Focus on Complement

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Issue 39

International Complement Society

European Complement Network

Dear Readers,

Welcome to the September 2015 issue of 'Focus on Complement'. This 39th issue of FoC contains:

- **News Flash** presenting two recent papers further solidifying the complement system as a major contributor to the pathogenesis and progression of macular degeneration including novel mechanistic insights
- **The Complement research teams around the world** series featuring the teams of Prof. Remuzzi, Drs. Noris, Benigni, Morigi and Zoja in Bergamo, Italy and of Prof. Erdei and Dr. Józsi in Budapest, Hungary.
- Part I 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

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

NEWS FLASH

NEWS FLASH 1: A local complement response by RPE causes early-stage macular degeneration. *Fernandez-Godino R, Garland DL, Pierce EA. Hum Mol Genet. 2015 Jul 21. pii: ddv287. [Epub ahead of print]*

In this paper Fernandez-Godino *et al*. report the development of a culture model to investigate the role of the complement system in macular degeneration (MD). They used primary retinal pigmented epithelium (RPE) cells from normal mice and mice carrying a EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1) mutation. Their hypothesis was that understanding how the RPE pathology develops in this *Efemp1*-associated MD model would provide insights into the early mechanisms responsible for RPE pathology in AMD. They show that this ex vivo model recapitulates the formation of the sub-RPE deposits that characterize *Efemp1*-associated MD in only 2 weeks. Interestingly, cultured RPE cells from *C3*$^{-/-}$ *Efemp1* mutant mice, like RPE cells from wt mice, did not develop deposits, which suggested that formation of deposits is complement dependent. Consistently, RPE cells were found to produce complement proteins, which localize in the sub-RPE deposits, supporting the idea that complement components produced by the RPE cells and activated locally are involved in the formation of basal deposits. The molecular mechanism by which the EFEMP1 mutant protein causes deposit formation was investigated next, finding that both secreted EFEMP1 and C3a, but not C5a, stimulate production of deposits through a mechanism that involves inhibition of MMP-2, a metalloproteinase required for normal turnover of the extracellular matrix (ECM) in the Bruch's membrane, and expression of IL-1B and IL-6 by the RPE cells. Using this *in vitro* model, the authors provide convincing evidence that basal deposit formation is a local inflammatory process, mediated primarily by C3b, which occurs in response to abnormal ECM. These findings may imply that local therapies against C3a in patients with early MD could avoid further basal deposit formation and ongoing complement activation, preventing progression of the disease to vision threatening sequellae. **Both NEWS FLASHES are reported by S. Rodriguez de Cordoba, Centro de Investigaciones Biologicas, Madrid, Spain**

Cus on NEWS FLASH 2: Regulation of age-related macular degeneration-like pathology by complement factor H. *Toomey CB, Kelly U, Saban DR, Bowes Rickman C. Proc Natl Acad Sci U S A. 2015 Jun 9;112(23):E3040-9. doi: 10.1073/pnas.1424391112.*

Evidence accumulated during the last decade implicates the complement system, and in particular factor H (FH) in the pathogenesis of AMD. However the lack of models that replicate the multifactorial complexity (genetic and environmental) of AMD is making it difficult to unravel the link between alterations in the complement system and AMD. In this paper, Toomey *et al.* have developed one of such models using aged *Cfh*$^{-/-}$ and *Cfh*$^{+/-}$ mice fed with high-fat cholesterol-enriched (HFC) diet. Using this model they found that decreased levels of FH increases sub-retinal pigmented epithelium (sub-RPE) deposits and demonstrated that this is a consequence of the competition between FH and lipoproteins for binding to heparan sulphate proteoglycans (HSPG) in the sub-PRE extracellular matrix. Aged *Cfh*$^{+/-}$ mice develop, in response to HFC diet, PRE dysmorphogenesis and visual function loss, with substantial Bruch's membrane (BrM) C3 staining. Extensive sub-PRE deposits were also observed in *Cfh*$^{-/-}$ mice, but notably and in contrast with *Cfh*$^{+/-}$ mice, these deposits develop without apparent damage to the RPE and photoreceptors. The authors hypothesized a complement response to sub-PRE deposits, which is not seen in *Cfh*$^{-/-}$ mice because they lack intact C3. In line with this, they observed complement breakdown products and RPE/choroid monocyte recruitment in the *Cfh*$^{+/-}$, but not in the *Cfh*$^{-/-}$ mice, concluding that detrimental complement activation caused by the deposits in the BrM leads to phagocyte recruitment, RPE damage and vision decline. This novel murine AMD model may help defining specific therapeutic targets for distinct phases during AMD development. In addition, the discovery that FH is a competitor for lipoprotein binding to BrM, justifies future experiments to test the role of FH variants, FH-related proteins and the extracellular matrix HSPG in regulating BrM deposit formation.

