Focus on Complement

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Issue 30

International Complement Society

European Complement Network

Dear members of the complement community,

Welcome to the June issue of the 'Focus on Complement' in 2013. This 30th FoC issue contains:

- A **Presidential Address** by the ICS President Zvi Fishelson.
- **Flash News** in this issue will present a publication that suggests that C5a plays a key role in airway remodeling and a paper that delivers a novel role for the factor H-related proteins in the *in vivo* control of complement alternative pathway activation.
- **Complement research teams around the world.** In this issue, we are introducing two research teams working on complement in Brazil: the groups of Drs. Anete Grumach and Iara Messias-Reason. We are looking forward meeting these teams 'in person' at the XXVth ICW in Rio de Janeiro in 2014!
- **Dr. Otto Götze Obituary**

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 Zvi Fishelson; Claudia.kemper@kcl.ac.uk or lifish@post.tau.ac.il .

PRESIDENTIAL ADDRESS

From the President:

Dear Colleagues:

About "Focus on Complement"- It is difficult to believe that we are already sending out Issue #30 of the bulletin. In April 2006 we established the bulletin and composed Issue #1. We published in it our "Mission and Vision" and I quote: "*The mission of Focus on Complement is to create a forum for tighter and more frequent interactions between the ICS and ECN Boards and Complementologists all over the world, a forum for rapid spreading of novel findings on Complement proteins and their activities and on novel reagents, a forum for open discussions of scientific and community matters (news and views) and as a platform to familiarize the Complement community about the past and present of Complement research and research teams at large. The vision is that Focus on Complement will disseminate a Complement passion to research groups around the world and will attract to the Complement community new members that will fertilize the Complement oriented research. Thus, the Bulletin will advance, even outside the Complement community, the perception of Complement as an important, and often essential, element in Innate and Acquired Immunity in health and disease.".* Since then numerous complement teams worldwide have been presented in the bulletin and we are planning to prepare an interactive world map that will allow each of us to reach any team with one mouse click. We have had several wonderful historical perspectives on the development of complement research and more will come. Highlights from the ICS and ECN meetings were and will be summarized for those unable to attend the meeting and for all of us to remember. If you have missed any of the past issues, you can link to our website www.complement.org and find it in the Archive. All this was made possible by a wonderful team of dedicated editorial board members whose names are shown on the first page of each bulletin issue. The conductor on issues #12-28 was the capable Berhane Ghebrehiwet from Stony Brook, New York, and the wheel is now in the talented hands of Claudia Kemper from London. Remember that this bulletin is for you, so please share with us your ideas and wishes for its development.

Complement workshops on the horizon- We have two attractive meetings on the near and more distant horizon. Please come and join us on August 17, 2013 for the "14th European Meeting on Complement in Human Diseases". This ECN workshop will be held in Jena, Germany and is promising to be scientifically rewarding and historically impressive. Peter Zipfel and his organizing committee members are busy now composing for us an exciting program, not forgetting of course, to include half a day of sightseeing of the interesting historical places in Jena and Weimar. Next year, is the "25th International Complement Workshop" of the ICS that will be held on September 14, 2014 in Brazil, the country that will also host the World Cup in the same year and then the Summer Olympics in 2016. The host city for the ICW will be the beautiful Rio de Janeiro - so if you have dreamed about hearing the breaking news of complement research while dancing samba in Copacabana, save this date! Denise Tambourgi and her organizing committee promise that it will be memorable and I believe it! The oral presentations in the ECN and ICS conferences are selected from the most innovative abstracts submitted. Students and postdoctoral fellows whose abstract is selected for oral presentation are eligible for Travel Awards, so waste no time and rush to the benches to make the next discovery in the complement field and increase your chance to win a Travel Award.

Sister Complement websites- The homepages of the ICS (www.complement.org) and the ECN (www.ecomplement.org) await your visits and we await your comments and suggestions for further development of the websites. To make sure that you are not transparent on the ICS website, click on "Become an ICS member" and register. If you have registered in the past, would like to update your info and forgot your login information, contact us (lifish@post.tau.ac.il) and we shall send you the details. Rest assured that this is no bother to us.

