December 1, 2008
Issue 12

Focus on Complement

EUROPEAN COMPLEMENT NETWORK

About this issue & More

<1> What's inside?

- Andrea Tenner presents two Flash News on the roles of C5a in T-cell suppression in cancer and of properdin in clearance of apoptotic cells.
- Two complement teams: one from Irvine, California and one from Houston, Texas are presented in Spotlights on Teams.
- Part one of a report on the 22nd International Complement Workshop held recently in Basel. The second part will appear in the next bulletin issue.
- Lilian Sotirov describes his findings on the alternative complement pathway of domestic animals.

<2> Special thanks again to Jürg Schifferli, Robert Rieben and Marten Trendelenburg, for organizing an exciting Workshop and a Teaching Day in Basel and for the summary report.

<3> The **12th European meeting on Complement in Human Disease** to be held in Visegrád, Hungary, September 5-8, 2009, is open for registration. For details visit the meeting website at: <http://www.chd2009.com/>.

<4> Many holiday greetings to you and your families and Happy and Successful 2009!

FLASH NEWS

C5a & Cancer Reporter: Andrea Tenner

Modulation of the antitumor immune response by complement

Markiewski et al. Nat Immunol 9:1225-1235, 2008

In this very interesting investigation Lambris and colleagues present evidence that complement activation may enhance tumor growth rather than contribute to tumor cell death. The scenario reported is that the generation of C5a recruits myeloid derived suppressor cells to the tumor and enhances their capability to suppress anti-tumor CD8+ T cell responses. This suppression of anti-tumor immune response contributes to increased tumor size. In C5aR knockout mice and in mice treated with a C5a receptor antagonist, the number of myeloid derived suppressor cells is reduced, CD8+ T cell tumor infiltration is greatly increased and tumor growth is inhibited. C5a-mediated regulation of suppressor cell function is attributed to the inhibitory effects of increased oxidative radicals on CD8+ T cell activity. While additional studies are needed to determine if these observations apply to other tumor types and/or locations, they are intriguing as drugs have been and are being assessed in the clinic that target C5a generation or receptor interactions. It should also be considered that while these data do not negate the efforts to enhance or facilitate complement mediated antibody dependent tumor killing, they do suggest that the "tumor cells bullets" postulated by others will have to be very effective if the T cell mediated antitumor activities are going to be suppressed in the area of the tumor due to C5a generation. All in all, these exciting data suggest a novel pathway to slow tumor growth.

Properdin & Apoptosis Reporter: Andrea Tenner

The complement protein properdin binds apoptotic T cells and promotes complement activation and phagocytosis

Kemper et al. Proc. Nat. Acad. Sci. 105:1093-1098, 2008

This report demonstrates yet another novel role for the alternative pathway complement protein, properdin. Purified properdin bound to apoptotic T cells (maximally to early apoptotic cells), but not to live resting or activated T cells. Addition of properdin depleted serum to apoptotic cells precubated with properdin resulted in rapid C3b deposition in contrast to apoptotic cells in the absence of prebound properdin and resulted in enhance ingestion of those apoptotic cells by phagocytic cells. Unexpectedly, pretreatment of apoptotic cells with properdin alone also enhanced apoptotic cell ingestion by mature DCs, immature DCs or macrophages. This was supported by the finding that these phagocytic cells also bound properdin and thus, suggests that properdin can act as a bridge between an apoptotic cell and a phagocyte even in the absence of C3b-C3b receptor interaction. Interestingly, properdin purified from serum or properdin released by degranulating activated neutrophils was much more efficient in binding to apoptotic cells than properdin in serum, suggesting a critical difference in the assembly state of the properdin from these sources or that binding is blocked by some other serum component that is removed during the purification. Since neutrophils at sites of injury are generally activated, the release of properdin provides a potential source of the "opsonin" at sites of injury and cell death. While not the only component facilitating rapid ingestion of apoptotic cells, a deficiency in properdin-mediated process could predispose individuals to inflammation and/or autoimmunity.

Xu, et al, J. Immunol. 180:7613-7621, 2008, provides additional insight on properdin interaction with apoptotic and necrotic cells.

SPOTLIGHT ON TEAMS - I

Teams at the University of California, Irvine

The Tenner and Anderson groups at UCI, have affiliations with the Center for Immunology, Institute for Brain Aging, Stem Cell Center and Spinal Cord Injury Research Center. The innate immune system recognizes deviations from the normal such as an infection or tissue injury and initiates a response that will provide protection and/or repair of the injured area. Our programs focus on how components of the innate immune system, in particular the complement system, influence immediate and induced protective responses to pathogens and injury thereby contributing to beneficial and detrimental processes following injury or dysfunction.

