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Issue 28

Focus on Complement

A newsletter of the International Complement Society

EUROPEAN COMPLEMENT NETWORK

About this issue & More

What's inside?

<1>Two flash news, are presented: (a) by Dr. Jörg Köhl on "the role of complement in intestinal tolerance, and (b) by Dr. B. Ghebrehiwet on " suppression by IgG1 antibodies of C5aR- mediated effector functions".

<2> Two "Complement Teams" are also presented: One team led by Dr. Denise Tambourgi in Brazil is presented by Dr. Jörg Köhl, and the second from the Institute of Molecular Biology in Armenia, is presented by Dr. Bob Sim.

<3>. Dr. Anna Blom also presents a delightful eyewitness account of the birth of "properdin". The author of this account, Dr. Wardlaw, was a member of Dr. L. Pillemer's team at Case Western Reserve in Cleveland.

<4>. A selected group of pictures from the recently held XXIV International Complement Workshop (ICW) in Crete is included. On behalf of the editorial board, I would like to thank Dr. A. Tenner for contributing these pictures.

<5>. This being the last issue I would edit, I should seize this opportunity to thank you **all** for making my job easy.

FLASH NEWS

Intestinal tolerance and C-Reporter: J.Köhl

Impaired intestinal tolerance in the absence of a functional complement system. Pekkarinen PT, Vaali K, Jarva H, Kekäläinen E, Hetemäki I, Junnikkala S, Helminen M, Vaarala O, Meri S, Arstila TP. J Allergy Clin Immunol 2012 October 22 pii:S0091-6749(12)01465-0 doi:10.1016/j.jaci.2012201209004 [Epub ahead of print]

The mechanisms underlying intestinal tolerance are incompletely understood. Dendritic cells have been shown to tip the balance between intestinal tolerance and immunity. The role of complement in this process has not been evaluated yet. Here, Petteri Arstila and colleagues describe a critical role for C3 in intestinal tolerance both in an experimental model and in patients. In the experimental intestinal tolerance model, the authors observed T cell proliferation in C3-deficient mice that was lacking in wild-type animals. This lack in tolerance induction was associated with a proinflammatory cytokine profile in the jejunum in C3-deficient mice as evidenced by high IFN-g and IL-17 levels. Importantly, this study shows human data that corroborate the findings obtained in the experimental tolerance model. In two C3-deficient patients, the authors found higher levels of IgG antibodies directed against commensal microorganisms as compared with age-matched controls. The shift of the B cell response was associated with a higher frequency of activated gut-homing CD4+ and CD8+ T cells. In summary, this is the first report showing a critical role for C3 in the regulation of intestinal tolerance. In future studies it will be important to delineate, which of the C3 and/or the C5 cleavage fragments and their corresponding receptors are involved in the C3-mediated regulation of intestinal adaptive immunity.

Suppression of C5aR by IgG1- Reporter: B.Ghebrehiwet

Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcgRIIB and Dectin-1. Karsten CM, Pandey MK, Figge J, Kilchenstein R, Taylor PR, Rosas M, McDonald JU, Orr SJ, Berger M, Petzold D, Blanchard V, Winkler A, Hess C, Reid DM, Majoul IV, Strait RT, Harris NL, Köhl G, Wex E, Ludwig R, Zilikens D, Nimmerjahn F, Finkelman FD, Brown GD, Ehlers M, Köhl J. Nat. Med. 2012 18:1401-1406

The C5a anaphylatoxin promotes inflammatory responses in autoimmunity, allergic asthma and sepsis, among other diseases. Strikingly, our knowledge about the regulation of C5a functions at the cellular level is scarce. In this study, Karsten et al. uncovered a novel anti-inflammatory feedback loop, by which IgG1 antibodies suppress C5a receptor-mediated effector functions in neutrophils and macrophages *in vitro* and in an experimental autoimmune skin blistering disease. Remarkably, they found that IgG1 require high Fc glycan galactosylation to exert their anti-inflammatory effect by engagement of the inhibitory FcgRIIB and the C-type lectin receptor Dectin-1. This is surprising, as FcgRIIB has been considered to pair only with activating FcgRs, whereas Dectin-1 is known as a pattern recognition receptor sensing fungi. Thus, galactosylated IgG1 and FcgRIIB exert anti-inflammatory properties beyond their impact on activating FcgRs. More than 20 years ago, researchers began to note that decreased Fc-glycan galactosylation is associated with increased inflammatory IgG properties in autoimmune diseases (RA, SLE, IBD), infections and tumors. The data from this study suggest that the increased inflammatory properties of agalactosylated IgG1 may result from their failure to inhibit C5a (and chemokine)-mediated inflammation.

