsten *et al*. (Abstract 195) uncovered a novel anti-inflammatory mechanism, by which plasma cell-derived IL-10 suppressed C5a-mediated functions in neutrophils. The authors induced a polyclonal plasma cell response and when they induced C5a-mediated peritonitis in such mice, they were almost completely protected from C5a-induced attraction of neutrophils to the peritoneum. IL-10 suppressed C5a-mediated migration of bone marrow-derived neutrophils and CD11b upregulation in such cells. This suggests that IL-10R1 signaling directly intersects with C5aR1 signaling that promotes neutrophil migration and may explain the immunosuppression observed in conditions of massive expansion of plasma cells such as B cell tumors or autoimmune diseases. Next, Schröder *et al*. (Abstract 079) identified a novel role for C5a in the induction of auto-Abs in an experimental model of collagen-induced autoimmune skin blistering disease. Wild type (wt) mice had skin blistering and inflammation as early as 4 weeks after immunization while $C5aR1^{-/-}$ showed later disease onset and a milder disease phenotype. Importantly, collagen VII-specific auto-Ab titers and the frequency of Ag-specific aut-Ab-producing plasma cells were significantly lower in $C5aR1^{-/-}$ mice. This reduction was associated with a decreased potency of such auto-Abs to drive ROS release from neutrophils and suggest that the C5a/C5aR1 axis does not only drive inflammation during the effector phase of autoimmune skin blistering disease but is also critical for the induction of auto-Ag-specific plasma cells. In the final presentation (Abstract 85), Alexander *et al*. showed that the adaptive immune response in focal glomerulosclerosis (FGS) depends on DAF and the C5a/C5aR1 axis. When they injected sheep anti-mouse podocyte IgG Abs into wt mice, they developed FGS, whereas $DAF^{-/-}$ mice were protected, which was associated with increased cellular but decreased humoral responses. Transplantation of either wt kidney into $DAF^{-/-}$ mice, or vice versa, revealed that systemic DAF was critical for protection. T cell transfers into nude mice and T cell depletion in wt and $DAF^{-/-}$ mice identified T cells as the critical cells type mediating FGS. Importantly, C5aR1 deficiency normalized the impact of DAF-deficiency on cellular and humoral immunity resulting in decreased nephrotic proteinuria in $C5aR1^{-/-}DAF^{-/-}$ mice. Mechanistically, C5a triggered T cell response in FGS by activation of DCs through the C5aR1 axis.

Session V: C3a, C5a and their receptors in health and disease II
(Chairs: Anna Blom, Lund, Sweden and Rick Wetsel, Houston, Texas)
In this session, Carmen van den Berg (Abstract 72) presented data showing that *in vitro* C5aR1 is more susceptible than the Il-8 receptors, CXCR1 and CXCR2 to degradation by neutrophil derived serine proteases, Elastase and Cathepsin G. This indicates that impairment of function in response to C5a is probably more important in neutrophil dysfunction than the responses to IL-8. This observation pertains to pathophysiology of cystic fibrosis. Next Axel Vater (Abstract 164) presented a study showing that in a mouse model of pneumonia neutralization of C5a with the specific Spiegelmer NOX-D19 (a PEGylated L-RNA aptamer) protects against lung and extrapulmonary injury in pneumococcal pneumonia-induced sepsis, indicating that NOX-D19 may have therapeutic benefit in the treatment of pneumococcal sepsis. Wuding Zhou (Abstract 35) described a study supporting the importance of the C3aR signalling for defence against kidney infection induced by uropathogenic *Escherichia coli*. For this purpose C3aR knockout mice were used. Administration of a potent C3aR agonist (WWTRRWRGDKLGLAR) significantly protected mice from kidney infection, suggesting that C3R agonism could be a potential treatment option for acute pyelonephritis in man. Rick Wetsel (Abstract 157) presented data on the protection provided by C5aR1 in infection with *Listeria monocytogenes*, a Gram positive intracellular bacterium capable of causing systemic infections. The mechanism of the protection was identified in prevention of *Listeria*-induced apoptosis of both myeloid and lymphoid cells by inhibiting expression of type I interferons and TRAIL. Finally, Veronica Schmitz (Abstract 154) presented data that examined the significance of cross-talk between C5aR1 and bradykinin receptor, B2R in the host response to infection with the intracellular protozoan, *Trypanosoma cruzi*. This study found synergy of C5aR1 and B2R signaling in induction of edema by trypomastigotes, internalization of trypomastigotes by macrophages, and IFN-γ production by antigen-specific T-cells, collectively indicating that C5aR1/B2R cross-talk is important in anti-parasite immunity.