The Tenner lab investigates the effect of C1q and other defense collagens on the phagocytosis of apoptotic cells and immune complexes and the induction of gene expression as a result of that stimulation. The second major area of focus is the investigation of the role of complement activation and subsequent inflammation in Alzheimer's Disease, with a goal of identifying critical detrimental pathways that could be targeted to prevent or slow the progression of this disorder. In addition, we are investigating the hypothesis that C1q may be a response to injury that could play a protective role in the early stages of disease by enhancing the clearance of cellular debris, altering the effects of the amyloid peptide on microglia, and/or providing direct neuroprotective effects.

The laboratory of Dr. Aileen Anderson which focuses on the role of complement in spinal cord injury and enhancement of stem cell therapies for these injuries contributes to the nidus of complement research at UCI. Complement research in Dr. Anderson's lab seeks to investigate the relative contributions of C1q, C3, and C6 to functional recovery, neuropathological injury, and local circuitry reorganization in animal models of central nervous system trauma, including spinal cord contusion. Additional work in the lab has focused on testing the roles of these complement proteins in the inhibitory microenvironment that evolves after traumatic central nervous system injury using cultured neuronal cells.

UC, Irvine is located in sunny southern California, between San Diego and Los Angeles, a 10 minute drive from the Pacific Ocean and beautiful beaches yearlong. We welcome visitors of all ages and complement research interests.

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SPOTLIGHT ON TEAMS - II

Teams at the Brown Foundation Institute of Molecular Medicine, Houston, Texas

The Hans J. Mueller-Eberhard and Irma Gigli Research Center for Immunology and Autoimmune Diseases is part of the Brown Foundation Institute of Molecular Medicine, University of Texas Health Science Center at Houston. Presently, there are five full-time faculty members in the center involved in complement research. Dr. Irma Gigli (Emeritus-Professor and Emeritus-Director), Dr. Rick A. Wetsel (Professor & Director), Michael Braun (Associate Professor), Dr. Scott Drouin (Assistant Professor), Dr. Scott Wenderfer (Assistant Professor), and Dachun Wang (Instructor).

Much of the focus of the center for the past several years has been on delineating complement's role in lung and kidney disease. Dr. Wetsel and his colleagues have generated several complement "knock-out" mice that have to facilitate these studies (C3, FB, C3aR, C5aR, C4BP, and CPN). Dr. Wetsel's group is currently examining complement's impact in allergic and infectious diseases of the lung, and in particular the regulatory role that the anaphylatoxins and carboxypeptidases play in pulmonary disease. Dr. Wang and Dr. Wetsel are also heavily involved in developing stem cell therapies to treat lung disease. Dr. Michael Bruan's group is focused on determining complement's role in modulating inflammatory diseases of the kidney such as lupus nephritis (SLE), membranoproliferative glomerulonephritis (MPGN), and other autoimmune renal diseases. Dr. Braun's research group also seeks to identify potential disease biomarkers using cutting edge proteomic tools. Dr. Scott Drouin's research interest is focused on complement and allergic lung disease, and in particular how complement regulates goblet cell formation and mucus production in the lung. Dr. Scott Wenderfer's research is focused on delineating the role of the complement anapylatoxins and immunoglobulin receptors in renal disease.

Since its conception in 1995, the center has been the training ground for numerous students and post-doctoral fellows. We thank them for their enthusiasm, energy, and research accomplishments. We expect that many of these young scientists will become the next generation of complement research leaders. The center always welcomes other interested students as well as our complement colleagues.

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XXII International Complement Workshop
September 28 - October 2, 2008
Basel, Switzerland

Written by Robert Rieben, Marten Trendelenburg and Jürg Schifferli

Summary of oral sessions (part 1 of 2)

