



International Complement Society



European Complement Network

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Dear Readers,

Welcome to the December 2015 issue of 'Focus on Complement'.
This 40th issue of FoC contains:

- **Season's Greetings** from the ICS and ECN Boards
- **Flash News** reporting on a new complement regulator hybrid protein observed in a HUS patient and a novel role for complement in metabolic reprogramming during Th1 induction
- **The Complement research teams around the world** series featuring the teams of Gregers Rom Andersen, Copenhagen, Denmark and Suzan Rooijackers in Utrecht, The Netherlands
- Part II of the **meeting report** on the European Meeting on Complement in Human Disease (EMCHD) that took place in Uppsala, Sweden, June 27-30, 2015
- **XXVIth International Complement Workshop** announcement
- **Obituary: Prof. Eckhard R. Podack**

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

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P.O. Box 30
5400 AA UDEN
The Netherlands

T +31 (0)413 251 335
F +31 (0)413 248 353
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W www.hycultbiotech.com



Complement
Technology, Inc.
4801 Troup Hwy, Suite 701
Tyler, Texas 75703
1-903-581-8284 (Phone)
1-903-581-0491 (Fax)
E-mail: contactCTI@aol.com
Website: www.ComplementTech.com

On behalf of the Board of the 'International Complement Society' and the 'European Complement Network', we would like to take this opportunity to wish you all a very joyful festive season.

We hope that the past year has been a happy and successful one and sincerely wish that the coming year will be prosperous, healthy and peaceful for you, your families and your friends.

*Wishing you a Happy
and Peaceful
New Year 2016*



NEWS FLASH

A De Novo Deletion in the Regulators of Complement Activation Cluster Producing a Hybrid Complement Factor H/Complement Factor H-Related 3 Gene in Atypical Hemolytic Uremic Syndrome. Challis RC, Araujo GS, Wong EK, Anderson HE, Awan A, Dorman AM, Waldron M, Wilson V, Brocklebank V, Strain L, Morgan BP, Harris CL, Marchbank KJ, Goodship TH, Kavanagh D. J Am Soc Nephrol. 2015 Oct 21.

The Newcastle group together with their collaborators reports on a young patient who developed HUS in combination with infections. The authors identified a *Factor H-CFHR3* hybrid gene and reverse CHR1-Factor H hybrid proteins in plasma. The genetic work-up identified a *de novo* deletion in the regulators of complement activation gene cluster in the *Factor H* gene through micro homology mediated end joining. Deletion of exons 21-23 *Factor H*, and of the 3' untranslated region of gene causes aberrant splicing and brings exon 20 of Factor H in proximity to exon 2 of CFHR3. After demonstrating expression of a *CFHR/CFHR3* hybrid transcript the authors further confirm expression of an aberrant complement Factor H-CFHR3 hybrid protein. This hybrid protein is composed of Factor H domains 1-17 and has the C-terminal recognition region replaced by all five SCR domains of CFHR3 (i.e. SCRs1-5). This hybrid protein was encoded by one allele and observed in plasma together with wild type Factor H derived from the second allele. Further functional analyses of the purified proteins showed for the hybrid protein defective or reduced cell surface cofactor regulative activity and lower decay accelerating activation on the surface of sheep erythrocyte. This new hybrid protein together with related proteins that have been identified so far define a new scenario for disease pathology of aHUS and for treatment. Given the overall genetic instability of the Human *Factor H-CFHR* gene cluster one might expect that in the near future other diseases are linked to variations in this particular unstable human gene cluster.

Both NEWS FLASHES are reported by Peter F. Zipfel, University of Jena, Jena, Germany

Complement Regulates Nutrient Influx and Metabolic Reprogramming during Th1 Cell Responses. Kolev M, Dimeloe S, Le Friec G, Navarini A, Arbore G, Povoleri GA, Fischer M, Belle R, Loeliger J, Develioglou L, Bantug GR, Watson J, Couzi L, Afzali B, Lavender P, Hess C, Kemper C. Immunity. 2015 Jun 16;42(6):1033-47. doi: 10.1016/j.immuni.2015.05.024.

