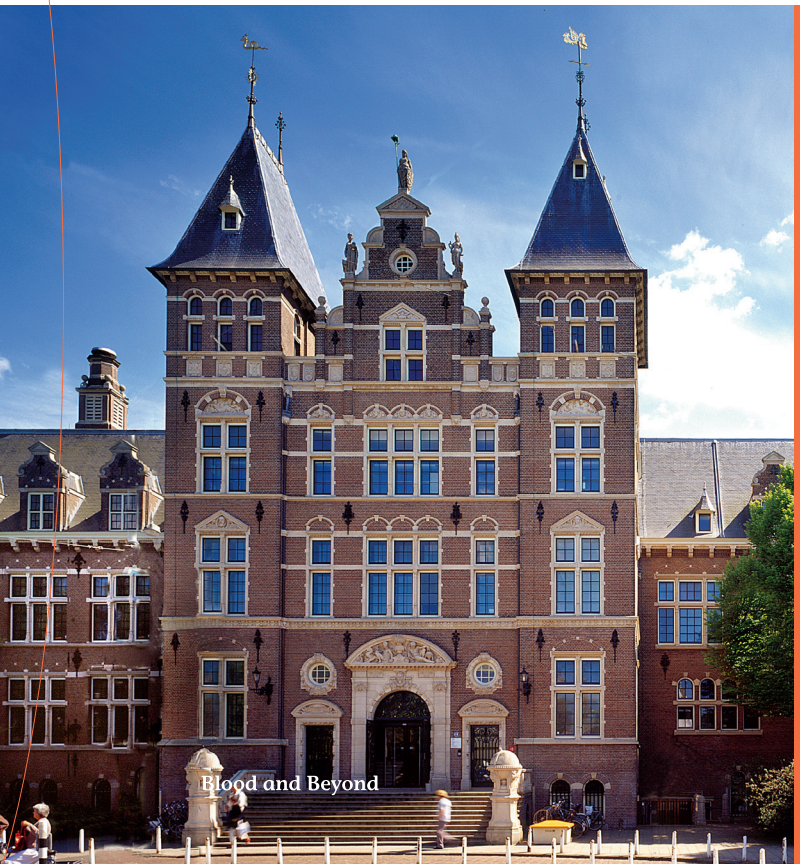
Sanquin

Spring Seminars 2015



Proteins, from discovery to therapeutics

23 & 24 April 2015,
Amsterdam, The Netherlands
Venue: Royal Tropical Institute

www.sanquin.nl/sss

Sanquin Spring Seminars

Proteins, from discovery to therapeutics

Conference chair: Prof Koen Mertens PhD,
Amsterdam, The Netherlands

23 & 24 April 2015

Royal Tropical Institute,

Amsterdam, The Netherlands

www.sanquin.nl/sss

Welcome message

On behalf of Sanquin Blood Supply Foundation we warmly welcome you to the fifth biennial Sanquin Spring Seminars. This year's theme is 'Proteins, from discovery to therapeutics'. The first day focuses on 'Proteomics and Structural Biology', while the second day addresses 'Therapeutics of Tomorrow'. Collectively, this program provides highlights of innovative research on the axis between fundamental protein science and the translation thereof into novel therapeutics.

We are delighted that so many top-notch speakers have been able to accept our invitation to present their work. They are all internationally recognized experts in their field, and their willingness to share their newest research with you provides the key asset of this meeting. Their contributions further set the stage for shorter contributions on related subjects by a variety of speakers from Sanquin. Finally, this program provides a platform for the younger generation of researchers who will present their work in the poster section. A few posters have been selected for oral presentation as well.

The presentations cover a wide spectrum of proteins, including antibodies, complement factors and hemostatic proteins, with particular reference to their therapeutic potential. At the end of the program, the focus shifts more specifically to hemophilia. This also includes the current status of gene therapy, which finally starts to fulfill its promise as a realistic alternative to protein substitution therapy.

We feel that the Scientific Committee succeeded in putting together an exciting program, and hope that you will share our enthusiasm!

Koen Mertens
Conference Chair

René van Lier
Sanquin Executive Board

Scientific Committee

Prof Koen Mertens PhD, Conference chair
Timo van den Berg PhD
Theo Rispens PhD
Maartje van den Biggelaar PhD

Organizing Committee

Prof Koen Mertens PhD
Anneke de Regt
Jan Willem Smeenk

Seminar Secretariat

MCI
Jan van Goyenkade 11
1075 HP Amsterdam
The Netherlands
Tel: +31 20-679 3411
sss2015@mci-group.com
www.sanquin.nl/sss

Venue

Royal Tropical Institute
Mauritskade 63
1092 AD Amsterdam
The Netherlands
Tel: + 31 20- 568 8698
Fax: + 31 20- 668 4579

Scientific program
Thursday 23 April 2015

09.00 Registration/coffee

09.45-09.50 Opening

Session I: Proteomics

09.50 - 10.35: Human Proteomes: From basic science to understanding drug action
Bernhard Küster, Munich, Germany

10.35 - 11.05: Phosphoproteomic analysis of coagulation protein-induced vascular signaling
Maartje van den Biggelaar, Amsterdam, The Netherlands

11.05 - 11.30: Coffee/tea break

11.30 - 12.00: Ins and outs of the platelet proteome in health and disease
Sander Meijer, Amsterdam, The Netherlands

12.00 - 12.45: Quantitative interaction proteomics for epigenetics
Michiel Vermeulen, Nijmegen, The Netherlands

12.45 - 14.15: Lunch/posters

14.15 - 14.45: Intermezzo 3 selected abstracts

Characterization of engineered IgG hexamers and their role in complement activation
Guanbo Wang, Utrecht, The Netherlands

Conformational activation of ADAMTS13
Brenda Luken, Amsterdam, The Netherlands

Factor Xa overcomes inhibition by the direct factor Xa-inhibitors following insertion of a structural element from the serine protease domain of snake venom Factor Xa
Daniël Verhoef, Leiden, The Netherlands

Session II: Structural Biology

14.45 - 15.30: Engineering immunity against HIV
Louise Scharf, Pasadena, U.S.A.