COMPLEMENT TEAMS AROUND THE WORLD

Complement in Italy, Bergamo: The teams at the Mario Negri Institute for Pharmacological Research

In the last 15 years several research groups at the Mario Negri Institute in Bergamo have actively worked on research linking complement activation to the pathogenesis of kidney diseases. The groups interact closely with each other as a unique team under the leader ship of **Prof. Giuseppe Remuzzi**.
The Clinical Research group has established the International Registry of Hemolytic Uremic Syndrome/Thrombotic thrombocytopenic purpura (HUS/TTP) that includes clinical data and biological sample from about 1,000 patients and the Registry of Primary Membranoproliferative Glomerulonephritis (MPGN) covering more than 250 patients. The team has pioneered innovative treatment strategies for these diseases, including combined kidney and liver transplantation. Clinical studies with complement inhibitors have been published and others are currently ongoing at the Center both in HUS and MPGN patients.

The Laboratory of Immunology and Genetics of Rare Diseases, under the responsibility of **Dr. Marina Noris**, is part of the Department of Molecular Medicine headed by **Dr. Ariela Benigni**. The group has greatly contributed to define the genetic causes of atypical HUS, describing the mutations in genes encoding complement factor H (CFH), membrane cofactor protein and thrombomodulin and genomic rearrangements between CFH and CFHR1 genes, and has also characterized the functional consequences of the mutations. The group further clarified that this specific genetic defect greatly impacts on clinical course of the disease and on the risk of disease recurrence after kidney transplantation.

Giuseppe Remuzzi (front right) and Ariela Benigni (front left) and co-workers of the Clinical Research Team and of the Department of Molecular Medicine

Finally, the Noris group has highlighted the role of common SNPs in CFH in determining susceptibility to aHUS in carriers of complement gene mutations. In 2010 the laboratory received the accreditation from the Italian National Health Service for complement biochemical and genetic diagnostic tests in patients with HUS. In collaboration with Dr. Miriam Galbusera and her Unit of Platelet-endothelial Cell Interaction, an *ex vivo* test has been developed that efficiently detects complement activation at endothelial cell level in aHUS, and allows to monitor the effectiveness of Eculizumab and to titrate doses and timing of administration. Other cellular tests have been developed to study the link between complement activation and pro-thrombotic transformation of endothelial cells and to test the effects of new complement inhibitors under clinical development.

Within the Department of Molecular Medicine, two other groups leaded by **Dr. Marina Morigi** and **Dr. Carla Zoja**, have highlighted the role of complement and particular of C3a, in the pathogenesis of endothelial damage and microvascular thrombosis of Shiga-toxin associated HUS, using both perfusion tests with cultured endothelial cells and a mouse model of HUS. They have also recently documented that Shiga toxin, via activation of the alternative pathway of complement and generation of C3a, promotes podocyte dysfunction and loss in the mouse model. Finally, they documented in protein-overload proteinuria, a mouse model of chronic proteinuric renal disease, that C3-deficient mice are protected against podocyte damage and sclerosis, indicating a role of complement also in common proteinuric diseases.
The interdisciplinary nature of the teams (see photos), spanning from cell biology and molecular biology to genetics and clinical studies, and the close collaboration and interaction among the groups created a number of synergies that allows rapid and efficient translation of new knowledge into treatment of patients, moving from rare diseases to common renal diseases and vice-versa.