Complement meets Nutrition in adaptive Immunity: Immune cells need energy to develop into effector cells during immune responses. Energy sources for cells include glucose, together with amino acids and ATP. The groups of Christoph Hess from the University of Basel and Claudia Kemper from King's College London joined forces to show that complement plays an important role in regulating nutrient influx metabolic programming necessary for Th1 induction in human CD4⁺ T cells. The authors demonstrate that, in CD4⁺ T cells, CD28 drives autocrine C3 activation leading to CD46 crosslinking by C3b and activation of C3aR via C3a. In conjunction with T cell receptor ligation, these events induce expression of glucose transporter GLUT1 and amino acid transporter LAT1 and initiate glucose and amino acid uptake. Through subsequent mTORC1 activation, CD46 (specifically via its CYT-1 intracellular domain) thus drives glycolysis and oxidative phosphorylation (OXPHOS) critical for IFN- γ production. In addition, by studying T cells isolated from CD46-deficient patients, the authors can confirm that CD46 signaling is essential for the Th1 response as cells derived from patients who lack CD46 show defective glycolysis, OXPHOS and Th1 responses – of which all can be restored by transfection of CYT-1. Thus, this study provides an interesting link between the complement system and immunometabolic adaptation which is essential for human CD4 T cell effector function. This novel complement/metabolome link raises additional interesting questions, such as whether autocrine C3b mediated metabolic signaling also exists in rodent immune cells, which lack CD46, and also whether CD46 plays a similar role in other human cells which express CD46.

COMPLEMENT TEAMS AROUND THE WORLD

Complement in Aarhus, Denmark:

The team of Gregers Rom Andersen

Research on the functional aspects of complement has a strong tradition in Denmark, in particular three Danish teams in Copenhagen (Peter Garred), Odense (Søren Hansen) and Aarhus (Steffen Thiel & Jens Christian Jensenius) have made seminal contributions to our understanding of the molecular mechanisms of the lectin pathway. Since 2005 Professor Gregers Rom Andersen at the Department of Molecular Biology and Genetics at Aarhus University has determined crystallographic structures of C3, C4 and C5 and his research team is now almost completely devoted to the structure determination of complement proteins.

The Andersen group at Aarhus University is one of eight groups in large and well equipped environment for structural biology which has state of the art equipment for X-ray crystallography, small angle X-ray scattering, bio-NMR and electron microscopy.

Besides the initial structure of bovine C3, almost all efforts until 2011 concerned structure determination of C5 alone or in complex with a bacterial protein and cobra venom factor. The latter structure led to a general model for how convertases recognize their substrates, which is also compatible with the known properties of the eculizumab and other convertase inhibitors. Lately the group has studied the structures of C3a and C5a and in this aspect collaborated with the German biotech company Noxxon in order to discover how their spiegelmer recognizes C5a and prevent it from binding to C5aR1. The Andersen group is now working on clarifying the structure of one of the most intricate molecules in complement system, the C5 convertase.

The group is also increasingly collaborating with the team of Professor Steffen Thiel at the Department of Biomedicine with the aim of elucidating structures representing all the steps from activation of MASP-proteases until the assembly and activation of the C3 convertase. An interdisciplinary approach is taken involving also Aarhus University experts (Jan S Petersen and Bjørn Sander) in small angle X-ray scattering and electron microscopy.

One example of successful collaborative work was solving the structure of a MASP-2 fragment bound to C4, which also involved collaboration with the group of Peter Gal group in Budapest. Together with the structure of C4, a rather complete picture of the C4 cleavage was obtained. It turned out that the MASP-2 recognizes C4 in a somewhat unexpected manner not resembling the substrate recognition mode of convertases. Recently, the group has also described the structure of C4b to complete the 'C4 cleavage movie'. Another contribution was the new structural study on the MBL:MASP-1 complex which, in combination with a functional study from the group of Steffen Thiel, strongly suggested that activation of MASP-proteases is an intermolecular reaction.

The Andersen group is now investigating the structure of convertase complexes downstream of C4b.

Another line of research has been opened in collaboration with Professor Thomas Vorup-Jensen from Aarhus University, who is studying complement receptors 3 and 4 and their role in leukocyte adhesion through interaction with counter receptors but also their how they mediate phagocytosis of complement opsonized objects. The two receptors are delicate and complex two-chain integrin receptors, but as a first approximation the ligand binding domain from the αM subunit was used to represent CR3. Through a combination of X-ray crystallography and functional studies it was shown that the thioester domain of iC3b was the minimal ligand for CR3, and that this is completely distinct from the CR4 binding site in C3c earlier observed by Tim Springer at Harvard. A very recent study by Daniel Ricklin confirmed the CR3 binding site study by showing that C3dg-opsonized erythrocytes could be phagocytosed by monocytes in a CR3 dependent manner. Ongoing studies in the Andersen group aim at elucidating how a much more complete CR3 fragment recognizes iC3b and counter receptors like ICAM-1 and RAGE.