15.30 - 15.55: Coffee/tea break

15.55 - 16.25: Regulation of integrin function
Coert Margadant, Amsterdam, The Netherlands

16.25 - 17.10: Molecular mechanisms of complement activation
Piet Gros, Utrecht, The Netherlands

17.10 - 19.00: Network reception

Scientific program
Friday 24 April 2015

Session III:	**Novel Therapeutics**
09.30 - 10.15:	Novel therapeutic antibody molecules - Inspired by nature Paul Parren, Utrecht, The Netherlands
10.15 - 10.45:	Potentiating antibody-dependent destruction of cancer cells by targeting CD47-SIRP-α interactions Hanke Matlung, Amsterdam, The Netherlands
10.45 - 11.10:	Coffee/tea break
11.10 - 11.40:	Therapeutic potential of complement inhibitor Factor H Diana Wouters, Amsterdam, The Netherlands
11.40 - 12.25:	Emerging concepts in gene therapy for hereditary diseases Thierry VandenDriessche, Brussel, Belgium
12.25 - 13.45:	Lunch/posters/stands
Session IV:	**Innovation in treatment of hemophilia**
13.45 - 14.30:	Gene therapy for haemophilia Amit Nathwani, London, United Kingdom
14.30 - 14.55:	Coffee/tea break
14.55 - 15.40:	Factor VIII / Von Willebrand factor: how to modify clearance of a complex that binds multiple receptors? Peter Lenting, Paris, France
15.40 - 16.25:	Engineering coagulation factors: can we improve on nature? Koen Mertens, Amsterdam, The Netherlands
16.25 - 16.30:	Poster award ceremony & closing remarks René van Lier, Amsterdam, The Neterlands
16.30:	Farewell reception

Sessions I-II

Session I Thursday 23 April 2015, 09.50

Bernard Küster
Technische Universität München, Germany

Human Proteomes: From basic science to understanding drug action

Berhard Küster studied chemistry at the University of Cologne, and obtained his doctorate in biochemistry at the University of Oxford. He subsequently worked as a postdoctoral researcher in Heidelberg, Germany, and in Odense, Denmark, and as Vice President of a biotech company in Heidelberg. He is currently professor at the Technische Universität München, where he is the Chair of the Dept of Biosciences and a member of the Graduate School for Experimental Medicine.

Professor Küster is an expert in the field of chemical and functional proteomics. His research focuses on questions relating to how proteins interact with each other and with active pharmaceutical ingredients, and which molecular mechanisms may provide targets for individual clinical treatment approaches. In his presentation, he will discuss the potential of proteomics to acquire understanding of drug action.

Session I Thursday 23 April 2015, 10.35

Maartje van den Biggelaar
Sanquin Research, Amsterdam, The Netherlands

Phosphoproteomic analysis of coagulation protein-induced vascular signaling

Maartje van den Biggelaar studied pharmaceutical sciences at Utrecht University and obtained her PhD degree at the same university on a thesis on the biosynthesis of coagulation Factor VIII. Since 2007 she is scientist at the Dept of Plasma Proteins of Sanquin, where she continued her Factor VIII-related research. She further was visiting scientist at the Beatson Institute for Cancer Research, Glasgow, UK (2011-2013) to expand her expertise in phosphoproteomics.

Dr Van den Biggelaar's research focuses on the biology of hemostatic proteins, and on the development of innovative protein therapeutics for hemostatic disorders and. Her current research interests are the interaction of hemostatic proteins with endocytic receptors and the identification of communication networks between the hemostatic system and the vasculature. The latter issue is the subject of her presentation.

Session I Thursday 23 April 2015, 11.30

Alexander B Meijer
Sanquin Research, Amsterdam, The Netherlands

Ins and outs of the platelet proteome in health and disease

Alexander B (Sander) Meijer studied molecular sciences at Wageningen University. He obtained his PhD at the Laboratory for Biophysics of the same University in 2001. He then continued his career at Sanquin, initially as postdoctoral investigator at the Dept of Plasma Proteins, and currently as head of the Laboratory for Proteomics.

Dr Meijer's research focuses on the structure of hemostatic proteins, and in particular on the inter- and intra-molecular interactions that drive their function. His expertise combines protein engineering with biophysics and biomolecular mass spectrometry. One of his interests is the use of proteomics for the identification of platelet dysfunction, and for the diagnostics of rare platelet disorders. This will be the topic of today's presentation.

Session I Thursday 23 April 2015, 12.00

Michiel Vermeulen
Radboud Institute for Moleculair Life Sciences (RIMLS), University of Nijmegen, the Netherlands

Quantitative interaction proteomics for epigenetics

Michiel Vermeulen studied medical biology at the Radboud University Nijmegen, where he also received his doctorate on biochemistry of gene expression in 2006. He spent 4 years as postdoctoral investigator at the Max Planck Institute for Biochemistry in Martinsried, Germany and then moved to Utrecht where he was scientist at the Dept Medical Oncology of the University Medical Center. Since 2014 he is professor Proteomics and Chromatin Biology at Radboud University Nijmegen.

Professor Vermeulen's research focuses on biochemical processes involved in epigenetics. Because pathological changes in gene expression may provide a suitable target for therapeutic intervention, detailed knowledge of these processes is essential for further exploring this area. In his presentation, he will address the potential of quantitative interaction proteomics in this field.

Session I Thursday 23 April 2015, 14.15

Intermezzo: 3 selected abstracts'
Abstracts to be found in het poster sector (page 42)

Guanbo Wang
Bijvoet Institute for Biomolecular Research,
Utrecht, The Netherlands

Characterization of engineered IgG hexamers and their role in complement activation To be announced

Brenda Luken
Sanquin Research, Amsterdam, The Netherlands

Conformational activation of ADAMTS13

Daniël Verhoef
Leiden University Medical Center, Leiden, The Netherlands

Factor Xa overcomes inhibition by the direct factor Xa-inhibitors following insertion of a structural element from the serine protease domain of snake venom Factor Xa

Session II Thursday 23 April 2015, 14.45

Louise Scharf
Howard Hughes Medical Institute, California Institute of Technology, Pasadena, USA

Engineering immunity against HIV

Louise Scharf received her scientific education at The University of Chicago, USA. In 2010 she obtained her PhD degree in Biochemistry and Molecular Biology. Since 2011 she is postdoctoral fellow at the California Institute of Technology (Pasadena CA, USA) where she works in the group of dr. Pamela Björkman.

In dr Björkman's laboratory, dr Scharf focuses on the recognition of the Human Immunodeficiency Virus (HIV) by antibodies. This research line has been very productive, and has yielded numerous publications in top-journals. In her presentation, dr Scharf will provide an overview of the issue as and will address the newest developments.

Session II Thursday 23 April 2015, 15.55

Coert Margadant
Sanquin Research, Amsterdam, The Netherlands

Regulation of integrin function

Coert Margadant studied Molecular Cell Biology at Utrecht University. Since then he has been working at several laboratories including Cell Biology (Utrecht University), the Hubrecht Institute for Developmental Biology and Stem Cell Research (Utrecht), the Beatson Institute for Cancer Research (Glasgow, UK), and the Netherlands Cancer Institute (Amsterdam). He received his PhD-degree at the University of Amsterdam for his work on integrin function and trafficking.

Dr Margadant's research focuses on the question how integrin function is regulated, and how the transport machinery of the cell coordinates trafficking of integrins and other cell-surface receptors to dictate cell behavior. This will be the subject of his presentation.

Session II Thursday 23 April 2015, 16.25

Piet Gros
Utrecht University, The Netherlands

Molecular mechanisms of complement activation

Piet Gros studied chemistry at the Rijksuniversiteit Groningen. He received his PhD degree at the same university, in the field of protein crystallography. Subsequently he worked as postdoctoral fellow at ETH Zürich (Switzerland) and Yale University, New Haven CT (USA). Since 1994 he is at the Dept of Chemistry of Utrecht University, where he currently is professor and director of the Bijvoet Center for Biomolecular Research.