We plan to add to both websites a webpage that provides information on complement clinical labs worldwide (with contacts), labs that can provide services and welcome collaborations, and links to selected clinical complement assays. Once the Complement Assays Standardization Committee, headed by Michael Kirschfink (Heidelberg) completes its analyses of the various assays, its main conclusions and recommendations will be posted on that webpage. Another committee that will soon end its discussions and reach resolutions to be approved by the ICS Board is the Complement Nomenclature Working Group headed by Claudia Kemper (London). The recommended complement nomenclature will be published and also posted on the ICS and ECN homepages.

Quo vadis: These are excellent days for complement research. Appreciation for the contributions of complement proteins, peptides and receptors to healthy and pathogenic processes is on the rise. We see an increasing number of papers on complement that are published by newcomers in the field; scientists and clinicians that have never attended our complement workshops. It is my main goal now to attract as many of them as possible to our workshops and activities and thus strengthen further the complement society. This can only be done with your help and support. So please do not think of us only in "workshop terms", but keep close contacts with the ICS and ECN Board members, share your ideas with us and by acting together our society will grow.

Best wishes to you all,

Zvi Fishelson

Flash News

Targeting complement component 5a promotes vascular integrity and limits airway remodeling.
Khan MA, Maasch C, Vater A, Klussmann S, Morser J, Leung LL, Atkinson C, Tomlinson S, Heeger PS, Nicolls MR. *Proc Natl Acad Sci U S A* 2013;110(15):6061-6.

Microvascular loss during acute rejection episodes causes local tissue ischemia and probably contributes to fibrotic remodeling and organ dysfunction in transplant recipients. Although decreasing complement component 3 activity greatly reduces the duration of tissue ischemia associated with allograft rejection, it also leads, paradoxically, to vasodilatation and increases microvessel permeability. Vasodilatation and increased capillary permeability can also be triggered by the anaplylatoxin C5a. Since the generation of thrombin is a well-established feature of both allograft and xenograft rejection, in the present study, Mohammad A. Khan and colleagues hypothesized that excessive C5a activation could occur if thrombin substituted C3-dependent C5 convertase at the graft site, via a recently described complement activation pathway. The authors demonstrate here, that $C3^{-/-}$ mice present with increased deposition of thrombin in graft microvessels, elevated systemic plasma concentrations of C5a, vasodilatation and increased vascular permeability. The use of a specific C5a inhibitor, NOX-D19, a PEGylated 44 nucleotide L-RNA oligonuleotide, also known as Spiegelmer, in the C3-deficient recipients of airway transplants significantly improved tissue oxygenation, limited microvascular leakiness and prevented airway ischemia, even in the absence of conventional T cell-directed immunosuppression. This study provides *in vivo* evidence for C5 cleavage in the absence of a classical C3b-containing C5 convertase and indicates a clinical benefit of simultaneously limiting both C5a and C3 complement activity during alloimmune injury.
Reporter: Denise V. Tambourgi

Dimerization of complement factor H-related proteins modulates complement activation *in vivo*.
Goicoechea de Jorge E, Caesar JJ, Malik TH, Patel M, Colledge M, Johnson S, Hakobyan S, Morgan BP, Harris CL, Pickering MC, Lea SM. *Proc Natl Acad Sci U S A* 2013; 110(12):4685-90.