Marina Noris (center) and the research group of the 'Laboratory of Immunology and Genetics of Rare Diseases'

Erica Daina (4rth from the left in second row) and the 'Rare Disease Group'

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Complement in Hungary, Budapest: The teams of Prof. Anna Erdei and Dr. Mihály Józsi

Complement research in Hungary has a long tradition. Researchers at the Department of Immunology (founded in 1973) at Eötvös Loránd University in Budapest worked mainly on the role of C3 bridging innate and adaptive immunity. Anna Erdei studied the role of C3 in B cell activation with Fritz Melchers in Basel and identified cell membrane structures binding C1q and factor H with Ken Reid and Robert Sim in Oxford.

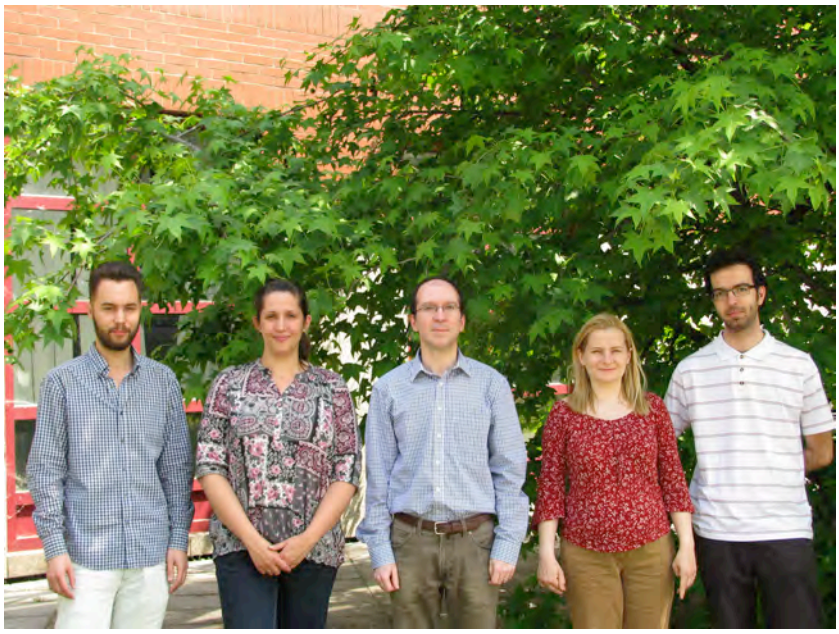
Today two research groups are working on related fields, mainly with the support of the Hungarian Academy of Sciences (MTA) and the Hungarian Scientific Research Fund (OTKA).

Prof. Anna Erdei's group studies the constructive and instructive role of complement to regulate adaptive immunity in human systems. In one line of research they study the involvement of the major component, C3 in the development of B and T cells and regulation of their responses under healthy and autoimmune conditions. In addition to the role of exogenous C3, locally produced C3-fragments are also in the focus of their interest. For several years they are intensively studying the expression and function of complement receptors CR1 (CD35) and CR2 (CD21). In humans these two receptors are encoded by two separate genes and mediate opposite effects on B lymphocytes – in contrast to mice. Namely, while CR2 – as the ligand recognition unit of the CD21/CD19/CD81 co-receptor complex – is involved in the enhancement of the B cell response, ligation of CR1 results in a strong inhibition of proliferation, antibody production and cytokine release by human B cells. They also investigate how the various cellular and molecular mechanisms participate in the pathogenesis of autoimmune diseases – particularly RA and SLE, and aim to reveal which B cell subpopulation is involved.
In another major line of research they study how various C3-fragments – including covalently fixed C3b – influence the differentiation and function of human monocytes, macrophages and dendritic cells. Recently they set out to study the „division of labour" between the integrin receptors CR3 and CR4 on human phagocytes. They also study the role of these receptors in the adherence of macrophages, neutrophils and dendritic cells using cutting-edge biophysical methods.
It should be noted that in the past ten years the group has developed a unique technology which is based on the monitoring of complement activation events on antigen arrays. By multiplex measurements they aim to generate comprehensive data sets that describe antibody initiated events upon antigen binding.