His research group uses protein crystallography to study bio-molecular recognition and regulation processes with emphasis on bio-medically important proteins, in particular in the area of infection and immunity. His research aims to reveal the mechanisms of tertiary and quaternary-induced activation in multi-domain proteins and multi-protein complexes that underlie these recognition and regulation processes. As an example thereof, he will present recent work of his group on the structure of the complement system and its constituents.

Sessions III-IV

Session III Friday 24 April 2015, 09.30

Paul Parren
Utrecht, The Netherlands

Novel therapeutic antibody molecules - Inspired by nature

Paul Parren holds MS and PhD degrees from the University of Amsterdam. He continues his career as associate professor in the Dept of Immunology at The Scripps Research Institute in La Jolla, CA (USA). In 2002 he joined the biotechnology company Genmab, where he was appointed Senior Vice President in 2008. He is further Adjunct Professor in translational cancer research at the University of Southern Denmark.

His main interest is in therapeutic antibodies, and he is author of numerous publications in this field, and is named inventor on over 70 patents and patent applications. His presentation will address novel developments in antibody engineering and design.

Session III Friday 24 April 2015, 10.15

Hanke Matlung
Sanquin Research, The Netherlands

Potentiating antibody-dependent destruction of cancer cells by targeting CD47-SIRP-α interactions

Hanke Matlung studied biomedical engineering at the University of Technology Eindhoven. She then worked in the field of vascular remodeling and atherosclerosis at the Dept of Biomedical Engineering and Physics at the Academic Medical Center in Amsterdam. She received her PhD in 2011. Since then she works as postdoctoral fellow at the Dept of Blood Cell Research at Sanquin.

Her current interest is in antibody-dependent cellular cytoxicity of neutrophils against cancer, and in particular of the role of CD47 and Signal Regulatory Protein alpha (SIRPα) therein. This will be the subject of her presentation.

Session III Friday 24 April 2015, 11.10

Diana Wouters
Sanquin Research, Amsterdam, The Netherlands

Therapeutic potential of complement inhibitor Factor H

Diana Wouters studied medical biology at the Free University of Amsterdam. As a PhD student, she worked at the Dept of Immunopathology at Sanquin. In 2008 she obtained her doctorate at the University of Amsterdam on a thesis entitled 'Assessment of complement activation in human disease'. Since then she is scientist in the Dept of Immunopathology at Sanquin.

Her research focuses on the pathophysiology of the complement system, and in particular on the delicate balance between complement activation and regulation under normal healthy conditions and in diseases such as atypical Hemolytic Uremic syndrome (aHUS) and systemic lupus erythematosus (SLE). Her presentation will focus on the function of the inhibitory protein factor H.

Session III Friday 24 April 2015, 11.40

Thierry VandenDriessche
Free University of Brussel and University of Leuven, Belgium

Emerging concepts in gene therapy for hereditary diseases

Thierry VandenDriessche studied Molecular Biology and Biotechnology at the Free University of Brussel. In 1992 he received his doctorate at the same university. He continued as postdoctoral fellow at the National Institutes of Health (Bethesda, USA) in the National Heart Lung and Blood Institute, and subsequently in the Heart Lung and Blood Institute. After his return to Belgium, he became group leader at the Flanders Institute for Biotechnology (VIB), Leuven. Since 2010, he is professor in Medicine at the University of Leuven and the Free University of Brussel.

Professor VandenDriessche's research interest is gene therapy as a way towards new treatments and cures for hereditary, acquired and complex diseases. His work comprises both applied translational research and hypothesis-driven fundamental studies. In his presentation he will address novel concepts in gene therapy for hereditary diseases, including hemophilia.

Session IV Friday 24 April 2015, 13.45

Amit Nathwani
UCL Cancer Institute, Royal Free Hospital, University College Hospital (UCH) and NHS Blood and Transplant (NHSBT), London, United Kingdom

Gene therapy for haemophilia

Amit Nathwani studied Medicine and Surgery at the University of Aberdeen (UK). After his PhD and training as hematologist he held a position as Wellcome Trust Advanced Training Fellow at St. Jude Children's Research Hospital, Memphis (USA). He currently is professor and honorary consultant hematologist at the UCL Cancer Institute and University College Hospital, as well as at the Royal Free Hospital, London (UK).

Professor Nathwani's research is driven by his interest in the development of novel treatment avenues for hematological disorders and cancer using gene transfer technology. His group has developed a distinctive approach for gene therapy of hemophilia B using a self-complementary adeno-associated viral vectors (AAV). The results of phase I/II studies in subjects with severe hemophilia B have recently been published, and a similar study for hemophilia A is currently in preparation. In his presentation, professor Nathwani will review these studies and discuss the prospects for the near future.

Session IV Friday 24 April 2015, 14.55

Peter Lenting
INSERM, Paris, France

Factor VIII / Von Willebrand factor: how to modify clearance of a complex that binds multiple receptors?

Peter Lenting studied biochemistry and started his career in 1989 as research technician at the Dept of Blood Coagulation of the CLB (now Sanquin) in Amsterdam. In 1996, he acquired his PhD degree at the University of Amsterdam on a thesis on blood coagulation factors VIII and IX. In 2000 he became associate professor at the Dept of Hematology at University Medical Center Utrecht. After a stay as Director at Crucell Holland (Leidein) he moved to France. He is currently Director of Research at the Institut National de Science Et de Recherche Medicale (INSERM), Unit 770, in Le Kremlin Bicêtre (Paris).

Dr Lenting's key interest is thrombosis and hemostasis, with particular reference to von Willebrand factor and its wide range of binding partners. In his presentation he will address the various mechanisms that contribute to the clearance of von Willebrand factor and factor VIII, and the complexity of modifying half-life.

Session IV Friday 24 April 2015, 15.40

Koen Mertens
Utrecht University and Sanquin Research, Amsterdam, The Netherlands

Engineering coagulation factors: can we improve on nature?

Koen Mertens studied biochemistry at Utrecht University. In 1985, he obtained his PhD at Leiden University on a thesis on the biochemistry of hemophilia. In 1982 he joined the CLB, now Sanquin, where he held various positions until he became head of the Dept of Plasma Proteins in 2000. In addition, he is professor in Pharmaceutical Plasma Proteins at Utrecht University since 1999.

Professor Mertens' research has been focusing on coagulation factors VIII, IX and X since 1978. In his presentation, he will discuss the developments in this field from a personal perspective. While the advent of protein engineering has brought many options to improve on nature, only a few have been translated in improved therapeutics for the treatment of patients with hemophilia. The various benefits and limitations of these innovations will be discussed.