Factor H (FH) is an abundant plasma protein, whose major function is to down-regulate C3 activation mediated by the alternative pathway and C3b amplification loops. FH mutations increase susceptibility to the renal diseases, atypical hemolytic uraemic (aHUS) syndrome and dense deposit disease (DDD). In addition, polymorphic variation of FH has been associated with additional important human disease states, including age-related macular degeneration (AMD) and meningococcal sepsis. It is now evident that variations in the complement factor H-related (FHR) genes also contribute to disease susceptibility and play a role in the pathology of HUS, DDD, AMD and systemic lupus erythematosus (SLE). The five FHR proteins (FHR1 to -5) with FH comprise a family of structurally related proteins. FH is a well-characterized negative regulator of complement C3 activation but the biological roles of the FHR proteins are poorly understood. In the present exciting article, Elena Goicoechea de Jorge and colleagues describe an important role of FHR proteins as competitive antagonists of FH to modulate complement activation *in vivo.* This process, which they termed as de-regulation, is mediated by the ability of FHR1, FHR2, and FHR5 proteins to form homo- and heterodimers. This structural property confers increased avidity for C3b, enabling these dimeric molecules to compete, at physiologically relevant concentrations, with FH for the ligand. Whether or not C3b will interact with FH or FHR proteins is influenced by microenvironmental factors, including C3b density, cell surface polyanions and local concentrations of FH and FHR proteins. An equally exciting and 'synergistic' publication by Dr. Santiago Rodrigues de Cordoba's group in JCI lends further insight into this novel mechanism, by which HFR proteins regulate complement activation *in vivo* http://www.jci.org/articles/view/68280).
Reporter: Denise V. Tambourgi

Focus on Complement Research Teams

Complement in Brazil: Prof. Iara Messias-Reason's Team

Our research group with a focus on Complement biology is directed by Iara J de Messias Reason. The team is situated in the Laboratory of Molecular Immunopathology (LIPM) at the Clinical Hospital of the Federal University of Paraná (HC-UFPR) in Curitiba, South Brazil. Since the 1990s, we have concentrated on delineating the role of complement activation in human infectious diseases. We initially performed functional studies on the interactions between the complement system and parasites such as *Strongyloides stercoralis* and *Leishmania braziliensis*. At that time, research grants in Brazil were scarce and a significant part of our work depended on successful collaborative studies, which played a fundamental role in the further establishment of our laboratory. Among our collaborators we wish to name are Profs. Michael Kirshfink (Institute für Immunologie, Heidelberg, Germany), Reinhard Würzner (University of Insbruck, Austria), Jens C. Jensenius (Aarhus University, Denmark) and Jürgen F. J. Kun (Institut für Tropenmedizin, University of Tübingen, Germany – in memorian). After the first decade of research, we expanded our area of expertise and initiated studies on polymorphisms in genes coding for complement proteins of the lectin pathway in conjunction with susceptibilities to neglected tropical diseases, including both purely infectious (Chagas disease, leprosy) and autoimmune diseases triggered by infections (rheumatic fever, pemphigus foliaceous). Joining into this new direction and becoming one of the leaders of our team is Prof. Dr. Shirley R. Utiyama: She has been working on the role of complement in autoimmune diseases and autoantibody generation, with special emphasis on celiac disease and rheumatoid arthritis. In line with the recent interest in complement in neurological pathologies and in development, Down Syndrome has also become a subject of increasing interest to our group. This was particularly triggered by the work performed by Dr. Renato M. Nisihara, who assesses the role of autoantibodies in patients with Down Syndrome. Dr. Angelica B. W. Boldt complemented the group in 2006 with her expert knowledge on the evolutionary and clinical investigation of complement gene haplotypes, at a time where the increase of research investments by the Brazilian government allowed significant advancements and expansions. So far, about fifty students performed their master, doctorate and/or post-doctorate studies in our group. Currently, our main areas of research puts further focus on the studies on the contributions of polymorphisms in genes of the lectin pathway, notably *MBL2, FCN2, FCN1, MASP2* and *MASP1* in the susceptibility to and clinical progression of infectious and autoimmune diseases. We are also working on the role of lectin pathway protein gene polymorphisms in HCV infections and HIV/HBV/HCV co-infections. More recently, our group has initiated a new and rather unusual line of research with emphasis on medicinal plants: We explore the popular use of typical Brazilian medicinal plants by indigenous populations in the treatments of allergies and autoimmunity. So far, we have achieved promising outcomes, which resulted in two patents for the application of these 'drugs' in the treatment of asthma and osteoarthritis. Our ambition is now to test in clinical studies these natural products whose effect has been demonstrated in experimental and in *in vitro* studies by us.