Jürg Schifferli opens the meeting

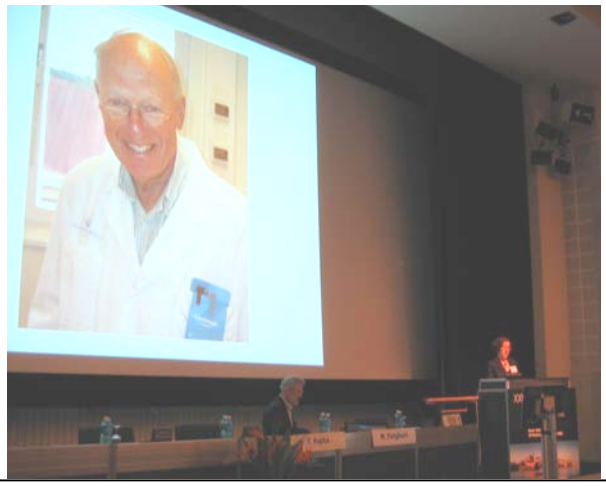
The XXII International Complement Workshop was held in Basel, Switzerland, from September 28 to October 2, 2008. Nice autumn weather seems to become a tradition at complement meetings, which we were able to maintain: The city of Basel presented itself in golden autumn weather most of the time! Another kept tradition was the start of the workshop by a teaching day for PhD-students and Postdocs. This was again a great success and attended by more than 80 students, for which a distinguished faculty provided an interesting, interactive teaching program. The ensuing four-day workshop, attended by more than 400 delegates, comprised 68 oral presentations, 280 posters, 3 clinical lectures by invited speakers, and a fabulous Müller-Eberhard lecture on interaction of pathogens with the host immune system by Jean Pieters.

All abstracts of the workshop and seven invited reviews are published in the ICW 2008 issue of Molecular Immunology (Volume 45, Issue 16, October 2008). Below follows a short, and of course incomplete, summary of the oral presentation sessions 3-5. The structure and function sessions 1 & 2 as well as sessions 6-9 will be published in a later issue of Focus on Complement. The many interesting posters, which were presented and discussed during a total of 6 h of poster sessions, cannot be included in these 'Focus' summaries. However, all poster abstracts are also published in the congress issue of Molecular Immunology and it's certainly worthwhile to have a look at them!

Session 3 (renal disease)

Kevin Marchbank presented data on anti-factor H autoantibodies in patients with atypical hemolytic uremic syndrome (aHUS). In contrast to earlier studies, suggesting an association between the presence of such antibodies and complete deficiency of factor H related proteins 1 and 3 (CFHR 1/3), his investigation found no correlation between the CFHR 1/3 gene copy number and the occurrence of anti-fH autoantibodies. Atypical HUS

was also the subject of a second talk in this session. Francesco Tedesco, together with collaborators from other groups, investigated the role of local complement activation in aHUS and indeed showed that complement activation products, including TCC, may be involved in renal damage in (some but not all) patients with aHUS.



Kristina Nilsson Ekdahl remembers the late Ulf R. Nilsson, one of the pioneers in complement research. Read more in Issue #10.

A mouse double-knockout model of membranoproliferative glomerulonephritis (MPGN) was then described by Michael Braun and colleagues. In mice, deficiency in C4BP alone is not sufficient for the development of MPGN, whereas fH and C4BP double-ko mice, which thus completely lack fluid phase C3 convertase regulation, develop progressive lethal MPGN resembling human MPGN type I. Finally, a clinical study on MPGN was presented by Aude Servais from Véronique Frémeaux-Bacchi's group. Histological data from 90 patients were compared with analysis of C3 nephritic factor and mutations screenings for factors H, I and MCP. The data show that a heterogeneous histological pattern of MPGN is associated with abnormalities of alternative pathway control proteins in humans.

The oral abstract presentations were followed by a **Clinical Lecture on Complement in Kidney Disease** with three invited speakers. Matthew Pickering gave an overview on glomerulonephritis, both in human patients and in animal models. The pathologist Michael Mihatsch then continued on "complement in the kidney in good and bad days", sharing some of his experience from >8000 normal kidneys and >5000 kidney transplant biopsies with us, and Manuel Pascual highlighted the importance of complement in human kidney allograft rejection and the use of C4d as a rejection marker in biopsies.

Session 4 (activation and regulation)

This session began with three talks about MBL-associated serine proteases. Mikkel-Ole Skjødt and coworkers investigated functional aspects of MASP3 and found that this protease, which is present in relatively high amounts in Danish blood donors (2000–12,900 ng/ml), is mainly associated with ficolin 3 and MBL in serum, and less with ficolin 2. Daisuke Iwaki from Teizo Fujita's group, presented studies on activation of MASP3. Mouse MASP1/3 knockout models and in vitro experiments were used to find the physiological substrate of MASP3. In vitro, this substrate seems to be MBL-A. Recombinant MASP3 restores the ability of MASP1/3$^{-/-}$ serum to opsonize Staph. A., putatively by direct activation of the AP

through MASP3. Minoru Takahashi, from the same group, then showed data on AP activation in MASP1/3$^{-/-}$ mice. These mice lack the AP due to deficiency of fD activation. MS-analysis reveals that fD in MASP1/3-/- mice is the pro-enzyme which still has the QPRGR activation peptide. The AP can be restored in these mice by recombinant fD, suggesting that MASP1 may cleave pro-fD directly.