Posters

Numbers refer to the poster board

Poster number	Title	Presenter
Topic	**Proteomics and structural biology**	
1	Conformational activation of ADAMTS13	Brenda Luken (oral)
2	Quantitative proteomics reveals subset-specific functional and metabolic programming in human regulatory t cells	Eloy Cuadrado
3	Unraveling PAR1 signaling in endothelial cells using quantitative phosphoproteomics	Bart van den Eshof
4	Regulation of Weibel-Palade body release by syntaxin-3 containing snare-complexes	Maaike Schillemans
5	Structural and functional changes associated with development of the platelet storage lesion	Maaike Rijkers
6	Igg subclass specific rheumatoid factor discriminates restricted RF responses from responses associated with isotype switching and ACPA positivity	Willem Falkenburg
7	Substrate induced rigidification of Factor IX protease domain examined by hydrogen-deuterium-exchange	Eduard Ebberink
8	Characterization of engineered IgG hexamers and their role in complement activation	Guanbo Wang (oral)
9	Endocytosis of coagulation Factor V by megakaryocytes	Annemarie Koornneef
10	Quantitative cell-surface proteomics to unravel the effects of pro-inflammatory cytokines on endothelial cells	Eelke Béguin Richard Pouw
11	The neonatal platelet proteome	Eva Stokhuijzen
Topic	**Therapeutics of tomorrow**	
12	Glyco-engineering of the IgG Fc Glycan	Gillian Dekkers
13	Systematic comparison of drug tolerant assays for anti-drug antibodies in a cohort of adalimumab-treated rheumatoid arthritis patients	Karien Bloem
14	Laboratory assessment of the reversal of the anticoagulant action of the new generation oral anticoagulants by prothrombin complex concentrate	Herm-Jan Brinkman
15	Unraveling the interaction and processing of VWF by human monocyte derived dendritic cells and its role in FVIII endocytosis and HLA-DR presentation	Robin Hartholt
16	SIRPα is expressed by human NK cells	Louise Treffers

Poster number	Title	Presenter
17	Inhibition of nucleosome releasing activity in serum of patients with systemic lupuserythematosus	Gerben Marsman
18	Factor VII-activating protease: a double-edged sword in inflammation?	Ingrid Bulder
19	HLA-DRB1*11 transgenic mice provide a model system for acquired thrombotic thrombocytopenic purpura	Fabian Verbij
20	Potentiating the functionality of a crucial complement regulator with a monoclonal antibody	Richard Pouw
21	Hemostatic phenotype of processed solvent detergent plasma	Herm-Jan Brinkman
22	The effectiveness of an allogeneic single donor fibrin sealant on functional knee recovery, a multicenter randomized controlled trial	Joost van Hilten
23	Factor Xa overcomes inhibition by the direct Factor Xa-inhibitors following insertion of a structural element from the serine protease domain of snake venom Factor Xa	Daniël Verhoef (oral)

1. Conformational activation of ADAMTS13

Luken, BM[1], Crawley JTB[2], Phillips R[2], Thomas M[2], Collins RF[3], Deforche L[4], Vanhoorelbeke K[4], Lane DA[5]

1) Sanquin Research, Immunopathology, Amsterdam, The Netherlands

2) Imperial College London, London, United Kingdom

3) University of Manchester, Manchester, United Kingdom

4) KU Leuven, Kortrijk, Belgium

5) Imperial College London, London, United Kingdom

Background

ADAMTS*13* regulates von Willebrand factor (VWF) platelet-binding function by cleaving unfolded von Willebrand factor (VWF) under flow.

Aim

Determine whether ADAMTS*13*-mediated VWF proteolysis depends on its own conformational activation.

Methods

We used ADAMTS*13*/VWF variants and antibodies for kinetic analysis/binding assays, and electron microscopy to determine ADAMTS*13* conformation.

Results

WT-ADAMTS*13* revealed ~2.5-fold reduced activity compared with ADAMTS*13* lacking its C-terminal tail (MDTCS) or CUB*1-2* domains (WTδCUB1-2), suggesting that the CUB domains limit ADAMTS*13* function. ADAMTS*13* activity was enhanced ~2.5-fold by preincubation with anti-CUB mAb20E9 or VWF-D4CK (which binds CUB*1-2*). The isolated CUB*1-2* domains bound MDTCS, and inhibited activity by 2.5-fold. A gain-of-function (GoF)-ADAMTS*13* spacer variant was ~2.5-fold more active than WT-ADAMTS*13*, but not further activated by mAb20E9 or VWF-D4CK and unable to bind/be inhibited by CUB*1-2*, suggesting that the inhibitory CUB domains interact with the spacer domain that is disrupted in GoF-ADAMTS*13*. Electron microscopy demonstrated a ‘closed’ conformation of WT-ADAMTS*13* and a more ‘open’ conformation for GoF ADAMTS*13*.

Summary/conclusion

ADAMTS*13* circulates in a closed conformation, which is maintained by a CUB-spacer domain binding interaction. ADAMTS*13* becomes activated on demand through interaction of its CUB domains with VWF, simultaneously exposing the cryptic spacer epitope as target for autoantibodies in TTP.

2. Quantitative proteomics reveals subset-specific functional and metabolic programming in human regulatory T cells

Cuadrado E[1], Van den Biggelaar M[2], Meijer A[2], Van Lier R[1], Borst J[3], Amsen D[1]

1) Sanquin Research, Hematopoiesis, Amsterdam, The Netherlands

2) Sanquin Research, Plasma Proteins, Amsterdam, The Netherlands

3) Netherlands Cancer Institute (NKI), Amsterdam, The Netherlands

Background

Regulatory T cells (Tregs) are critical for the maintenance of self-tolerance and immune homeostasis and are of great clinical interest for many medical applications. It is increasingly recognized that distinct subpopulations of Tregs exist.

Aims

We aim to better characterize human Treg subpopulations through untargeted label-free proteomics on highly purified human Treg cells.

Methods

Human Treg subpopulations were isolated from buffy coats and FACS-sorted based on the membrane expression of CD*4*, CD*127*, CD45RA and CD*25*. Isolated cells were lysed and proteins were subjected to reduction, alkylation and in-solution digestion with trypsin. Purified peptides were analyzed on an Orbitrap Fusion mass spectrometer.

Results

MS data identified 5,220 unique proteins, of which 355 are differentially expressed amongst the different T cell subsets. Interestingly, we find that there are major differences between different Treg subpopulations and that only a small subset of proteins defines a Treg 'core signature'. The majority of this core proteome has not previously been recognized by widely used transcriptome analyses. Moreover, our data infers remarkable metabolic differences between the different Treg cell subsets.

Conclusion

Newly identified Treg-specific targets may provide guiding targets for more in-depth signaling studies and new means to manipulate these important cells for clinical applications.

3. Unraveling PAR1 signaling in endothelial cells using quantitative phosphoproteomics

Van den Eshof BL, Van Alphen FPJ, Mertens K, Meijer AB, Van den Biggelaar M
Sanquin Research, Plasma Proteins, Amsterdam, The Netherlands

Thrombin-induced endothelial signaling has been recently dissected, revealing thousands of regulated phosphorylation sites. Proteinase-activated receptor 1 (PAR1) is the predominant thrombin receptor in endothelial cells (ECs), however it is unclear whether it accounts completely for this extensive phosphoregulation.
The aim of this study is to dissect the contribution of PAR*1*, additional PARs and other receptors in thrombin signaling using an unbiased phosphoproteomic approach.
Blood outgrowth endothelial cells (BOECs) and platelets were stimulated with PAR*1*-peptide(10µM) or thrombin(1U/ml) in presence/absence of PAR*1* antagonist(100nM Vorapaxar). Platelet aggregation and endothelial barrier function were measured using aggregometry and ECIS. BOECs were metabolically labeled using SILAC and stimulated for 2/10 min as described above. Phosphorylated peptides were enriched using TiO*2*, detected by Orbitrap Fusion Tribrid MS and analyzed using MaxQuant.
In addition to inhibiting PAR*1*-mediated platelet aggregation, vorapaxar completely blocked thrombin- and PAR*1*-mediated endothelial barrier disruption. Principal component analysis of quantified phosphosites(>3300) and hierarchical clustering of regulated phosphosites(>200) showed that thrombin and PAR*1*-peptide induced identical phosphoregulation, which was completely blocked by vorapaxar.
This study indicates that only PAR*1* mediates thrombin-induced phosphoregulation in ECs. This approach will now be applied to further dissect signaling networks induced by different PAR*1* agonists.