Dr. Mihály Józsi's group is interested in the function and regulation of the alternative complement pathway, particularly the structure and function of factor H and factor H-related proteins. Because alternative pathway dysregulation, polymorphisms and mutations in factor H or anti-factor H autoantibodies play a role in various diseases (e.g., age-related macular degeneration, atypical hemolytic uremic syndrome and dense deposit disease), the aim is to unveil the physiological roles of factor H and factor H-related proteins, and the reason for their association with certain diseases. In contrast to factor H, the function of the FHRs is less characterized and controversial. The group studies how the FHR proteins interact with certain host and non-host ligands, as well as their role in complement activation and regulation. Factor H autoantibodies and C3 convertase binding autoantibodies are also characterized, in collaboration with several other groups in Hungary and abroad.

In addition to being a major regulator of the alternative complement pathway, factor H binds to receptors and influence cellular functions. This non-canonical role of factor H beyond complement regulation is, however, poorly characterized. Therefore, the group investigates the interaction of factor H with particularly innate immune cells, such as neutrophil granulocytes, macrophages and dendritic cells, and studies how factor H modulates the activation and function of these cells.

Anna Erdei's group:
Standing: Katalin Török, Noémi Sándor, Krisztián Papp, Barbara Uzonyi, Mariann Kremlitzka.
Sitting: József Prechl, Zsuzsa Bajtay, István Kurucz
In front: Anna Erdei.



Mihály Józsi's group:
Ádám Csincsi, Andrea Schneider, Mihály Józsi, Zsóka Weiszhár, Marcell Cserhalmi

Contact: Anna Erdei (anna.erdei@elte.hu); Mihály Józsi (mihaly.jozsi@gmx.net)
Further information: http://immunologia.elte.hu/ and http://abc-arrays.com

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. Below, we provide a summary of the first 5 sessions of the meeting – as usual, composed by the respective session chairs. A summary of the last 4 sessions will be published in the next issue of the Focus on Complement (Issue 40).
We thank all contributors to the Teaching Day. The Conference Dinner was served during a boat trip in the Stockholm Archipelago. Finally, during the closing ceremony, a total number of 24 travel awards and 12 poster prizes were granted to young scientists.

Session A: Genetics, Structure and Functions
Chairs: Zvi Fishelson (Tel Aviv/Israel) Peter Gál (Budapest/Hungary)
Bärbel Blaum from University of Tübingen (Abstract 19) analyzed the interaction of factor H (fH) with sialic acid and C3b. The crystal structure of a ternary complex consisting of the two C-terminal domains of fH, a sialylated glycan and the C3b TED domain was solved. NMR was also used to study the fH-glycan interaction. Key residues in the sialic acid binding site are linked to aHUS.
Gregers Andersen form Aarhus University (Abstract 129) presented the crystal and solution structures of C4b. The solution structures of C2, the pro-convertase C4bC2 and the C3 convertase C4bC2a were also obtained by SAXS. Based on the structural data he proposed molecular models for the classical pathway C3- and C5 convertases.
Steffen Thiel from Aarhus University (Abstract 97) reported the solution structure of an initiation complex of the lectin pathway consisting of a MASP-1 dimer and a tetrameric MBL molecule. The catalytic region of MASP-1 is positioned outside the MBL framework indicating an intercomplex, clustering-based mechanism of lectin pathway activation. Stephen Perkins from University College London (Abstract 130) also focused on the initiation steps of the lectin pathway. He presented crystal and solution structures of the N-terminal region of MASP-1 and MASP-2, as well as, solution structures of the full-length MASPs. He concluded that there is a significant flexibility in the region containing the four C-terminal domains which may play a role in the lectin pathway activation.
Daniel Ricklin from the University of Pennsylvania (Abstract 157) described a rare loss-of-function C3 mutant, M373T, found in a Swedish family and associated with episodic symptoms but not with a familial disease. This point mutation in the C3 b-chain led to conformational changes that altered its fH and C5 binding. Binding of the mutated C3 to surface bound C3b was reduced, leading to impaired C3 activation and opsonization.
Johan Rockberg from KTH Royal Institute of Technology (Abstract 248) presented an analysis of the C5 conformational epitopes identified by Eculizumab. Using a combinatorial gram-positive cell-surface display of folded domains, they mapped the Eculizumab epitopes within the MG7 domain of C5. Patients carrying a mutation in this epitope are likely to respond poorly to Eculizumab treatment.