Spotlight on teams-I

Complement Research in Brazil: The Butantan Institute in São Paulo.

Located at Butantan Institute, a 112-year-old institution, in São Paulo, the sixth biggest city in the world, the laboratory of Denise V. Tambourgi sets its focus on the interaction of the complement system with poisonous animals' venom and the role of this interaction on the genesis of systemic and local envenomation reactions.

She has started in the Complement field during her PhD with Professor Wilmar Dias da Silva, the father of the complement area in Brazil. In the 60's, in the laboratory of Irvin Lepow, Western Reserve University, Cleveland, USA, Prof. Wilmar's studies have provided the first evidence that anaphylatoxins were a product of the complement system. Back in Brazil, his studies have encouraged young students to start in the field, which was the case of Denise. Denise has done her PhD with Dr Wilmar, at the University of São Paulo, Brazil, and also at the School of Medicine of Yale University, New Haven, USA, under the supervision of Dr Keith Joiner. The subject of her PhD thesis was: "the analyses of *Trypanosoma cruzi* mechanisms of evasion to the lytic action of the Complement system".

In 1994, Denise moved to the Butantan Institute, which is the center for production of vaccines, anti-venoms and anti-toxins in Latin America. There, she started to study the action mechanisms of venom toxins from spiders, snakes and caterpillars and their interaction with the human complement system, in order to establish the basis for new therapeutic strategies for envenomation. This theme is of high relevance, since envenomation resulting from bites from poisonous animals is a particularly important public health problem in rural areas of tropical and subtropical countries. As an example, a recent study estimates that at least 421,000 envenomation and 20,000 deaths occur worldwide from snakebites alone each year, but these figures may be as high as 1,841,000 envenomation and 94,000 deaths per year. Because of this, WHO has included this condition in the list of "Neglected Tropical Diseases". In 1997, Denise conducted some studies on the action of spider venom toxins on complement regulators in Prof. Paul Morgan's lab, in Cardiff, UK. Since then, Denise and the Cardiff Complement group, including Dr Carmen van den Berg, have established a fruitful collaboration that continues to this day. Besides her interest in toxins, she has made efforts to develop the Complement field in Brazil, including the organization of the "Brazilian Meeting on Complement in Disease" in 2000, which had the participation of several established investigators from the ICS. Several students some of whom are presently working in Brazil and abroad attended this meeting. Following this endeavor of developing the field in South America, she is now more than happy to host the next Complement Workshop in Rio de Janeiro, Brazil, in 2014.

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***In the photograph*:** back row from left to right: Dr Cynthia Okamoto, Felipe França, Prof. Wilmar Dias da Silva, Dr Denise V. Tambourgi, Daniele Myamoto, Dr Carla Squaiella Baptistão, Priscila Hess Lopes. In the front row from left to right: Dr Gabriela Tanaka, Dr Daniel Manzoni, Mariana Torrente Gonçalves, Marie Delafontaine, Isadora Villas Boas, Dr Danielle Paixão Cavalcanti, Dr Giselle Pidde Queiroz.

SPOTLIGHT ON TEAMS-II

Complement Research in Armenia: The Institute of Molecular Biology in Yerevan.

The complement research team is a unit of the Laboratory of Macromolecular Complexes of the Institute of Molecular Biology located in Yerevan. The Institute belongs to the National Academy of Sciences of the Republic of Armenia. The team leader and head of laboratory is Prof. Anna Boyajyan. The complement-related studies of the group are focused on the role of the complement system in different diseased conditions including stroke, schizophrenia, posttraumatic stress disorder (PTSD), diabetes mellitus, familial Mediterranean fever (FMF), and radiation injury. The investigations mainly include human studies on functional activities of the complement pathways, complement proteins, receptors, regulators, and their genes. For a period of 10 years the team published 5 book chapters, contributed to about 50 journal articles, and attended 60 conference proceedings related to complement. In addition, 8 PhD theses focused on complement study were defended (supervisor Prof. Anna Boyajyan).

Recent studies of the team demonstrated the involvement of the alterations in the functional activity of the complement system in pathomechanisms of schizophrenia-associated immune system abnormalities including: increased activity of the alternative pathway and the level of membrane attack complex (MAC); increased expression of CR1 on erythrocytes, lymphocytes, monocytes, and neutrophils, increased levels of complement derived triggers of inflammation and apoptosis (Dr. Aren Khoyetsyan, Dr. Arsen Arakelyan, Andranik Chavushyan). In addition, Roksana Zakharyan and Hovsep Ghazaryan found that the rs291982*G allele of the *C1QB* gene and rs424535*A allele of *CFH* gene represent risk factors for schizophrenia, whereas rs12614*T allele of *CFB* gene might has a protective role in this disorder.