4. Regulation of Weibel-Palade body release by syntaxin-3 containing snare-complexes

Schillemans M, Van Breevoort D, Wahedi M, Voorberg J, Van den Biggelaar M, Bierings R
Sanquin Research, Plasma Proteins, Amsterdam, The Netherlands

Background

Vascular endothelial cells contain unique rod-shaped secretory granules, called Weibel-Palade bodies (WPBs), which contain the hemostatic glycoprotein von Willebrand factor (VWF) as their main component. Several components that are critical for regulated WPB exocytosis have been identified, but the mechanism remains unclear. We have previously identified syntaxin binding protein 1 (STXBP*1*) as a positive regulator of WPB release.

Aim

In this study we investigate the role of a STXBP*1* binding partner, the t-SNARE syntaxin-*3*, in the regulation of WPB exocytosis.

Methods

We characterized the subcellular location of syntaxin-*3* in endothelial cells using immunocytochemistry. To further explore its role in WPB biology we mapped the endothelial interaction partners of syntaxin-*3* through an unbiased mass spectrometry approach using pull downs of lentivirally expressed mEGFP-syntaxin-*3* with anti-GFP nanobeads. Additionally, VWF secretion assays were performed after the depletion of syntaxin-*3* in endothelial cells.

Results

Syntaxin-*3* was primarily associated with WPBs. Among its interaction partners are various SNAREs and associated proteins such as syntaxin binding proteins 2 and 5 (STXBP*2/5*), N-ethylmaleimide-sensitive factor (NSF), SNAP*23* and α-SNAP. Preliminary results indicate that depletion of syntaxin-*3* leads to decreased Ca^{2+};-mediated VWF secretion.

Conclusions

Our data position syntaxin-*3* as a WPB-linked SNARE-protein that regulates secretion of VWF.

5. Structural and functional changes associated with development of the platelet storage lesion

Rijkers M[1], Voorberg J[1], Leebeek FW[2],Jansen AJG[1]
1) Sanquin Research, Plasma Proteins, Amsterdam, The Netherlands
2) Erasmus University Medical Center, Rotterdam, The Netherlands

Background

Storage of platelet products is limited to 7 days and during storage platelets undergo modifications that alter the functionality and structure, called the platelet storage lesion (PSL). The limited shelf life of platelets provides a major limitation for current platelet transfusion approaches.

Aim

We want to investigate the mechanisms that contribute to development of the PSL, focusing on loss of GPIba and P-selectin exposure.

Methods

Isolated platelets were stored under conditions that allowed for rapid development of the PSL. The ristocetin-induced binding of VWF to platelets was studied. We also monitored surface expression of sialic acid/GlcNAc, GPIba, GPV, GPIX, P-selectin and Annexin V using flow cytometry.

Results

During storage the surface expression of GPIba declined after 48h; also ristocetin-induced VWF binding started to decline at this time-point. Loss of sialic acid was only observed after prolonged storage (6 days). Gradually increase of P-selectin exposure was observed indicating continuous release of α-granules during platelet storage. Annexin V positive cells were observed after 2 days of storage and gradually increased over time.

Conclusion

Our findings suggest that loss of GPIba as well as continuous release of α-granules contribute to the development of the PSL.

6. Igg subclass specific rheumatoid factor discriminates restricted RF responses from responses associated with isotype switching and ACPA positivity

Falkenburg WJJ[1], Van Schaardenburg D[2], Ooijevaar-de Heer P[1], Wolbink G[2], Rispens T[1]

1) Sanquin Research, Immunopathology, Amsterdam, The Netherlands

2) Dept of Rheumatology, Amsterdam Rheumatology and immunology Center, Reade, Amsterdam, The Netherlands

Background

Evaluation of the rheumatoid factor (RF) response is often limited to measurement of IgM-RF recognizing polyclonal IgG. Distinguishing between polyspecific RF responses reactive with all four IgG subclasses and restricted responses with specificity for only some subclasses could be useful to discriminate pathological from non-pathological RF.

Aims

Investigate the presence and patterns of specific IgG subclass recognition by RF with a newly developed ELISA, thereby discriminating between polyspecific and restricted RF responses.

Methods

Our ELISA uses individually coated recombinant IgG subclasses instead of polyclonal IgG as target antibodies.

Results

In 93 sera previously tested IgM-RF positive in a conventional RF-assay we could discriminate between sera containing polyspecific IgM-RF responses and restricted IgM-RF responses with low or absent relative reactivity against IgG*2*, IgG*3* or IgG*4*. Polyspecific RF responses more often showed RF response maturation, with more isotype switching to IgA-RF. Also in a validation cohort of RF+anti-citrullinated protein antibody(ACPA)+/- arthralgia patients we found restricted RF responses in 35% (49/140) of RF+ACPA- patients, while RF+ACPA+ patients, who have a much higher risk of future arthritis, virtually always (123/128 (96%)) showed a polyspecific RF response.

Conclusion

IgG subclass specific RF distinguishes between immature restricted RF responses and potentially more pathogenic, ACPA-associated polyspecific responses.

7. Substrate induced rigidification of factor ix protease domain examined by hydrogen-deuterium-exchange

Ebberink AHTM, Meijer AB, Mertens K
Sanquin Research, Plasma Proteins, Amsterdam, The Netherlands

FIX needs to become activated to form an effective serine protease. However, activated FIX (FIXa) remains catalytically inactive in absence of cofactor FVIII. To date, it has remained unclear which structural changes enables the assembly with FVIII and its subsequent increase in catalytic activity. To address this issue we have used biomolecular mass spectrometry and hydrogen-deuterium-exchange (HDX). With HDX we could measure the stability and solvent exposure of the amino-acid backbone chain of FIX in different states: FIX, FIX without calcium, FIXa and FIXa containing a substrate inhibitor (FIXa-EGRck). Calcium addition to FIX indicated stabilization of amino-acids within a region spanning from the calcium binding site towards a FVIII interaction site. Surprisingly, FIX and FIXa (containing calcium) did not display any differences in structure dynamics. Possibly, FIXa is rather flexible, suggesting a role for FVIII binding in order to rigidify the FIXa protease domain. FIXa-EGRck did show a rigidification of a surface loop (Ala*390*-Tyr*397*) situated between the FVIII binding site and the active site. The homologous loop in thrombin and FXa is known to bind a sodium ion important to maintain catalytic activity. Our HDX results indicates an important role for the putative sodium binding site in FIXa as well.