Although the past 10 years have resulted in an explosion in our structural knowledge regarding the complement proteins, the topic is by no means exhausted. In addition, the very recent progress in especially cryo electron microscopy opens the doors for structural studies of large and delicate non-crystallizable complexes of complement proteins. Such new structures will most likely be decisive in designing new complement inhibitors for therapeutic intervention. Gregers Rom Andersen welcomes any suggestions for collaborative efforts with the aim of determining complement relevant structures.



From left: Trine Gadeberg, Janus Asbjørn Schatz-Jakobsen, Gregers Rom Andersen, Sofia Mortensen, Rasmus Kjeldsen Jensen, Nick Laursen, Dennis Vestergaard

Contact: Prof. Gregers Rom Andersen, Aarhus University, Department of Molecular Biology and Genetics; Phone: + 45 87155507; E-mail: gra@mbg.au.dk

Complement in Utrecht, The Netherlands: Team of Dr. Suzan Rooijackers

Resistance to antibiotics among clinically important bacteria increases rapidly and alternative antimicrobial strategies are necessary to stop the rise of multi-drug resistant infections. Since the complement system is an extremely potent mechanism to kill bacterial cells, our research at the Department of Medical Microbiology, University Medical Center Utrecht is aimed at understanding the molecular basis of complement activation on bacteria. This way, we hope to improve desired complement activation by therapeutic antibodies or vaccination strategies in infectious diseases.

Previously, our work mainly focused on deciphering the mechanisms exploited by pathogenic bacteria to block complement. We discovered that bacterial pathogens secrete several small proteins to block the complement cascade. In collaboration with Prof. Piet Gros, we used one of these inhibitors (SCIN) to reveal the structure of the alternative pathway C3 convertase enzymes. Guided by the microbial inhibitors that others and we discovered, we have recently established novel methods to study complement activation in highly purified model systems allowing us to provide insight into complement activation processes on bacteria. We hope that these methods allow us to overcome previous technical limitations and characterize certain processes in the complement cascade that appear highly surface-specific (such as formation of C5 convertases [Berends et al, BMC Biology, 2015]).

In our lab we use a combination of microbiological, immunological and biochemical approaches to tackle these questions. At the University campus in Utrecht, we have a strong focus on molecular systems. We are very grateful to our local collaborations with strong complement experts (Piet Gros, Paul Parren) and many other (inter)national collaborators on complement and microbiology.



Research group (left to right): Top: **Maxime Duijst** (student), **Dani Heesterbeek** (PhD Student), **Anneroos Velthuisen** (student), **Ronald Gorham** (Post-doc). Below: **Bart Bardoel** (Post-doc), **Annemarie Kuipers** (PhD student), **Maartje Ruyken** (research technician), **Suzan Rooijackers** (P.I.), **Shi You Fu** (student), **Seline Zwarthoff** (PhD student). Missing: **Fernanda Paganelli** (Post-doc)

Contact: Dr. Suzan Rooijackers, Department of Medical Microbiology, University Medical Center Utrecht, The Netherlands; Phone: + 887556535; E-mail: s.h.m.rooijackers@umcutrecht.nl

ANNOUNCEMENTS



On behalf of the organizing committee, Dr. Teizo Fujita (Chair) and Dr. Nobutaka Wakamiya (President of the Japanese Association for Complement Research, JACR) invite members of the complement community and beyond to the 26th International Complement Workshop in Japan. The meeting will take place in the historical city of Kanazawa from September 4th to 8th 2016.

For further information, please see www.icwkanazawa2016.com

ALEXION PHARMACEUTICALS



Title: Research Scientist III, Protein Sciences

Location: Cheshire, CT, USA

Position Summary:

Provides leadership in identifying and prosecuting discovery research programs, specifically in the field of complement biology, and also in other disease pathways as needed; participates in proposing, identifying, evaluating new targets/programs for the research portfolio; provides leadership in designing screening cascades in aid of lead identification, in developing cellular and PK/PD assays in support of the discovery projects; participates in performing diligence activities in support of Business Development initiatives and in performing competitive intelligence analyses; establishes and manages external collaborations as needed.