Session VI: Complement and Disease II
(Chairs: Santiago Rodriguez de Cordoba, Madrid, Spain and Wenchao Song, Philadelphia, USA)
A novel mouse model of atypical hemolytic uremic syndrome (aHUS) carrying a prototypical factor H (FH) C-terminal mutation and recapitulating the most severe forms of the human disease, with thrombotic microagiopathy events affecting multiple organs, was presented by Wenchao Song's group (Abstract 54). The identification of the putative binding site for the anti FH autoantibodies associated with autoimmune aHUS in FH and FH-related 1 (FHR1) and subsequent analyses revealed structural differences that suggested to Sakari Jokiranta's group (Abstract 40) a hypothetical pathogenic mechanism for the generation of these autoantibodies. An interesting study from Peter Zipfel's group (Abstract 25) reported additional functionalities of the FHR5 and the FHR2::FHR5 hybrid protein associated with Dense Deposit Disease. Their data demonstrated an interaction of FHR5 with properdin and suggested a function for FHR5 in the AP amplification on cellular surfaces, which in the case of the FHR2::FHR5 hybrid protein is pathogenically elevated due to multimerization. The identification of C4 nephritic factor (C4Nef), alone or in combination with C3Nef, in a significant number of C3-glomerulopthy (C3G) patients by R. Smith's group (Abstract 31) illustrates further etiological heterogeneity in C3G.

Interesting data from the analysis of a $CD59^{-/-}ApoE^{-/-}$ mouse chemically induced to develop diabetes was presented by Dr. Halperin's group (Abstract 19) to support earlier findings that functional inactivation by glycation of CD59 confers high risk for CVD in diabetic patients. Lastly, the recognition by Dr. Lupu's group (Abstract 147) that the Gram + peptidoglycan causes a strong complement activation via all three pathways in baboons offered an important model to evaluate therapies based on complement inhibition in severe sepsis.

Session VII: Complement and Inflammation
(Chairs: Michael Holers, Denver, Colorado and Leendert Trouw, Leiden, The Netherlands)