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Dr. Anete Grumach's Team

Our laboratory is located at the Faculdade de Medicina da Universidade de São Paul, Department of Dermatology, São Paulo/SP, Brazil. The specialty of our group is the development of a national register for the identification and further characterization of patients with complement deficiencies in Brazil (Grumach *et al*. Brazilian report on primary immunodeficiencies in children: 166 cases studied over a follow-up time of 15 years. J Clin Immunol 1997). Our group has been following patients with primary immune-deficiencies now for almost two decades and the very first studies were developed at the Faculty of Medicine, University of São Paulo. Brazil is a huge country and we had increasing numbers of patients and cases with suspected congenital or acquired defects in proteins of the complement system referred to us. Thus, we have had the opportunity to assess and discuss these patient groups over the last years and among the first studies and cases that firmly established our interest in this area were the descriptions of a family with congenital C3 deficiency (Grumach *et al*. Inherited C3 deficiency of the complement system. Braz J Med Biol Res 1988) as well as several subsequent studies connecting mutations in and defects of proteins of the complement system to other immunologic diseases and clinical conditions, including IgA deficiency, agammaglobulinemia, diabetes and 21OH-hydroxylase deficiency. We furthermore reported on very rare conditions such as congenital factor I and H deficiencies (Leitão *et al*. Complement factor I deficiency in a family with recurrent infections. Immunopharmacology 1997; Falcão *et al*. Deficiency of the human complement regulatory protein factor H associated with low levels of component C9. Scand J Immunol 2008). Excitingly, these initial years of research have cumulated in the establishment of a Brazilian network named BRAGID (Brazilian Group of Primary Immunodeficiencies). This network now makes it much easier to share pertinent information fast among researchers and clinicians and fosters collaborative interactions among immunologists. Our initial years as well as our ongoing efforts in this direction have been and are supported generously by other groups throughout the world and we have established a long-lasting exchange program with Prof. Michael Kirschfink from the Institute of Immunology, University of Heidelberg, Germany. In the last years, our group has been focused on applying our knowledge clinically and we have developed tools for the earlier and better diagnosis of Hereditary Angioedema patients so that their chances of a success upon therapy are increased (Grumach *et al*. Hereditary angioedema: first report of the Brazilian registry and challenges. J Eur Acad Dermatol Venereol 2012). We are now extending these clinically relevant studies and our knowledge to other Latin American research groups and hospitals.

Our Clinical Immunology group currently consists of a PhD Biologist, Dr. Rosemeire Navickas Constantino-Silva and several graduate and post-graduation students that are working on complement-related projects in a specialized laboratory. On the clinical side, we train and mentor medical residents (2nd, 3rd and 4th year) as well as a medical assistant. Our invaluable connection to a large outpatient group is established via interactions with the Departments of Pediatrics and Dermatology and Pneumology, both members of the Faculty of Medicine ABC, São Paulo, Brazil.

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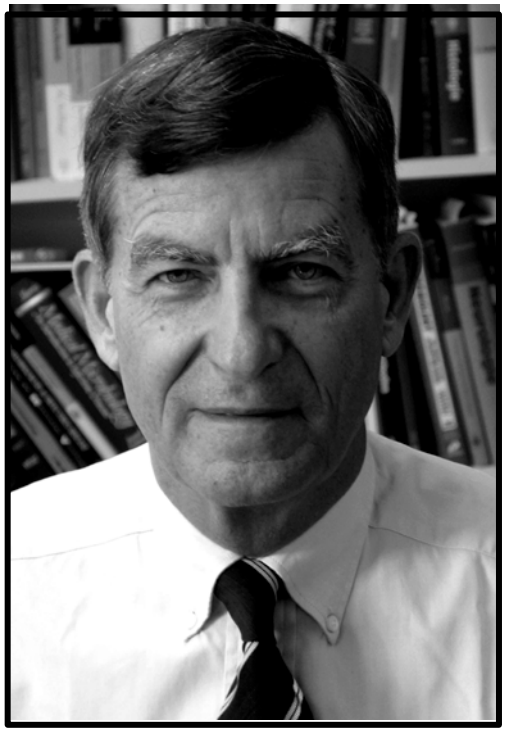
TRIBUTE to OTTO GÖTZE (1935 – 2013)

The following tribute was authored by Martin OPPERMANN and Reinhard WÜRZNER.