Qualifications:

- Ph.D. in biochemistry/cell biology /molecular biology /pharmacology/structural-biology with 5-6 years of relevant industrial/academic research experience
- Extensive knowledge in complement biology, structure-function relationships, disease areas related to complement dysregulation
- A sound understanding of the theory governing macromolecular behavior
- Experience in research programs towards identifying therapeutic lead molecules is a plus
- Experience in collaborating/managing/directing within a matrix research organization desirable
- Ability to effectively allocate efforts amongst multiple projects and drive to aggressive timelines
- Good oral and written communications skills

MEETING REPORT – EMCHD 2015 in Uppsala, Sweden

The 15th European Meeting on Complement in Human Disease took place in Uppsala, Sweden, June 27-30 and was hosted by Bo Nilsson and Kristina Nilsson Ekdahl. According to tradition, the meeting started with a Teaching Day which was very well attended, and which also included specially invited lecturers. The conference had 360 participants who had submitted more than 250 abstracts, out of which 54 were selected for oral presentations. More than 2/3 of the submitted abstracts were directly related to the clinic and to diagnostic and therapeutic aspects of complement. The meeting was organized in 9 scientific sessions comprising oral presentations. The last issue of the Focus on Complement (issue 39) contained a summary of the first 5 sessions of the meeting – as usual, composed by the respective session chairs. This current issue now completes the summaries with an overview over the last 4 sessions of the meeting.

Session F: Therapeutics

Chairs: Daniel Ricklin (Pennsylvania/USA), Richard Smith (Iowa/USA)

The session included six talks exploring novel anti-complement strategies to address a variety of pathologies. Activation of complement is a key determinant of neural pathology and clinical disability after traumatic brain injury (TBI) and its inhibition is neural protective. The Cardiff group (Abstract 69) reported on a novel complement inhibitor made by fusing CRIg with CD59 to inhibit MAC at sites of C3b/iC3b deposition. In a mouse model of traumatic brain injury, this agent significantly reduced MAC deposition, suggesting that anti-complement strategies should be explored in TBI patients. Ra Pharmaceuticals (Abstract 156) reported on a potent cyclic peptide inhibitor of C5 that inhibits its cleavage to C5a and C5b. At low nanomolar concentrations, this agent blocks formation of the terminal complement complex and in cynomolgus monkeys is effective following single and multi-dose subcutaneous administration, potentially making it a novel therapeutic for patients with PNH. Christoph Schmidt and colleagues (Abstract 175) also reported on the treatment of PNH, comparing a variety of mini factor H constructs and a FH-CR2 fusion protein (TT30). The FH-CR2 fusion protein was more effective in preventing complement activation induced by LPS; however the mini FH variants were substantially more effective than FH-CR2 in preventing complement-mediated lysis of PNH-cells. These results show that variability amongst the C3-opsonin targeted inhibitors likely reflects the nature of the activator.

Surface-targeted complement inhibition was also at the center of the study presented by Fang Xiao and colleagues (Abstract 219). The group from King's College London evaluated the clinically developed convertase inhibitor APT070 (Mirococept), which combines a regulatory region of CR1 with a membrane-binding tether, both *in vitro* and in a humanized mouse model. They could show reduced inflammation and enhanced protection of islets from early damage, thereby potentially paving the way for improved cell therapies in type I diabetes. A different therapeutic use of CR1 was presented by Xue Xiao and colleagues from the University of Iowa (abstract 220): Whereas soluble CR1 (sCR1) had shown promise in C3 glomerulopathy (C3G), the Iowa group took the approach to the next level by establishing a transposon-based gene therapy strategy for constitutively expressing sCR1 *in vivo* and circumventing challenges with parenteral delivery of sCR1. In a mouse model of C3G, sCR1 was detected over the experimental period of 6 months and a significant impact on disease parameters by restoring C3 levels and reducing dense deposits in the kidneys.

Finally, Alnylam Pharmaceuticals provided insight into the data from preclinical and clinical evaluation of their C5-silencing RNAi therapeutic ALN-CC5 (Abstract 246). Preclinical studies in various animal models had shown an almost complete reduction in plasma C5 levels with marked impact on hemolytic activity and other disease markers after biweekly or monthly subcutaneous administration. Results from phase I trials showed a similar reduction of C5 levels in human volunteers and good safety/tolerability profiles after subcutaneous injection for this therapeutic candidate with suggested applications in PNH, aHUS and other diseases.