The session on complement and inflammation was kicked off by a presentation by Gaelle Le Friec (Abstract 88), discussing isoform splicing and differential compartmental localization of CD46 which impacts on Th1 responses. Even though in resting T cells four isoforms of CD46 are expressed they are residing in different compartments suggesting different functions. CD46 isofoms can actively localize to the nucleus suggesting a role in interacting with intracellular complement fragments. In her presentation on the effects of intra-cellular factor H, Myriam Martin (Abstract 69) explained that following apoptosis factor H is actively transported to the intracellular space. In this intracellular environment factor H is functional and impacts on the degree of complement activation. During apoptosis C3 mRNA was upregulated and increased levels of iC3b were observed. Here the authors indicate that factor H may be important in the generation of the opsonin iC3b. The presentation of Katarine Bröker (Abstract 78) focused on the peritoneal B1 cell compartment. The B1 cells provide humoral protection via T-cell independent antibody responses. C5a was previously shown to impact on the B1 cell compartment and here the authors described where the C5a comes from and how it signals towards the B1 cells. Several stimuli (via TLR2 and IL-10) induced C5 production from macrophages that led to the generation of C5a via macrophage proteases. The C5a finally induced CXCL13 via C5aR2. Kristina Ekdahl (Abstract 74), the host of next years EMCHD meeting in Uppsala, Sweden, provided insight into their novel data on the contact activation of C3. This contact activated C3 now allows the tethering between activated platelets and neutrophils via complement receptors. Platelet derived microparticles from human serum expose membrane bound C3(H2O), which allows the interaction with PMN via CD11b/CD18. Importantly, this interaction takes place without the involvement of proteases. Adam Blatt (Abstract 70) explained the role of two opposite regulators of the alternative pathway, factor H and properdin on the formation of aggregates between platelets and leukocytes. Activated platelets serve as a platform for the initiation as well as the amplification of the alternative pathway. Both properdin and factor H can bind to the surface of activated platelets and impact on the degree of complement activation and on the degree of platelet leukocyte aggregation. Finally Dr. Rus (Abstract 71) showed us how initial findings made in complement, the identification of response gene to complement-32 (RGC-32), can now be translated into the shaping of the adaptive immune response. In this case specifically Th17 differentiation mediated by TGF-beta. RGC-32, initially cloned as a gene induced by C5b9 plays important roles in cell cycle regulation. Because of the relationship between RGC-32 and TGF-beta the authors now analyzed the effect of RGC-32 on one of the functions of TGF-beta, the induction of iTreg or Th17 cells. Pronounced effects were especially observed for Th17 cells differentiation.

Session VIII: Complement Diagnostics and therapy
(Chairs: Bo Nilsson, Uppsala, Sweden and Michael Kirschfink, Heidelberg, Germany)
This session was devoted to diagnostics and complement therapeutics. In the first contribution Frank Beursken (Abstract 117) reflected on the fact, that IgG forms a hexamer by non-covalent Fc interactions when it binds to antigens on a surface, thereby allowing C1q to dock efficiently to the complex. He presented data on monoclonal antibodies to various tumor antigens such as CD20, CD38 and CD52 that were mutated in the Fc region in order to increase the natural Fc-Fc interaction. By doing so they could increase the complement activating ability of the antibodies and the complement-dependent cytotoxicity on various tumor cell lines. Since the authors were able to also significantly improve CDC on cells from Chronic Lymphocytic Leukemia patients, this novel approach appears to be suited to increase the efficacy of antibody-based immunotherapy of hematological tumors. In her presentation, Marieta Ruseva (Abstract 113) focused on the efficacy of targeted complement inhibition in experimental C3 glomerulopathy (C3G). She investigated the efficacy of TT30, a recombinant mouse protein comprised of CR2SCR1–4 and FHSCR1–5, in two models of C3 glomerulopathy (C3G). By specific targeting tissue bound C3, TT30 protected the GBM from spontaneous or triggered C3 deposition in vivo, suggesting that TT30 might be therapeutically beneficial in C3G, where alternative pathway dysregulation determines disease development. Two new anti-C5 compounds were presented which are well under way to the clinic. The first one (SOBI002), now subjected to phase 1 trial, was presented by Patrik Strömberg (Abstract 114). This C5 inhibitor is from Sobi, Stockholm, Sweden, and combines the Affibody® technology with a technique to fuse the protein with an albumin-binding domain in order to improve half-life in the circulation. Wynne Weston-Davies (Abstract 119) introduced a protein called Coversin (Volution Immuno Pharmaceuticals, Geneva. Switzerland), which in addition to C5 also binds with high affinity to LTB4. Phase 1 results were promising and immunogenicity studies are still ongoing. The last presentation by Michael Kirschfink (Abstract 62) addressed the problem of tumour cell complement resistance, which to a large extend is due to an over-expression of the membrane complement inhibitors DAF, MCP and D59. The authors demonstrated that on HER2 positive tumors a significant antibody (Trastuzumab, Pertuzumab)-induced complement-dependent cellular cytotoxicity by NK cells, macrophages and PMNs could be achieved upon knockdown of membrane complement regulators. To enable specific tumor targeting, chemically stabilized siRNA was encapsulated in novel lipoplex structures (AtuPLEX). This strategy provides a valuable adjuvant to antibody-based cancer immunotherapy.