This obituary was originally published in the American Association of Immunologists (AAI) Newsletter [May-June 2013] and is published here with the kind permission of the AAI.



Professor Dr. Otto Götze, who devoted his scientific and medical life to innate immunity, and in particular to the alternative pathway of complement, died February 4th 2013 in Göttingen after a long illness, aged 77. He was head of the Department of Immunology at the University of Götttingen from 1979 – 2003.

Otto Götze was born on August 2, 1935 in Recklinghausen in the Ruhr area, but his family very soon moved to Hamburg where he spent his entire childhood and adolescence. He was always proud of his roots and his attitude was indeed typical for a “hanseatic” character, straightforward, but always a bit stiff like the winds in that region. Otto, as he was addressed in the lab, but not in his presence, where he was “Herr Götze”, was authentic, open-minded and well-respected by his co-workers and students, who enjoyed him as enthusiastic teacher and thoughtful mentor. His peers and professorial colleagues respected him as constructive and reliable partner endowed with a strong personal and scientific integrity which was probably the main reason for his high international reputation. Two of his scholars from his time in Göttingen, Martin Oppermann (MO) and Reinhard Würzner (RW), would like to review his scientific and medical career by dividing Otto Götze’s scientific life into four periods.

The first period, the sixties, or, to be more specific, from 1960, when he finished his medical studies at the Universities of Hamburg, Frankfurt, Paris, Munich and Freiburg with a doctoral thesis on an immunohematological topic, till 1968: During this time he received a basic clinical training and gained expertise in the laboratory of Herbert Fischer, one of the founding directors of the Max-Planck-Institute for Immunobiology in Freiburg. It was probably a first-author paper in ‘Nature’ on immune hemolysis of terminal complement components [1] which served as an entrance ticket to his second period.

In the second period, the seventies, 1968-1979, he joined Hans-Joachim Müller-Eberhard, one of the most prominent figures in the field of complement during that time, at the Department of Molecular Immunology of the Scripps Clinic in La Jolla, California. Otto was recipient of a DFG stipend and therefore brought his own money. He and his family often recalled memories from this stimulating and scientifically productive time at the pacific coast of Southern California. Müller-Eberhard's expanding and "roaring" research group was likely to be a perfect setting for an ambitious young scientist and complement was a hot topic revealing a number of mostly biochemical secrets during this time, often by the work of Müller-Eberhard's group. A number of complement factors were fractionated and characterised during this time and a complement "cascade" really became evident. Otto's personal merits are mostly represented by his work on the "alternative" pathway of complement, a concept of antibody-independent activation which at that time was not readily accepted by the scientific community [2]. With factors B and D he characterized central components of this pathway and his findings formed the core of a model detailing that this pathway was neither "alternative" nor an escape route, but actually the evolutionary much older, conserved, and thus true "classical" pathway [3-5]. A similar way of danger sensing by nonspecific recognition mechanisms was rediscovered more than 20 years later with the concept of pattern recognition molecules. At the end of that period Otto spent one year as visiting associate professor at the Rockefeller University in New York. During this second period he performed his seminal work on the alternative pathway of complement.

The third period, the eighties, 1979-1990: His first decade as professor and head of the Department of Immunology at the University of Göttingen, which can be summarized as a period of construction. He took the first chair in this at that time novel discipline "immunology" in Göttingen which had just previously been dissolved away from microbiology and generated a new research institute which quickly adopted its own identity. His scientific interests at that time focussed on two topics. First, in continuing earlier work from his time at Rockefeller he hypothesized that some complement proteins, e.g., C6, may not only be present in the fluid phase, but are also expressed on the cell surface as integral membrane proteins which upon activation-induced aggregation relay signals into the cell in a manner similar to immunoglobulins [6,7]. This fascinating concept, however, could not be verified.