Session G: Mechanisms and Biosurfaces

Chairs: Jörg Köhl (Lübeck/Germany), Robert Rieben (Bern/Switzerland)

The first talk in this session was by Arun Cumpelik from Basel, Switzerland (Abstract 43). He presented data suggesting that ectosomes shed from platelets during sub-lytic MAC attack are responsible for the hypercoagulability seen in patients treated with anti-thymocyte globulin. Prasad Dasari, Jena, Germany (Abstract 45), then asked the question whether secreted fungal proteins could directly regulate T cell function. He found that complement regulator acquiring surface protein 12 (CRASP12) from *C. albicans* dose-dependently blocks CD3+CD28 induced IL-2 production and by this down-regulates T cell mediated anti-fungal reactivity. Nicolas Merle from Paris, France (Abstract 121), showed data on the influence of G protein-coupled receptors and TLR4 on heme-induced activation of endothelial cells. Marcela Pekna from Gothenburg, Sweden (Abstract 187), tried to convince the audience that not the kidney but the brain is the most interesting organ, also for complementologists. Her data support the idea of an anti-inflammatory effect of C3a by showing that C3a is neuroprotective in a mouse model of neonatal hypoxic-ischemic encephalopathy. The next Swedish speaker, Simone Talens, Malmö (Abstract 192), described a novel link between complement and the rest of the plasma cascade systems: C4BP, in particular the acute-phase variant of C4BP lacking the beta-chain, binds to and activates plasminogen, leading to an increased degradation of fibrin by plasmin. Finally, Wai-Hong Tham from Melbourne, Australia (Abstract 194), presented data on complement evasion strategies of the blood stage (merozoites) of the Malaria parasite *P. falciparum*. She found evidence for fH recruitment to the merozoite surface by the blood-stage specific Pf92 protein.

Session H: Infectious Disease

Chairs: Christine Skerka (Jena/Germany), Reinhard Würtzner (Innsbruck/Austria)

In this session, new evasion strategies used by different human pathogens were reported in four of the six talks and specific complement activities as response to microbial infections were discussed in the remaining two presentations.

New evasion strategies were presented by the group of Christoph Schmidt from Ulm, Germany (Abstract 68), who defined the interaction of the malaria protein PfRh4 within CR1, which is the docking molecule for malaria parasites on erythrocytes. They showed that PfRh4 did not interfere with the cofactor activity of CR1, but affected the decay accelerating activity, thereby identifying the active domains for complement regulation in CR1. Christian Meinel from the complement laboratory of Peter Zipfel in Jena, Germany (Abstract 119), showed that a new variant of PspC, isolated from pneumococcal aHUS isolates, was structurally and functionally different from the known PspC molecules. The new PspC variant bound significantly more factor H suggesting an enhanced complement immune evasion. David Emert from the laboratory of Anna Blom in Lund, Sweden (Abstract 51) reported on a novel virulence mechanism by *Streptococcus pyogenes*. They found an enhanced binding of complement regulator C4bp to the bacterial surface in the presence of exclusively human IgG. Finally, Jörg Köhl from the University of Lübeck, Germany, presented data from a collaborative work with Claire Chougnet from the Cincinnati Children's Hospital, USA (Abstract 128). They identified C5aR1 as enhancer of CCR5 mediated HIV infections. Interestingly C5aR1 can form hetero-dimers with the HIV-co receptor CCR5. Targeted reduction of C5aR1 expression in ThP1 cells reduced HIV infection by about 50%, thus introducing C5aR1 as a new target to reduce HIV infections. This may open a unique way for the development of novel therapeutics.

Investigating the human immune responses to infections, Per Nilsson, on behalf of Morten Harboe, from the Tom Erik Mollnes laboratory in Oslo, Norway (Abstract 67), reported on the dependence of properdin on C3b to bind to myeloperoxidase (MPO) and *N. meningitides*. By using complement inhibitors to block C3 cleavage or using C3-depleted serum they demonstrated that properdin is not a recognition molecule of these targets and rather depends on previous deposition of C3b to bind to *N. meningitides*. Kjetil Egge from the same complement laboratory in Oslo (Abstract 50) showed generally preserved effects upon combined complement and CD14 inhibition on Gram-negative and Gram-positive bacterial-induced inflammation during an escalating bacterial load.