Further, the investigations fulfilled by the Population Eco-genetics group leader Dr. Karine Mayilyan, demonstrated that schizophrenia is associated with increased activities of the complement classical and lectin pathways, as well as decreased level of the C4B complement protein and haplodeficiency of *C4B* gene (*C4BQ0*) due to the absence of the C4 short gene locus in *RP-C4-CYP21-TNX* modules. A protective effect of the rare alleles of the *C2* rs1042663*A and factor B rs641153*A (32_Q) in schizophrenia was revealed. Studies performed by Dr. Gohar Mkrtchyan and Lilit Hovhannisyan demonstrated that pathogenesis of chronic PTSD is characterized by systemic alterations in functional activity of the complement cascade including hyperactivation state of the classical pathway, hypoactivation state of the alternative pathway, and deficiency of the C3 protein. Also this group demonstrated upregulation of the complement classical pathway in autoinflammatory FMF disease.Investigations implemented by Dr. Violetta Ayvazyan, Dr. Elina Arakelova, Dr. Gohar Tsakanova, and Ani Stepanyan demonstrated the involvement of classical, alternative, and lectin pathways of the complement in postischemic inflammatory response developed after human stroke onset and hyperactivation of the terminal complement pathway resulting in excess formation of MAC. The association of ischemic stroke with factor H functional polymorphism was also found.

Legend to picture: ***First row (L/R):*** Roksana Zakharyan, Dr. Gohar Tsakanova, Dr. Violetta Ayvazyan, Prof. Anna Boyajyan, Dr. Karine Mayilyan, Dr. Aren Khoyetsyan, Dr. Arsen Arakelyan; ***Second row (L/R):*** Andranik Chavushyan, Dr. Elina Arakelova, Dr. Lusine Zhamharyan, Ani Stepanyan, Tigran Hovsepyan, Lilit Hovhannisyan, Dr. Gohar Mkrtchyan

Dr. Meri Hovsepyan demonstrated hyperactivation of the complement classical pathway, as well as increased activities of the complement C1, C2, C3 and C4 components in long-term diabetes mellitus type 1 and type 2. In addition, the study revealed increased levels of MAC in type 2 diabetes patients, which probably results from a presence

of inactive glucated form of the negative regulatory protein CD59 that normally inhibits a formation of MAC. Studies performed by Dr. Lusine Zhamharyan and Tigran Hovsepyan using animal models of radiation injury indicated that pathologic processes induced by ionizing radiation strongly influences functional state of the complement system promoting inflammation and increasing severity of radiation injury. This group developed radioprotectors, cyclic amino acid derivatives, targeting the complement cascade, and diminishing cytotoxic effects of irradiation.

The complement research team has a long history of collaboration with the Oxford University immunochemistry unit leaded by prominent scientists, the expert in complement research, Prof. Robert Sim. This collaboration has been realized through implementation of joint projects, organization of joint conferences, and exchange visits and is reflected by a number of joint publications. The team members express sincere thanks to Prof. Sim for his kind continuous support, assistance and help.

The team invites collaborations and visits from other labs at any time.

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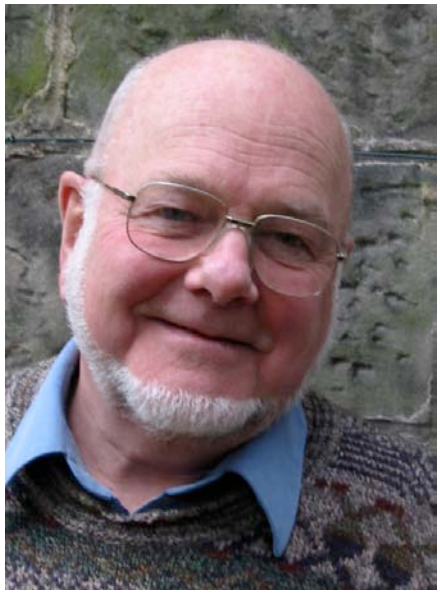
Birth of Properdin 1954
Reminiscences: by Alastair Wardlaw

Complement has four components: C-prime one, C-prime two, C-prime three and C-prime four". I can still hear in my mind's ear, nearly 60 years later, the voice of the late Louis Pillemer booming out in 1953, in Cleveland, Ohio. He was a big man, then in his mid-forties, which seemed ancient to me at age-23. He spoke with a bad stammer such as one rarely hears today. Typically he would be in his white lab-coat, beside the coffee urn, with a mug in one hand and gesticulating with a cigarette in the other. Around him on the hardwood floor were the stubbed-out butts of the several cigarettes he had already chain-smoked that morning. All the researchers, but not his secretary or the technicians, addressed him as "Pill". His main scientific co-worker was the late Irwin H. Lepow, whom I greatly admired and respected, and who was profiled in *Focus on Complement* (issues 3 and 4).