8. Characterization of engineered IgG hexamers and their role in complement activation

Wang G[1], De Jong RN[2], Van den Bremer ET[2], Beurskens FJ[2], Schuurman J[2], Parren PWH[2], Heck AJR[3]

1) Utrecht University, Biomolecular Mass Spectrometry, Utrecht, The Netherlands

2) Genmab, Utrecht, The Netherlands

3) Utrecht University, Utrecht, The Netherlands

Complement contributes to the natural immune defense against pathogens, tumors and self-antigens. The classical complement pathway is triggered by the interaction between antibodies and the initial complement component C1q. The biophysical characterization of this complex has been elusive due to the low affinity of the IgG/C1q interaction. We recently reported complement is activated by IgG hexamers (IgG(*6*)) assembled at cell surface via Fc:Fc interactions, and described an IgG variant that dynamically hexamerized and activated complement. Here we demonstrate this model IgG(*6*) enables detailed characterization of complement initiation by native MS and other techniques. By monitoring the oligomeric states of different IgG variants in solution, we determined the impact of Fc mutations. Complement activation in solution correlated with the level of hexamerization, while both glycan and Fab were dispensable for hexamerization and complement activation. Addition of C1q yielded a stable IgG(*6*)-C1q complex, implying only one C1q-binding site in IgG(*6*) was occupied. IgG(*6*) bound maximally 12 antigens, even in the presence of C*1*, using the capability of native MS to accurately measure MW of protein assemblies. To our knowledge, this is the first time that the stoichiometry of an antigen-antibody-complement complex (40 subunits; > 2 MDa) has been demonstrated experimentally in solution.

9. Endocytosis of coagulation Factor V by megakaryocytes

Koornneef JM, Zappelli C, Koch S, Van der Zwaan C, Mertens K, Meijer AB

Sanquin Research, Plasma Proteins, Amsterdam, The Netherlands

The α-granules of platelets contain a large variety of proteins with diverse and opposing functions. The current paradigm is that most α-granule proteins originate from megakaryocyte (MK) biosynthesis. Fibrinogen and Factor V (FV), however, are completely derived from endocytosis by MKs and/or platelets. Interestingly, distinct uptake pathways appear to exist for these two proteins. While integrin αIIbß*3* mediates the uptake of fibrinogen, endocytosis of FV is suggested to be LRP-*1* dependent. In the present study, we employed MK-like cell lines to further elucidate the LRP-*1* dependent uptake mechanism of FV. To this end, we assessed the uptake of fluorescent FV in the presence and absence of an antagonist of LRP-*1*.
Our results revealed, however, that the uptake mechanism used by the MK-like cell lines is incompatible with a role for LRP-*1*.
We next evaluated the uptake of FV by cells that very effectively internalize ligands in an LRP-dependent manner. We observed that activated FV (FVa), rather than full-length FV was endocytosed.

Thus, our findings suggest that conversion of FV to its activated form FVa may be a prerequisite for LRP-*1* mediated endocytosis..

10. Quantitative cell-surface proteomics to unravel the effects of pro-inflammatory cytokines on endothelial cells

Béguin EP, van den Eshof BL, Mertens K, Meijer AB, Van den Biggelaar M
Sanquin Research, Plasma Proteins, Amsterdam, The Netherlands

During acute inflammation, vascular endothelium responds to pro-inflammatory signals by mediating leukocyte extravasation and promoting vascular leakage of plasma proteins. Literature is available on transcriptional profiling upon stimulation, however, the precise cell surface alterations during these processes, key in cellular interactions, have remained unclear. The aim of this study is to probe cytokine-induced changes on the cell surface of endothelial cells using quantitative proteomics. Blood Outgrowth Endothelial Cells were metabolically labeled using Stable Isotope Labeling with Amino acids in Cell culture (SILAC) and treated with 10 ng/ml IL1ß or TNFafor 24 hours. The cell surface was labeled using a non-membrane permeable NHS-biotin. Pooled cell lysates were processed into peptides using Filter Assisted Sample Preparation (FASP). Biotinylated peptides were enriched, measured at the Orbitrap Fusion Tribrid Mass Spectrometer and analyzed using the MaxQuant and Perseus computational platform.

Our quantitative approach identified >2600 biotinylated peptides, of which 174 were significantly regulated, corresponding to 126 proteins, including the established ICAM1 and VCAM1 and various proteins not previously associated to inflammation.
In conclusion, a novel mass spectrometry approach was developed to quantify cell surface proteome perturbations. Using this approach, various putative novel inflammatory effectors were identified and will be verified employing functional studies.

11. The neonatal platelet proteome

Stokhuijzen E[1], Van Alphen FPJ[2], Van den Biggelaar M[2], Koornneef AJN[2], Van der Zwaan C[2], Kuijk C[3], Mertens K[2], Fijnvandraat K[4], Meijer AB[2]

1) Emma Children's Hospital, Academic Medical Center, Pediatric Hematology, Amsterdam, The Netherlands

2) Sanquin Research, Plasma Proteins, Amsterdam, The Netherlands

3) Sanquin Research, Hematopoiesis, Amsterdam, The Netherlands

4) Emma Children's Hospital, Academic Medical Center, Amsterdam, The Netherlands

It has been suggested that neonatal platelets are less reactive than platelets from adults. A reduced expression of specific platelet integrins or glycoproteins has been proposed to cause this phenomenon. However, decreased platelet functioning has not been directly associated with an increased risk for bleedings in healthy newborns.

This study aims to obtain an overview of the differential protein expression between neonatal and adult platelets.

A label free quantitative mass spectrometry approach was used to compare protein expression of platelets derived from healthy newborns (cord blood) and adults. Platelet proteins were processed by in-solution trypsin digestion and the resulting peptides were analyzed using a nano-LC Orbitrap Fusion mass spectrometer.

Little variation was observed in the level of critical platelet integrins, glycoproteins, a-granule proteins and proteins that contribute to integrin activation. The vitamin K-dependent protein S was among the down-regulated proteins in the neonatal platelet proteome. The upregulated proteins included thromboxane A*2* synthase and enzymes involved in metabolic processes.

There were no apparent differences in the neonatal platelet proteome compared to adults. We propose that a mechanism that is independent of the level of expression of integrins and/or glycoproteins causes the reduced reactivity of neonatal platelets.

12. Glyco-engineering of the IgG Fc Glycan

Dekkers G[1], Plomp HR[2], Koeleman CAM[2], Visser R[1],
Van der Schoot CE[1], Wuhrer M[2], Vidarsson G[1]

1) Sanquin Research, Experimental Immunohematology, Amsterdam, The Netherlands

2) Biomolecular Mass Spectrometry Unit, Leiden University Medical Centre, Leiden,The Netherlands

Background

IgG functions through interaction with the immune system through IgG-Fc receptors (FcγR), the neonatal Fc receptor (FcRn), TRIM21, DC-SIGN and complement (C1q). These interactions are influenced by several factors, including IgG subclass, allotype, and N-linked glycosylation of Asn*297* in the IgG Fc-part. The invariant core of this glycan can contain additional fucose, galactose, sialic-acid, and/or bisecting N-acetylglucoseamine (bisection), resulting in approximately 14 prevailing glycoforms of each subclass. Their relative functional capacity is largely unknown, mostly because of the complex assembly of the glycan structures.