Session I: Autoimmunity: Clinical and Diagnostic

Chairs: Christoph Schmidt (Ulm/Germany), Leendert Trouw (Leiden/The Netherlands)

Sophia Thanei (197) Abstract showed elegant work indicating that anti-C1q autoantibodies when bound to C1q can change the phenotype of macrophages *in vitro*. Incubating human monocyte derived macrophages with either C1q alone or with C1q and anti-C1q autoantibodies induced a pro-inflammatory phenotype with enhanced cytokine production upon LPS activation and decreased phagocytosis of apoptotic cells. Macrophages derived from SLE patients and healthy controls reacted in a rather similar fashion. The stimulation of macrophages was suggested to exacerbate the underlying pathological process of nephritis in lupus patients. Arun Cumpelik (Abstract 42) and colleagues set out to unravel the mechanism that governs the spontaneous resolution of the inflammatory joint disease gout. The study appears very relevant since the authors presented both: *in vivo* data derived from a murine model of monosodium urate crystals (MSU) induced peritonitis as well as *in vitro* data with patient material. The elegant work by Cumpelik et al showed that at early time points C5a production upon complement activation on MSU crystals primed the inflammasome for IL-1 β release. However, neutrophils that infiltrate in response to C5a signalling then released micro-vesicles which suppressed C5a-mediated inflammasome priming and thus inhibited further IL-1 β release and neutrophil influx in an auto-regulatory fashion. Mariann Kremlitzka (Abstract 101) explained that there may be more cross-talk between complement, Toll-like Receptors and cells of the adaptive immune system than previously anticipated. Clustering of CR1 on B cells had profound inhibitory effects on the combined stimulation of the BCR and several TLR (1/2, 7, 9) whereas in the absence of BCR signalling only the TLR9 stimulation was inhibited. Whereas B cells of SLE patients may have a reduced level of CR1 molecules on the cell surface, they responded equally well as B cells of healthy donors to CR1-induced inhibition of BCR-TLR combined stimulation. Reduced complement dependent signalling via CR1, due to hypocomplementaemia, may impact on B cell activity in SLE. To utilise microRNA (miRNA) as a novel biomarker of disease states in atypical atypical haemolytic uremic syndrome (aHUS) was the ambitious objective of the fascinating study presented by Elena Goicoechea de Jorge (Abstract 153). miRNA's act intracellularly as post-transcriptional regulators of gene expression, but are also secreted and found in peripheral body fluids. Goicoechea de Jorge and colleagues found that miRNA secretion was dysregulated in plasma samples of aHUS patients and identified a common miRNA signature among the tested aHUS patients. It is still early days, but hopefully, this interesting assay will prove its robustness and one day may help to predict disease progression and outcome in aHUS.

It seems that at this meeting the organizers have saved a very interesting study for last. In a very convincing presentation Diana Wouters (Abstract 238) discussed data on the destruction of erythrocytes in Autoimmune Haemolytic Anemia. Both IgM and IgG autoantibodies that target the erythrocytes have been described but some patients appear antibody negative. In this study it was shown that even in patients where it was not possible to demonstrate IgM on the surface of these cells that the complement activation inducing property was in the IgM fraction of size-separated patient serum. Next the authors demonstrated that the complement activation induced by these IgM antibodies only utilized the classical pathway with minimal involvement of the alternative pathway amplification loop.



OBITUARY

Remembering Prof. Eckhard R. Podack, the architect of polyC9 and perforin polymers



The field of Immunology lost two months ago **Eckhard R. Podack (1943- 2015)** who has his signature on the discovery of C9 and perforin polymerization. Four years ago Eckhard published in *Focus on Complement* issue #22 an obituary on Jürg Tschopp who was his and Hans Muller-Eberhard's post-doctoral fellow at the Research Institute of the Scripps Clinic in La Jolla, CA. Together, they performed pioneering experiments on the structure and functions of the C5b-9 complex and later on, on the perforin polymers of cytotoxic lymphocytes.