I had finished my PhD in Bacteriology at the University of Manchester in September 1953, and then travelled to America by steamship to take up a post-doc. research fellowship in Pillemer's lab. Transatlantic passenger jets were not yet in the skies, and only the very rich or influential could afford to fly in a Boeing Stratocruiser, the state-of-the-art propeller plane. For admission to the USA I had to present three medical documents: a valid vaccination certificate against smallpox, a full-size chest X-ray film to show I didn't have TB, and a negative result of a Wasserman test for syphilis. Arriving in downtown Cleveland by overnight train, I found my way along to the Institute of Pathology at Western Reserve University (as it then was). Peering into one of the labs, I saw Pillemer already at the bench, well before 8 a.m. He was all alone, pipette in hand, setting up a complement titration. That was how he worked: long hours, and doing crucial tests and purifications with his own hands. All the complement work was with human serum.

Immunology labs in 1953 were almost plastic-free. Pipettes, test tubes and other vessels were of Pyrex glass and were reused repeatedly. They were cleaned in a mixture of concentrated sulphuric acid and potassium dichromate and then rinsed exhaustively. There were no automatic pipettes with disposable plastic tips, no plastic vials or microtiter plates or ELISA readers or acrylamide gels, or indeed any electrophoresis equipment. Complement titrations were done in 4 x 0.5-inch glass test tubes, in volumes typically of 1.5ml, the fluids being delivered by mouth-pipetting with glass pipettes. Likewise bacterial cultures were grown in repeatedly-reused glass petri dishes. In 1953, HIV had not yet hit mankind but we were aware of hepatitis as a health hazard of human serum. Equipment-wise, we were well fitted out with controlled-temperature water baths, and refrigerated centrifuges. There was a Spinco preparative ultracentrifuge, and a Beckman spectrophotometer. We had a large -80^{o}C, 2-stage, mechanical, deep-freeze for storage of sera and

complement reagents. Although Lowry *et al.* had described their method for measuring proteins in 1951, I don't recall it being used in Pillemer's lab in 1953-55. Instead, protein was assayed by the Kjeldahl procedure.

Antibodies were already known from ultracentrifuge studies to exist in two molecular sizes, but the H and L-chain structure and the Immunoglobulin types still lay in the future, as did B-cells, T-cells, cytokines, HLA, monoclonals, protein and DNA sequencing, and PCR.

The four then-known complement components were abbreviated as C´1, etc, spoken as 'See-prime-one', rather than just C1. They were assayed by the serum reagents R1, R2, R3 and R4 in which respectively C´1, C´2, C´3 and C´4 were individually absent, but with all the other components present. The belief that complement had only these four components was asserted with total dogmatic conviction in 1953, just as DNA having four bases is stated today. The 3 sub-components of C1, and the esterase activity, had still to be discovered. The roles of Ca^{2+} and Mg^{2+} for complement action was well-established from treating serum with cation-exchange resin. Barbitone buffer with Ca^{2+} and Mg^{2+} was the standard diluent for complement.

Pillemer, for his main experimental variables, was steeped in the accurate manipulation of pH, ionic strength, temperature, time and centrifugal force. He showed that treatment of serum with zymosan, at the unusual temperature of 17°C for 1h didn't inactivate C3. However, it removed a serum component, soon afterwards called properdin, which was needed for any subsequent inactivation of C3 by fresh zymosan at 37°C. The properdin-depleted serum from the 17°C-zymosan treatment was called RP, meaning Reagent for Properdin. RP contained the four components of complement, but lacked the capability of having its C3 inactivated by zymosan at 37°C. Pillemer eluted the properdin from the 17°C-zymosan-serum pellet by raising the ionic strength to 0.6. Then by adding the much-purified properdin back to RP he reconstituted a serum, which allowed C3-inactivation by zymosan to proceed at 37°C, as initially.

My contribution in 1953-55 was to discover that the bactericidal activity of serum towards *Shigella dysenteriae*, and some other Gram-negative bacteria, depended on properdin acting with the then-four components of complement, and requiring Mg^{2+}. I had chosen the dysentery bacillus in the belief that Shiga dysentery didn't occur much in Cleveland, and that normal human serum from local blood donors would be unlikely to contain antibodies to it.