Session B: Renal Disease

Chairs: Veronique Fremeaux-Bacchi (Paris/France), Matthew Pickering (London/UK)

This was a highly interesting session where we discussed new developments in complement-mediated disease that included biomarkers, mechanisms and therapies. Biomarker data included the prognostic value of (1) the ratio between serum factor H-related protein 1 to factor H levels and outcome in IgA nephropathy (Jaouad Anter et al., Abstract 4) and (2) the relationship between complement activation and reduced ADAMTS13 activity in secondary haemolytic uremic syndrome (aHUS)/thrombotic thrombocytopenic purpura (Zoltan Prohaszka et al., Abstract 53). Mechanistic data included the novel observation that renin can cleave complement C3 and that inhibition of renin could ameliorate C3 glomerulopathy (Zivile Bekassy et al., Abstract 13). The glycosaminoglycan binding profile of complement factor H-related protein 5 was presented together with how this might relate to competition with factor H along the glomerular basement membrane (Frederick Gyapon-Quast et al., Abstract 64). Further, Marina Noris' group demonstrated that inhibition of the C5a receptor 1 using CCX168 was effective in reducing the thrombogenic effect of serum from patients with aHUS on microvascular endothelial cells (Abstract 59). This suggested that the deleterious effect of C5 activation in aHUS is due to C5a-mediated pathways rather than C5b-9 effects. An update on genetic screening in C3 glomerulopathy was presented and the difficulty in interpreting the sequence changes in these data sets was outlined (Bertha Martin et al., Abstract 117).

Session C: Deficiencies and Dysregulation

Chairs: Pilar Sanchez-Corral (Madrid/Spain), Seppo Meri (Helsinki/Finland)

This session included three presentations about lectin pathway involvement in viral infections, pre-eclampsia or ischemia, three others concerning alternative pathway and renal/ocular damage, and one presentation on a CD59 mutation.

Angelika Bolt (Abstract 21) showed data stressing the implication of the lectin pathway in HIV/HBV/HCV infections. HIV patients presented reduced MASP-2 levels and higher frequency of CDV haplotype, while other MASP-2 variants conferred either risk or protection to HIV+HBV+ status or HIV/HCV coinfection. Roberta Bulla (Abstract 26) presented interesting data on complement in pre-eclampsia. Stronger deposits of C1q and MBL were found in endothelia of pre-eclamptic placentas. MBL-A was linked to pregnancy loss in a mouse model, where miscarriage could be prevented with MBL or C5 inhibitors. Simon Clark (Abstract 39) emphasized the role of FHL-1 in AMD. FHL-1 is expressed in the eye and can diffuse through Bruch's membrane, while FH cannot and was only found coating drusen (the AMD local lesions). The AMD-associated Y402H variant may thus act via FHL-1 as well as via FH itself.

Moglie Le Quintrec (Abstract 115) analyzed a large cohort of C3G and MPGN I patients, where they found anti-C3b antibodies in 5/230 and anti-Bb antibodies in 11/230 patients of C3G or MPGN I. Anti-Bb antibodies promoted C3bBb formation, whereas anti-C3b antibodies prevented C3b binding to CR1, but not to FH. Dror Mevorach (Abstract 123) reported observations on the very interesting North African patients with homozygous p.Cys89Tyr mutation in CD59. Devastating polyneuropathy syndrome, stroke or infections were common, and the mean survival was only 5±3.1 years. Fortunately, eculizumab has changed the disease course, and no other medications were required to prevent disease progression.