Eckhard received his medical degree from Johann Wolfgang Goethe University in Frankfurt (Germany) in 1968, and his doctoral degree from Georg-August University of Göttingen (Germany) in 1972. He also completed in 1974 a postdoctoral fellowship in biochemistry at the same University. He then joined the Department of Immunology of the Research Institute of Scripps Clinic in 1974, as a staff member, where he collaborated with the late Hans Muller-Eberhard on the characterization of the complement terminal complexes. In 1984, Eckhard moved to the New York Medical College, Valhalla, NY to accept a position as Professor of Microbiology and Immunology, and in 1987, he was recruited to the University of Miami Miller School of Medicine as Professor of Microbiology and Immunology and Professor of Medicine and Oncology. In 1994, he became the Chairman of the department and in 1997, he also received the Sylvester Distinguished Professor Award from the Sylvester Comprehensive Cancer Center (Miami).

In the past four decades, Eckhard wrote or contributed to more than 300 professional articles, book chapters and monographs. In his post-Complement research he investigated the regulation of viral infection and cancer by perforin-1 and the control of intracellular bacteria by perforin-2. One of his last published studies, "Perforin-2 is essential for intra-cellular defense of parenchymal cells and phagocytes against pathogenic bacteria," appeared September 2015 in the *eLife Journal*. In a 2013 review article (*Immunol. Res.* 57: 268, 2013), Eckhard summarized briefly and elegantly his major findings on polymerization of C9, perforin-1 and perforin-2. Even though Eckhard strongly adhered to basic research, he was visionary in translating such findings into applied biomedicine to advance clinical care.

In the early 1990s, Eckhard created a monoclonal antibody to seek out and attach to CD30, a receptor on lymphoma cells. He sold the technology to Seattle Genetics, which developed Brentuximab vedotin, an antibody-drug conjugate designed to destroy specifically Hodgkin's lymphoma cells. Another of his accomplishments was developing a novel vaccination technology using secreted gp96, a chaperone of the endoplasmic reticulum, linked to specific antigenic peptides. This vaccine generates CD8 potent cytotoxic T lymphocyte responses that may be targeted against cancer, HIV or malaria. In 2008, Heat Biologics, Inc. was established to translate the Heat's IMPACT technology Eckhard invented into cancer therapeutic protocols. They now conduct stage I and II clinical trials using these therapeutics in patients with non-small cell lung cancer and bladder cancer. Eckhard chose to remain in his laboratory and was quoted saying "I'm not a businessman. I'm a scientist. I want to see what works and how it works". Eckhard and his team finally discovered that the TNF receptor/ligand pair TNFRSF25/TNFSF15 (TNFR25/TL1A) is one of the prime regulators of T regulatory cells (Treg). These findings have important implications in modulating the balance between immune activation and immune suppression.

Eckhard E. Podack: The Complement angle

Between 1974 and 1984 Eckhard's research was devoted to the terminal complement complexes. This work resulted in more than 45 papers on this subject and on vitronectin (S-protein), 27 of them together with Hans Müller-Eberhard, and with some ambitious post-docs who later became independent and highly productive senior scientists, including Jürg Tschopp, Björn Dahlback and Klaus T. Preissner. *Klaus is describing below his personal accounts on his collaboration with Eckhard below.*



Eckhard's first of a series of original and seminal papers on the purification of C6 and C7 and on the composition of the C5b-9 complex were published in 1976 in "The Journal of Immunology". In 1982, Eckhard, Tschopp and Müller-Eberhard published their first two papers on the polymerization of C9 in "Nature" and the "The Journal of Experimental Medicine".

I vividly remember Eckhard and his "eternal" smile from my 3 years at the Scripps Research Institute as a post-doctoral fellow of Hans Muller-Eberhard. Eckhard's office was just next to my lab bench and even though we investigated different "ends" of the complement cascade (I studied the C3 convertases), we met on a daily basis and had many scientific and other discussions. He was a gentle, pleasant, smart and wise person. Discussing science with him was always rewarding. It was a privilege and fun to be there when he showed to me very excited (and smiling) the first electron-microscopical images, just "out of the oven" of the poly-perforin rings that resembled the poly-C9 rings.

Eckhard is survived by his wife Kristin and daughters Verena and Eilika. On behalf of all us, fellow complementologists, I extend our heartfelt condolences to Kristin and the entire Podack family.