Aims

To assess the functionality of different IgG-glycoforms we aimed to setup methods for production of recombinant antibodies with specific glycoforms.

Methods and results

Using decoy substrates for fucose and galactose we can reduce their incorporation from 95% to 8% and 20% to 6%, respectively, determined by mass spectrometry on the glycopeptide level. Co-transfection of glycosyl-transferases for galactose, sialic-acid and bisection results in an increased incorporation, from 20% to 85%, 0.4% to 37% and 1 to 52%, respectively. These techniques can be combined to create even more IgG glycoforms.

Summary and conclusion

All possible IgG-glycoforms can be created and functionally tested. These glyco-engineering methods might also be transferred to production of other (therapeutic) proteins.

13. Systematic comparison of drug tolerant assays for anti-drug antibodies in a cohort of adalimumab-treated rheumatoid arthritis patients

Bloem K[1], Van Leeuwen A[1], Verbeek G[1], Nurmohamed MT[2], Wolbink GJ[1], Van der Kleij D[1], Rispens T[1]

1) Sanquin Research, Immunopathology, Amsterdam, The Netherlands

2) Jan van Breemen Research Institute/Reade, Amsterdam, The Netherlands

Monoclonal antibodies against TNFα are often used as a treatment for patients with auto-immune diseases. In a portion of the patients, the therapy fails because of decreased drug concentrations in the serum, caused by the presence of anti-drug antibodies (ADA). Although the currently used assays nicely correlate with treatment failure due to ADA responses, the detection of ADA in these assays is hampered by the presence of drug itself, making these assays less suitable for studying the mechanisms underlying immunogenicity. Drug-tolerant assays have the potential to overcome such limitations. In this study, we compared the measurement of ADA to adalimumab in 94 adalimumab-treated rheumatoid arthritis patients using the traditional antigen binding test and four different drug-tolerant assays of which three were newly developed assays for this study. Our results indicate that drug-tolerant assays provide a fairly consistent view on the antibody formation: quantitatively, the results from all four assays correlate well (Spearman $r > 0.9$). However, the percentage of ADA-positive patients ranges from 51 to 66% between assays. The different drug-tolerant assays provide reasonably consistent view on ADA responses, which however, breaks down at the lower end of the detectable range, and highlight that ADA is best reported quantitatively.

14. Laboratory assessment of the reversal of the anticoagulant action of the new generation oral anticoagulants by prothrombin complex concentrate

Brinkman HJM[1], Dinkelaar J[2], Patiwael S[1], Harenberg J[3], Leyte A[4]

1) Sanquin Research, Plasma Proteins, Amsterdam, The Netherlands

2) Onze Lieve Vrouwe Gasthuis, Amsterdam, The Netherlands

3) Ruprecht-Karls-University, Heidelberg, Germany

4) Onze Lieve Vrouwe Gasthuis, Amstedam, The Netherlands

Background

Controversial data are reported on the usefulness of prothrombin complex concentrate (PCC) as reversal agent for the non-vitamin K direct oral anticoagulants (NOACs) dabigatran, rivaroxaban and apixaban.

Aim

Evaluation of the applicability of potential appropriate coagulation assays in analyzing the reversal of NOAC anticoagulation by PCC.

Methods

NOAC anticoagulated plasma or whole blood spiked with PCC was subjected to a variety of coagulation tests including the thrombin generation assay (TGA).

Results

Assays triggered by contact activation did not show inhibitor reversal by PCC. Assays triggered by tissue factor (TF) showed NOAC type and NOAC concentration dependent anticoagulation reversal ranging from partial normalization to overcorrection. Extent of reversal appeared also dependent on the TF concentration and the presence of blood cells. TGA area under the curve (AUC) was the only parameter showing complete reversal of anticoagulation by PCC for all NOACs in the (supra)therapeutic dose range.

Conclusion

TGA-AUC fits with the concept that reversal assessment of NOAC anticoagulation should be based on measurements on the clotting potential or thrombin generating potential of the plasma or whole blood patient sample. Low sensitivity of TGA-AUC for NOACs and its correlation with bleeding are issues that remain to be resolved.

15. Unraveling the interaction and processing of VWF by human monocyte derived dendritic cells and its role in FVIII endocytosis and HLA-DR presentation

Hartholt RB[1], Sorvillo N[1], Sedek M[1], Ten Brinke A[2],
Van der Zwaan C[1], Alphen F[1], Meijer S[1], Voorberg J[1]
1) Sanquin Research, Plasma Proteins, Amsterdam, The Netherlands
2) Sanquin Research, Immunopathology, Amsterdam, The Netherlands

Background

Factor VIII (FVIII) circulates in complex with von Willebrand Factor (VWF), affecting FVIII stability and half-life. Several studies propose that VWF affects FVIII immunogenicity by reducing its uptake by dendritic cells (DCs).
Aim: Here we determined the role of VWF in FVIII endocytosis and MHC class II presentation by DCs.

Methods

Human monocyte-derived DCs were incubated with FVIII, VWF or FVIII/VWF complex. Binding/uptake was analyzed by flow cytometry and confocal microscopy. HLA-DR-peptide repertoire of pulsed DCs was determined using mass spectrometry.

Results

Interestingly, VWF is not internalized by DCs but remains tightly bound to the cell surface. FVIII derived-peptides were still abundantly presented on MHC class II, even when in complex with VWF. This suggests that efficient peptide presentation of FVIII proceeds independently of VWF. When in complex, a repertoire-shift in presented FVIII peptides was found, indicating that VWF influences FVIII presentation.
Surprisingly, few VWF derived peptides were identified when cells were incubated with FVIII/VWF, suggesting that FVIII endocytosis promotes/facilitates uptake of VWF by DCs. No VWF peptides were detected in cells treated with VWF alone.

Conclusions

VWF is not endocytosed by DCs. We also show that VWF has a modulatory effect on HLA-DR-FVIII peptide presentation.

16. SIRPα is expressed by human NK cells

Treffers LW, Van Houdt M, Schornagel K, Kuijpers TW, Matlung HL, Van den Berg TK
Sanquin Research, Blood Cell Research, Amsterdam, The Netherlands

Background

Cancer therapeutic antibodies are used in patients for the treatment of various types of cancer. However, the clinical efficacy of antibody therapy is generally too low to apply in the absence of chemotherapeutics. We and others have previously demonstrated that CD*47*-SIRPα interactions restrict the efficacy of cancer therapeutic antibodies, and that targeting such interactions can substantially potentiate antibody-dependent destruction of tumor cells by phagocytes both *in vivo*; and *in vitro*. Studies in rodents have shown that the inhibitory immunoreceptor SIRPa is selectively expressed on myeloid cells.

Aim

It is important to identify methods for improving the clinical efficacy of cancer therapeutic antibodies by potentially targeting CD*47*-SIRPα interactions.