Another immunological paradigm in 1953 was for complement to be fixed only by an antigen-antibody complex. Pillemer's discovery of properdin seriously challenged that dogma. It indicated that the need for anti-bacterial antibody could be bypassed, with properdin substitution. The first paper on properdin, published in *Science* (**120**: 279-285, 1954) and on which I was a co-author, was entitled rather grandly 'The Properdin System and Immunity'. It became highly-cited, but also attracted a lot of adverse criticism from those committed to antibodies as necessary initiators of complement action. There was a period of eclipse and dismissal, especially after Pillemer's untimely death at age 49 in 1957. Then a further-dissected version of the Properdin System eventually re-surfaced under the label of "The Alternative Pathway".

I had to leave Pillemer's lab in 1955, after 2 years, because of visa limitations. I later worked in London, Toronto and finally Glasgow. My last original-research papers on complement were in the mid-1960s. These dealt with C5-deficient mice and were published with Hardi Cinader and Stan Dubiski, as the lead investigators, in well-respected journals (*J. exp. Med.* **120**: 897-924, 1964; *Nature* **205**: 97-98, 1965; and *Genetics* **7:** 32-43, 1966).

Information-processing was vastly different in 1953. It relied on paper, pen and ink, and secretaries with typewriters. The transistor may have been invented in 1947, but the low-cost silicon chip didn't come on stream until several decades later. Thus in 1953 there were no desktop or laptop computers, and therefore no word-processing or Powerpoint; there was no worldwide web, internet or e-mail. There was no Google or Wikipedia. There were no digital cameras, nor pocket calculators nor cell-phones. There wasn't even a photocopier in the department – none! - *can you believe that*! Nor had English then established itself as the world language of science. We also needed French and German. Life in 1953 obviously had narrower horizons and opportunities than today. On the other hand, a lot of biomedical investigations could be done without external funding by those working in diagnostic, teaching, or research-dedicated laboratories. These had automatic provision of budgets for support. These budgets generally contained some slack, which allowed for unscheduled researching on the side. The present imperatives for external grants, patents, citations and impacts are highly pressurized and judgmental, as compared with 60 years ago. Much more is expected now - and is delivered! Today's operator in a combine harvester cuts more corn than yesterday's peasants with their simple implements.

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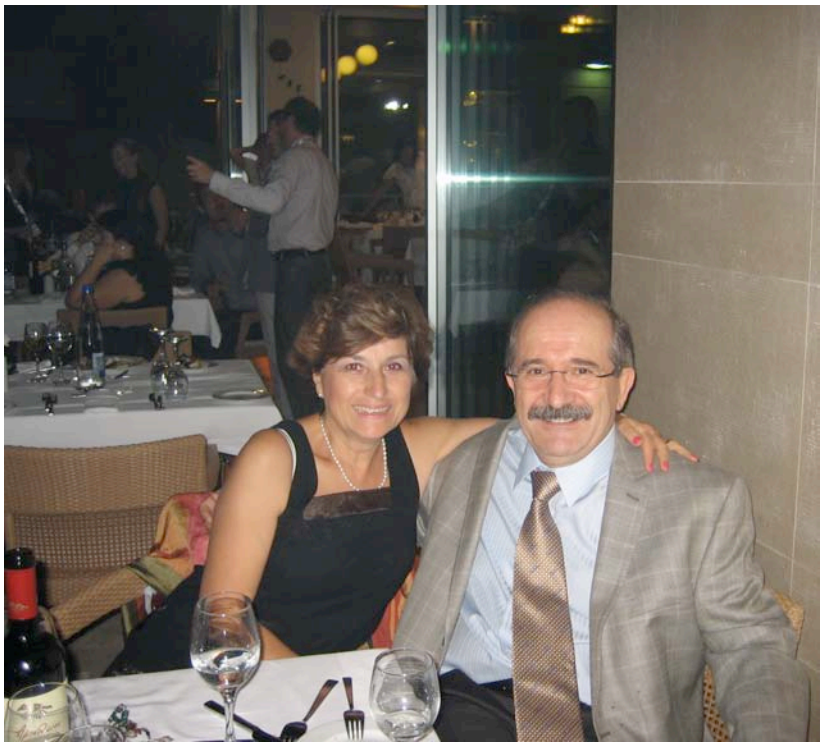
Souvenirs of the XXIV ICW in Chania, Crete



Rodanthi and John Lambris

Sir Peter Lachmann

David Isenman and the Toronto group

The U-Penn Group

Philippe Frachet and Gerard Arlaud

Complement Reagents Corner

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