The subject of the next two talks was properdin. Viviana Ferreira (Michael Pangburn group) presented an analysis of the complement functions of human properdin. Separation of native polymeric forms from non-physiological aggregates of properdin revealed that physiologic dimers, trimers and tetramers specifically bind to necrotic Raji and Jurkat cells and activate the AP, whereas the larger properdin polymers, which are formed as freeze-thaw artifacts, also bind live cells. Dennis Hourcade then talked about inhibition of properdin-directed complement activation by serum amyloid P (SAP) component. The latter competitively inhibits binding of properdin to certain surfaces. Serum may thus contain SAP (and possibly other inhibitors), which inhibits properdin binding to certain surfaces.

Christine Skerka and coworkers showed that fH related protein I (CFHR1) is a regulator of the human AP by controlling the C5 convertase activity. This finding may explain why deletion of CFHR1 and -3 is predisposing for the development of aHUS. New data on the membrane attack complex, in particular about the involvement of dynamin and lipid rafts in MAC endocytosis, were presented by Oren Moskovich from Zvi Fishelson's group. From the same group, Muhammad Masarwa, showed that silencing of the mitochondrial hsp70 mortalin can be used as an adjuvant in cancer therapy. Mortalin is overexpressed in colorectal adenocarcinomas and correlates with poor prognosis. Silencing of mortalin by siRNA sensitizes cells to complement-mediated lysis, both in vivo in mice and in vitro with Raji, K562 and EL4 cells.

Session 5 (innate and adaptive immunology)

Two presentations of Wen-Chao Song's group were at the beginning of this session. Xinhua Zhang talked about the TLR3 ligand poly-inosinic-cytidylic acid (polyI:C), which induces liver injury and systemic inflammation in vivo. Using DAF, C3, and C4 knockout mice and combinations thereof the authors showed that poly I:C seems to act mainly as an AP activator and that TLR3 binding is less important for the inflammatory effect of the substance. Wen-Chao Song then presented data on the promotion of the development of inflammatory Th-17 cells by complement, which synergizes with TLR4 to induce IL-6, TNFα, IL-1β, and IL-10. Coincidental activation of complement can thus regulate TLR4-mediated cytokine production and pathogenic Th-17 stimulation.

Paramita Baruah (Marina Botto's lab) showed data suggesting that C1q enhances IFNγ production by antigen specific T cells via the CD40 co-stimulatory pathway on DC, obviously via augmentation of the production of IL-12p70 by DCs following CD40 stimulation.

Two studies on B cells and antigen trafficking then followed by Michael Carroll's group. Uncoupling of the complement receptor CD21 and CD19 of the B cell co-receptor in specific knockout mice leads to impaired humoral immunity, confirming the importance of interaction of CD21 and CD19 (Carroll). Santiago Gonzalez then presented a study on trafficking of complement-tagged antigens in lymph nodes. Small lymph-borne antigens enter the follicles via conduits composed of a collagen rich core and wrapped by a basal membrane, whereas large antigens are excluded from follicular conduits. B cells then 'sample' the antigens from the conduits. B cells were also the subject of the talk of Amanda Jacobson (Weis lab, Salt Lake City). She showed that complement regulation by CD21/CD35 on B cells is independent of the B cell receptor, highlighting the role of CD35 as complement inhibitor.

Ilse Jongerius (Jos van Strijp / Suzan Rooijakkers lab) presented data on the inhibition of the C3 and C5 convertases by extracellular complement binding proteins (Ecb) of Staph. A. Finally, Gus Dalmasso, described how IL-4 and IL-13 induce resistance of vascular endothelial cells against the membrane attack complex. Metabolic protection of the cells is achieved by sterol receptor element binding protein-1 activation, fatty acid synthase expression, fatty acid/phospholipid synthesis, and preservation of mitochondrial integrity.

Levels of Alternative Pathway of Complement Activation (APCA) in domestic animals