Session IX: Therapeutic complement inhibitors
(Chairs: Tom Mollnes, Oslo, Norway and Peter Garred, Copenhagen, Denmark)
It has previously been shown that the combined regimen of inhibiting complement and CD14 attenuates the inflammatory reaction induced by bacteria and improves survival in mice sepsis. In this session we learned that the same treatment, using a combination of the C5 inhibitor coversin (OmCI) and an anti-pig CD14 antibody improved survival in a pig model of polymicrobial sepsis (Abstract 171). Death was closely related to increase in pulmonary pressure and to the degree of complement activation, both substantially attenuated by the treatment. The same C5 inhibitor, which also binds LTB4, markedly reduced pig myocardial infarction size and improved cardiac function (Abstract 165). It was postulated that the reason for previous failing of complement inhibitors to be effective in human infarction was due to delayed treatment and/or insufficient complement inhibition. LTB4 neutralization might have an additional beneficiary effect.

Factor H (FH) is an efficient inhibitor of the alternative complement pathway. Different modifications of FH, including "miniFH" with enhances activity, have been produced. Here the authors have engineered a novel FH variant by deleting the central 10-15 CCP domains, resulting in encryptation of domains 19-20 (Abstract 173). This variant had a substantially increase binding affinity to C3 activation products, whereas is regulatory role on the surface of vulnerable cells was comparable to normal FH. These data extend our knowledge of FH in the regulation of AP and might lead to novel therapeutic constructs with therapeutic potential in a number of conditions since the amplification loop of the alternative pathway contributes to the complement-mediated tissue damage in many disease conditions. Recently an endogenous regulator of lectin pathway activation was discovered. This molecule known as MAP-1 or MAp-44 is a truncated splice variant expressed from the MASP1 gene. Previously it has been shown that the lectin pathway is important for the development of inflammatory arthritis in the collagen antibody induced arthritis (CAIA) mouse model of human rheumatoid arthritis using MASP knockout mice. Using adenovirus expressing MAP-1/Map-44 in the joints of wild type mice it was documented that MAP1/MAp-44 was able to inhibit joint inflammation and decrease complement activation (Abstract 162 Banda et al). This suggests a crucial and quite unexpected role of the lectin pathway in the pathophysiology of the CAIA model and illuminates a possible novel option for MAP-1/MAp44 in the treatment of inflammatory arthritis. By expressing chimeric molecules comprising full-length MAP-1/MAp44 and the first *N*-terminal SCR domains of FH it was shown that the fusion protein was a very potent inhibitor of both the lectin and the alternative pathways as well as the alternative amplification loop (Abstract 169). However, the orientation of whether FH was fused either with the *N*- or *C*-terminal part of MAP-1/MAp44 appeared to be crucial for the synergistic effect on the pathway activity. Last, but not least a new therapeutic approach against multidrug resistant gonorrhea using sialic acid analogues based on the knowledge how carbon substitutions on sialic acid were important for complement inhibition on gonococci were presented (Abstract 166).

Session X: Complement crosstalk with other systems

(Chairs: Berhane Ghebrehiwet, New York, USA and Claudia Kemper, London, UK)