Maria-Grazia De Simoni (Abstract 143) investigated the pathogenetic role of MBL in ischemia-induced endothelial damage. By using *in vitro* and *in vivo* models, she demonstrated a toxic effect of MBL deposits on brain endothelial cells, thus leading the way to MBL inhibiting treatments. Bärbel Rohrer (Abstract 161) described the effect of the alternative pathway inhibitor CR2-FH to prevent or ameliorate the ocular damage associated to smoke. Using control and smoke-exposed mice, she demonstrated a significant reversion of ocular morphological and functional changes in treated animals.

Session D: Cardiovascular Disease and Miscellaneous

Chairs: Peter Garred (Copenhagen/Denmark), Marten Trendelenburg (Basel/Switzerland)

The first two presentations of this session focussed on experimental models of I/R injury. In a xenoperfusion model, Robert Rieben (Bern/Switzerland) could nicely demonstrate that the transgenic (over)expression of human CD46 in pig limbs leads to marked inhibition of complement activation, endothelial damage and procaogulating/anti-fibrinolytic effects (Abstract 22). Afterwards, Castellano (Bari/Italy) reported on the role of complement in the expression of anti-aging factor Klotho in a mouse model of renal I/R injury and in patients that underwent renal transplantation (Abstract 31). Interestingly, the application of C1 Inhibitor led to a preserved expression of Klotho. In the following presentation, Emilie Holmquist (Lund/Sweden) reported on a striking association between tumor expression of Cartilage Oligomeric Matrix Protein (COMP) in breast cancer and mortality/disease recurrence in patients (Abstract 74). In an experimental model, COMP-expressing breast cancer cell lines were found to be more aggressive. After this, Huda Kozarcanin (Uppsala/Sweden) provided further evidence for the interplay between blood clot fomation and lectin pathway activation (Abstract 99), and Yves Laumonnier (Lübeck/Germany) analysed in a mouse model of allergic asthma the direct effector cells downstream of C5a among which DCs seem to be most critical (Abstract 106). Finally, Ronald Taylor (Virgina/USA) demonstrated the sequence of molecular events leading to complement-dependent cytotoxicity by hexameric anti-CD20 antibodies with the help of beautiful four-colour confocal microscopy movies (Abstract 193).

Session E: Activation, Regulation and Experimental Models

Chairs: Marina Noris (Bergamo/ Italy), Steffen Thiel (Aarhus/Denmark)

Novel insights into the role of complement in regulating the function of T cells, neutrophils, macrophages and dendritic cells have been presented in this session. A study in a mouse kidney allotransplant model presented by M. Noris (Bergamo, Italy; Abstract 30) documented that the alternative complement pathway in the recipient mediates post-transplant inflammation and T cell alloimmune response. N. Sandor (Budapest, Hungary; Abstract 166) showed evidence that in dendritic cells and macrophages CR3 (CD11b) dominates iC3b-mediated phagocytosis, while CR4 (CD11c) mediates adherence to fibrinogen. O.A. Hamad (Uppsala, Sweden; Abstract 65) reported data demonstrating that C3 on activated platelet membranes and microparticles (PMP) acts as a ligand for CD1b on PMNs and promotes the formation of platelet-PMN and PMP-PMN complexes. M. Jozsi (Budapest, Hungary; Abstract 173) showed that by binding to CD11b, FH supports neutrophil migration and IL-8 release, but it also inhibits NET formation and ROS production, thus potentially influencing local inflammatory reaction and tissue damage. In a baboon model of sepsis, F. Lupu (Oklahoma, USA; Abstract 111) found that complement inhibition at the C5 level with a short peptide molecule exerts similar anti-inflammatory, organ protective and survival benefits as complement C3 inhibition. Inhibition of C5 decreased bacterial lysis but phagocytosis and bacterial clearance was well preserved. Finally, by using a mutant lipodystrophic mouse strain that lacks adipose tissue and does not produce FD, and partial lypodistrophic mice that have 10% of normal FD, X. Wu (Washington, USA; Abstract 218) showed that about 1 to 10% normal FD is enough to restore the full AP activity.

COMPLEMENT REAGENT RESOURCES