Lilian Sotirov, Ph.D.,D.S., Stara Zagora, Bulgaria

We have studied the levels of alternative pathway of complement activation (APCA) in several domestic animals and human using a method developed in our lab (Sotirov; Phenotype characteristic and inheritance of lysozyme and complement activity in swine, Ph.D. thesis, Stara Zagora, Bulgaria, 1991). Briefly, each serum sample was diluted in U bottomed plates (Flow Laboratories, UK) with veronal-buffered saline (final buffer concentrations: 146 mM NaCl, 1,8 mM 5,5-diethylbarbituric acid sodium salt, 3,2 mM 5,5-diethylbarbituric acid, 1 mM EGTA and 0.8 mM $MgCl_2$). First, the serum was diluted as follows: 80 µl serum + 20 µl buffer, 70 µl serum + 30 µl buffer, 60 µl serum + 40 µl buffer, 50 µl serum + 50 µl buffer, 40 µl serum + 60 µl buffer, 30 µl serum + 70 µl buffer and 20 µl serum + 80 µl buffer. 350 µl veronal - veronal Na buffer was then added to each well, resulting in the final serum dilutions of 8/45, 7/45, 6/45, 5/45, 4/45, 3/45 and 2/45. Then, 50 µl buffer and 100 µl of 1% rabbit erythrocyte suspension were added to each well. After

incubation for 1 hour at 37°C, samples were centrifuged at 150 g for 3 minutes at room temperature (23°C). Thereafter, 150 µl of each supernatant were removed and placed in flat bottomed plates for measurement of optical density at 540 nm by "Sumal-PE2" ELISA reader (Carl Zeiss, Germany). The final APCA activity was calculated using special computer programs developed in the Trakia University, and expressed as AP50 units (AP50 units correspond to 50% of complement-induced haemolysis of applied erythrocytes). This modification was for studies on human and sheep sera. Later, this method was modified for other animal species and described in our articles published in Rev. Med. Vet., 2004, 155, 4, 221-225 (for horses, donkeys and mules); 2005, 156, 7, 405-408 (for sheep); 2006, 157, 3, 143-148 (for pigs); 2007, 158, 239-243 (for cattle). The differences were only in the dilutions of the sera. The following results were obtained:

Animals	APCA (mean ± SE) AP50	CV*	n
Cattle (7 breeds)	599.25 ± 10.44	3.89	210
Horses (8 breeds)	68.92 ± 2.32	10.46	143
Donkeys (local breed)	62.42 ± 2.32	15.38	61
Mules (local animals)	59.11 ± 1.49	8.34	12
Sheep (5 breeds)	150.4 ± 3.89	13.51	561
Goats (4 breeds)	210.04 ± 2,25	4.93	51
Swine (7 breeds and 4 crosses)	82.21 ± 2,24	50.92	1164
Turkeys (8 breeds)	566.75 ± 8.89	10.73	442
Hens	381.24 ± 12.51	-	116
Broilers	232.67 ± 0.10	29.42	347
Humans	227.0 ± 12.43	-	18

CV* - coefficient of variation

As is shown in the table, the APCA activity of humans is intermediate as compared with other animal species; cattle and turkeys have the highest APCA activity. We noticed in our studies that animals that possess the highest APCA activity were more resistant to infections than animals with low APCA activity (under 200 AP50). For example, non inbred pigs have about 20% higher APCA activity than inbred ones (coefficient of inbreeding Fx = 0.25) and correspondingly, non inbred pigs suffer less from traditional diseases than inbred pigs. Broiler chickens carriers of allele B21 (genotype B21B21 – Major Histocompatibility Complex in birds) have 27% higher APCA activity than genotype A2A5 and are more resistant to Marek's disease; the same broiler chickens from the same genotypes but raised on hard floor have also higher (10%) APCA activity than those raised in batteries and are also more resistant to diseases. Horses vaccinated against influenza and herpes virus have 9% higher APCA activity than controls, suggesting that APCA is actively participating in viral immune defense in horses. Pigs vaccinated against red wheat show a dramatic decrease in APCA 2 weeks after inoculation (from 131.91 AP50 to 27.87 AP50 units) that is restored to normal values after 3 weeks. On the basis of those findings we conclude that: 1) there are significant differences in alternative complement system activity between various animal species, and 2) the alternative complement pathway takes a significant part in health defense of domestic animals.

Complement Reagents Corner

Job Advertisement

LUND UNIVERSITY

Postdoctoral position to study mechanisms of immune evasion developed by bacterial pathogens.

Postdoctoral position (2 years) available at Lund University, Sweden to study mechanisms developed by bacterial pathogens for defense from killing by the complement system.

The applicant will join an ongoing project in a dynamic group of prof. Anna Blom, who focuses on the complement system in physiology and pathology. Applicants should be highly motivated, communicative and inventive with a Ph.D. in biochemistry, molecular biology, immunology, microbiology or related discipline. Particularly valuable experience: microbiology, molecular biology, protein chemistry. Applicants must have completed their PhD by the start of the appointment and preferably within the last 5 years.

Applicants should submit by e-mail (anna.blom@med.lu.se) curriculum vitae including publication list and e-mail address of two references at latest January 20th 2009. Starting date is flexible. Salary approx 1700 euro/month (tax-free stipend).

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