Written by Zvi Fishelson, Department of Cell & Developmental Biology, Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel

The following contribution has been composed by Klaus T. Preissner, Department of Biochemistry, Medical School, Justus-Liebig-University, Giessen, Germany

Some anecdotes from the scientific work and life as a postdoc in La Jolla during the early 1980ies

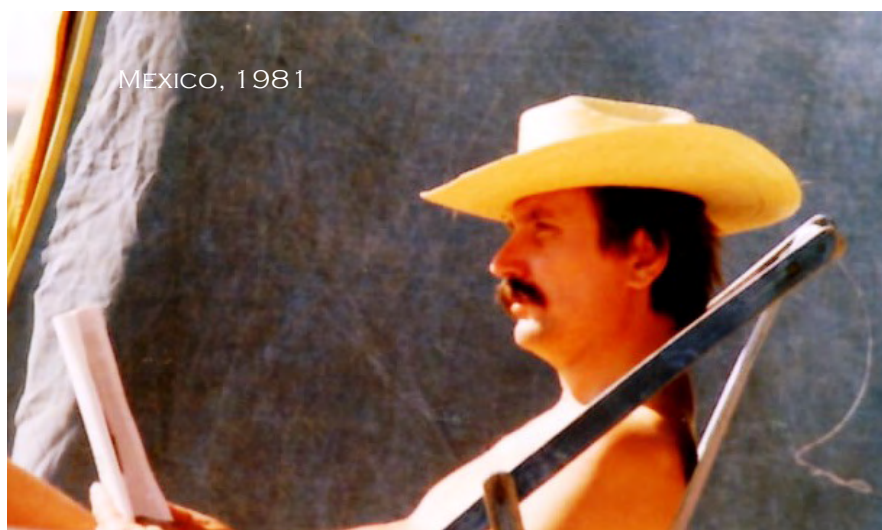
On the occasion of a seminar invitation in 1979, Eckhard Podack visited the Institute for Physiological Chemistry in Cologne (Germany), where I just completed my PhD in Biochemistry. Our meeting not only influenced but completely oriented my scientific career towards the field of immunology, inflammation and blood vessel research: After Eckhard's talk about the terminal complement components and their assembly within the "membrane attack complex" (poly-C9 had not been recognized), I really fell in love with this biomedical topic as such but also with the combinatorial technological approaches he presented to solve biological questions.

Above all, Eckhard not only had an ingenious imagination and molecular feeling to plan and execute the next “right” experiments, but he was an excellent researcher who loved to visualize the results of his work, at best (and many times a day) by “eye inspection” with the help of an electron microscope. This led him to discover the molecular structures of the poly-C9 pore of the membrane-attack-complex as well as the perforin pores and much more.

When I moved to La Jolla in 1980 to join Eckhard’s group at Scripps (after being accepted as a post-doc by Prof. Müller-Eberhard), he not only picked me up at the San Diego airport and invited me to my first Mexican dinner in Del Mar, but he helped me and my family a lot to get acquainted with the scientific and social life in Southern California. While a large number of dedicated international scientists worked on literally each complement factor in the department of Hans Müller-Eberhard, some postdocs and established researchers from Germany and Switzerland, including Eckhard, Alfred Esser, Charlie Vogel, Jürg Tschopp (who sadly passed away in 2011), Rolf Horstmann and myself, brought some European fresh air into the group.

Based on the great assistance from all members of the lab, even disappointing data turned out to be of instrumental help for future experiments to come. Here, Eckhard shared a great portion of the temporary disappointment and always had constructive suggestions for alternative solutions to “compose” a nice scientific story that could be wrapped up for publication. The daily communications and battle of words between the C1-, the C3-, the alternative pathway- or the membrane attack-labs were really stimulatory for everyone, but never disgusting. In my memory, after a while, complement numbers transformed into individual lab members who served to represent the respective character of a given complement protein, such that the entire complement group grew into a big family adventure. As complement “terminator”, Eckhard was a role model in sure instinct, motivation and teaching and became a real friend.

After returning to Germany in 1983 and establishing my own group in Giessen, I imported one of the complement inhibitors, S-protein (vitronectin), into the lab and, among other vascular biology topics, remained engaged with this multifunctional adhesive protein and its receptors for quite some time. Out of this endeavor, the first International Vitronectin Workshop was organized 1993 in Marburg, and among many new and old friends and colleagues in the field, Eckhard and his wife Kristin joined us for this unforgettable meeting. With his wholehearted engagement and internationally recognized performance in science as well as his warmth and sense of humor he will be missed by all of us.



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