The last session of the XV ICW, was yet another confirmation of the long-held, but tacit belief, that complement is ubiquitous and its functions may go deep into territories that were thought to be the forbidden domain of cellular immunologists. In fact we may just be beginning to scratch the surface. The crosstalk between complement proteins and basic cellular processes was underpinned by the work of the Kemper group, (Martin Kolev, Abstract 189), which showed that CD46 is critical in the metabolic re-programming required for acquisition of effector Th1 function. This involves CD46-driven expression of nutrient channels GLUT1 and LAT1 as well the mTORC1-mediated induction of high levels of glycolysis and oxidative phosphorylation in human $CD4^+$ T cells. The significance and impact of this observation in turn is strengthened by the observation that C3 or CD46-deficient patients fail to mount normal Th1 responses and consequently suffer from recurrent infections and possibly autoimmune diseases. The biological relevance of the crosstalk between complement and cellular pathways and its emerging role in intracellular signaling events was further demonstrated by Ulrika Krus (Abstract), who showed, that intracellular CD59 is required for pancreatic beta cell insulin exocytosis through formation of the exocytotic SNARE complexes, which allow fusion of insulin granules with the cell surface and the eventual release of insulin to the extracellular environment. Furthermore, while CD59 was found to be highly expressed in human and rat pancreatic islets, it was significantly lower in the islets of rodent models of diabetes.

The extrahepatic and *in situ* synthesis of complement proteins by various cell types, and the ability of these proteins to modulate the function of the cell that expresses or secretes them is a biological phenomenon that is catching on rapidly. The presentation of Mariana de Lucena Palma (Abstract 197) represents a continuation of this trend. Using functional polarization, this group developed two types of dendritic cells (DC): DC_1, which induces Th1 and DC_2, which induces Th2 responses. These cells were then examined for their ability to express/secrete a wide range of complement proteins and receptors. While DC_1 cells were found to produce almost all of the complement proteins and complement receptors tested (C1q, C1-INH, Properdin, C3, fD fB, fH, fI, CD55, CD46, CD97 and C5aR), DC_2 failed to secrete C1q, C3, fI and C1INH, regardless of the activation status. These results seem to suggest that the ability of DC_1 and DC_2 to activate and polarize Th cells may be influenced in part by complement proteins secreted in situ. The presentation by Mikkel-Ole Skjødt (Abstract 7), who showed that the MBL/Ficolin-associated protein-1 (MAP-1) can inhibit platelet aggregation, attenuate fibrin formation and inhibit the intrinsic pathway of blood coagulation is yet another example of the intimate and intertwined relationship between the coagulation pathway and the complement system and is likely to have a far-reaching significance in health and disease. HUS is induced by shiga toxin 2 (Stx2) producing *E.coli*, which has a direct effect on the integrity of podocytes and the impairment of fH. The data presented by Reinhard Wurzner (Abstract 14) now show that the deleterious effect of Stx2 on podocytes can be inhibited by serum amyloid P component (SAP), through upregulation of the anti–apoptotic Bcl2. Moreover, since proteins of the factor H (fH) family of proteins, FHL-1 and FHR-1 can compete with factor H, these molecules could have a therapeutic potential since free factor H would be predicted to fulfill its complement regulatory function and thus prevent kidney damage. That administration of an antigen with an antibody enhances the immune response to the antigen via antibody feedback mechanism has been known for some time. Along these lines, the presentation by Zhoujie Ding (Abstract 175) not only confirmed that administration of IgG3 together with ovalbumin induces a high antibody titer but also provided evidence that this enhancement is due to the ability of the IG3 to facilitate binding of the antigen to CR1 and CR2 expressing follicular B cells. When the experiment was repeated in mice lacking CR1/CR2, the antibody production was significantly impaired suggesting that IgG3-mediated enhancement of antibody production requires the expression of CR1 and CR2 on both B cells and follicular dendritic cells.

Last but not least, Robert Rieben (Abstract 10) underscored the biological relevance of the crosstalk between the complement and coagulation cascade. To prevent pig-to-human xenograft rejection, they generated a CD46 and HLA-E transgenic model to prevent complement activation and NK cell reactivity. These experiments showed that, while the transgenic CD46/HLA-E expression clearly showed reduced terminal complement activation and NK cell infiltration, complement inhibition by hCD46 was associated with down-regulated activation of the coagulation pathway.

COMPLEMENT REAGENT RESOURCES