The second topic of this time was the "quantitation of complement activation". He successfully used the monoclonal antibody technology, which was still in its infancy, together with J. Hinrich Peters and an arsenal of anti-complement monoclonal antibodies was raised in his lab in that decade [8]. These reagents, originally generated to characterise membrane complement proteins, later served as valuable tools for complement quantitation in various clinical settings. The methods in hybridoma technology were later published in the form of a laboratory manual [9]. This book became known as the "green book" when it was first published in German language, and sort of remained the green book, even when the cover of the internationally distributed English version later changed to blue.

Two highlights from his scientific achievements with therapeutic implications may be illustrated as examples for this period: First, the generation of blocking anti-C5a antibodies and their successful application in a pig sepsis model [10] and the characterisation of an anti C5 monoclonal antibody (N19-8) which was the first to successfully block both C5a release and activation of the terminal pathway [11]. This antibody was the fore-runner of Eculizumab, the humanised monoclonal complement blocker which was used to treat almost 400 German critically ill patients during the EHEC O104:H4 outbreak [12].
These research activities were accompanied by setting up a modern routine diagnostic laboratory for immunological parameters at the university clinics. Furthermore, even in administration of the medical faculty he quickly made his footprint - as both dean and vice dean and member of numerous university and clinical boards.

The 80s – that was the time when CD59 was not known yet, the role of complement in xenotransplantation and cell regulation completely neglected and no anti-complement drugs around. Many scientists in immunology were convinced that complement was dead! Otto instead constantly motivated his co-workers to remain faithful to the originally chosen field of complement, but at the same time advised the junior members of his group to build up an independent qualification in a related field such as nephrology, dermatology or microbiology. At the end of this period he received the gold medal of the European Complement Network (ECN) for his life-long achievements for both complement and the European complement society and one of us (RW) had the pleasure to deliver the laudatory speech.

The fourth scientific period ran from 1990 to Otto's retirement in September 2003. This event was marked by an international symposium held in his honour. During these last twelve years, a coincidential rather than planned exchange of reagents led to a fruitful cooperation with Otto's good friend from his Freiburg period, the late biochemist Kurt Jungermann. Together they identified the liver as a target of complement activation and thus established the modulatory effect of complement on metabolism [13].

Unlike other colleagues of his he basically stopped working in science almost immediately after becoming emeritus professor, so this symposiums was also the final point in a successful career as an active scientist, which is well documented in more than 120 publications, including several 'citation classics'. Less than 10% of his publications were not directly related to complement which illustrates the life-long focus of his scientific interests. The German Research Foundation (DFG) continuously supported his research throughout his career. Apart from numerous medical students who completed their dissertation in his lab, nine PhD students graduated and three post docs habilitated under his guidance. Two of his scientists fell in love with each other in his lab and started a family. Together with 23 other co-workers, colleagues or scholars they signed the obituary notice.

Otto influenced quite a number of scientists which today work not only in the field of immunology, but also in related fields including dermatology, nephrology, transplantation medicine, and microbiology. He fostered international exchange of his staff, attendances to international conferences and stays abroad. Above all he kept his scholars on a long leash and was confident that they do not require a day-to-day guidance to achieve scientific success. He was a fair senior advisor, fostering, above all, the autonomy of his scholars.
Otto was an outstanding scientist, devoted physician and engaged teacher and it was very sad seeing him passing away in the true sense of these words, gradually losing control and orientation over a period of several years. In his memorial address one of us (MO) detailed that, by seeing his demise, he was commemorated by Otto's own final point in his valedictory lecture: **"We are as confused as ever, but we are now confused on a higher level and about more important things"** – an expression Otto had often used earlier at various occasions. Otto positively influenced so many scientists, most of them still confused with daily scientific problems and less important things of life, whereas he is now on a "higher level" – he will not be forgotten.

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Complement reagent resources