Methods and results

We have created a new panel of antibodies against human SIRPα and have found that SIRPα is, in addition to human myeloid cells, also expressed on human natural killer (NK) cells. We are investigating whether blocking the interaction between CD*47* expressed on cancer cells and SIRPα on NK cells can, like neutrophils, enhance ADCC and potentially also spontaneous killing.

Conclusion

These findings could provide further support to the idea that targeting of CD*47*-SIRPα interactions can be used to potentiate the clinical efficacy of cancer therapeutic antibodies in cancer patients.

17. Inhibition of nucleosome releasing activity in serum of patients with systemic lupus erythematosus

Marsman G[1], Stephan F[1], De Leeuw K[3], Bulder I[1], Ruinard J[1], Zwart B[1], De Jong J[1], Westra H[2], Bultink I[4], Voskuyl A[3], Aarden L[1], Kallenberg C[2], Zeerleder S[2]

1) Sanquin Research, Immunopathology, Amsterdam, The Netherlands

2) University Medical Center Groningen, Groningen, The Netherlands

3) VU University Medical Center, Amsterdam, The Netherlands

The pathogenesis of Systemic Lupus Erythematosus (SLE) predominantly involves inefficient removal of apoptotic cells. Inadequately removed apoptotic cells expose the immune system to harmful intracellular contents such as (ds)-DNA and histones (nucleosomes), which may result in auto-antibody formation. We have previously described that serum removes nucleosomes from late apoptotic cells. The plasma serine protease factor-VII activating protease (FSAP) is responsible for this activity. Since nucleosomes play a prominent role in the pathogenesis of SLE, we investigated nucleosome release by serum from SLE patients. We show that nucleosome release by sera of SLE patients with active disease is significantly decreased as compared to healthy subjects ($p<0.01$). This decrease during active disease cannot be explained by decreased serum levels or disturbed activation of FSAP. Decreased nucleosome releasing activity correlates with SLEDAI ($r = 0.52$, $p<0.001$) and the level of anti-DNA antibodies ($r = 0.64$, $p<0.001$). Removal of IgG and IgM antibodies from sera of SLE patients restored nucleosome releasing activity. Addition of monoclonal antinuclear antibodies to sera from healthy subjects resulted in inhibition of nucleosome release. In conclusion, SLE patients in exacerbation show decreased nucleosome release which is mainly due to anti-nuclear antibodies. This inhibition may contribute to the propagation of disease.

18. Factor VII-activating protease: a double-edged sword in inflammation?

Bulder I, Marsman, Aarden LA, Luken BM, Zeerleder S
Sanquin Research, Immunopathology, Amsterdam, The Netherlands

Upon incubation of primary or secondary necrotic cells with serum, nucleosomes are released into the extracellular environment. Nucleosome complexes consist of important damage-associated molecular patterns (DAMPS), such as dsDNA, histones and HMGB1, each with well characterized pro-inflammatory properties. In the past we have shown that a single protease in serum is responsible for the efficient release of nucleosomes from dead cells, and have identified this protease as Factor VII-activating protease (FSAP). FSAP is a 76kDa serine protease that circulates in plasma as zymogen that is activated upon contact with primary and secondary necrotic cells. In the present study we set out to characterize the detailed molecular mechanism by which FSAP mediates nucleosome release, and to investigate how the release of nucleosomes from necrotic cells modulates the inflammatory response of monocytes. Our results show that after activation, FSAP cleaves histone H1 and HMGB1, suggesting an anti-inflammatory role for FSAP. In contrast, we find that stimulation of primary human monocytes with FSAP-treated necrotic cells strongly induced IL-6 production, whereas necrotic cells or FSAP alone did not. Our results suggest an important immunomodulatory role for FSAP, but further experiments are required to delineate its precise effects upon necrotic cell clearance.

19. HLA-DRB1*11 transgenic mice provide a model system for acquired thrombotic thrombocytopenic purpura

Verbij FC[1], Kaijen P[1], Fijnheer R[3], Ten Brinke A[2], Sorvillo N[1], Voorberg J[1]

1) Sanquin Research, Plasma Proteins, Amsterdam, The Netherlands

2) Sanquin Research, Immunopathology, Amsterdam, The Netherlands

3) UMC Utrecht, Utrecht, The Netherlands

Background

Thrombotic thrombocytopenic purpura (TTP) is a rare and life-threatening disorder characterized by the systemic aggregation of platelets within the vasculature. Acquired TTP is due to the development of inhibitory antibodies directed towards the von Willebrand factor (VWF) cleaving protease ADAMTS*13*. As yet our knowledge on the etiology of acquired TTP is limited. Three independent studies have shown that HLA DRB*1*11* is overrepresented in patients with acquired TTP. We have recently shown that ADAMTS*13* derived peptides with the core sequence FINVAPHAR are preferentially presented on HLA-DRB*1*11*.

Aim

To determine whether the FINVAPHAR-peptide contributes to the onset of auto-immune TTP in DRB*1*11* transgenic mice.

Methods

HLA-DRB*1*11* transgenic mice were generated and back-crossed to C57Bl6/J mice. HLA-DRB*1*11* transgenic mice were injected for 5 weeks with peptide containing the FINVAPHAR core sequence or a control peptide in conjunction with Incomplete Freunds' adjuvance (IFA).

Results

Significant levels of CD4+ T cell proliferative responses of splenocytes against the infused FINVAPHAR-peptide were observed. No response was seen in mice injected with a control peptide in which the anchor residues required for binding to HLA-DRB*1*11* were modified.

Conclusion

Together our results support a role for HLA-DRB*1*11* binding peptides containing the FINVAPHAR-motif in the onset of auto-immune TTP.

20. Potentiating the functionality of a crucial complement regulator with a monoclonal antibody

Pouw RB[1], Brouwer MC[1], Schmidt CQ[3], Kuijpers TW[2], Wouters D[1]

1) Sanquin Research, Immunopathology, Amsterdam, The Netherlands

2) Sanquin Research, Blood Cell Research, Amsterdam, The Netherlands

3) Institute of Pharmacology of Natural Products & Clininical Pharmacology, University of Ulm, Ulm, Germany

Background

The indispensable complement regulator factor H (FH) protects host cells from complement-mediated damage. Severe diseases like atypical hemolytic uremic syndrome (aHUS) and the most common cause of blindness, age-related macular degeneration, are often associated with heterozygous mutations in FH. Here we describe an unique monoclonal antibody (moAb) that potentiates FH leading to rescue of complement regulation in diseases with impaired FH function.

Methods & results

Complement activation by zymosan could be dose dependently inhibited by addition of the anti-FH moAb, as determined by ELISA. Using a FH-dependent hemolytic assay with sheep erythrocytes, the anti-FH moAb increased FH-mediated complement inhibition. Most strikingly, the decreased functionality of FH in serum of three aHUS patients with known FH mutations could be completely restored by the moAb, as shown in the hemolytic assay. Potentiation was not caused by moAb-dependent FH dimerization as Fab' fragments gave similar results. Instead, the moAb greatly increased FH binding to C3b, as determined by SPR.

Conclusion

We identified an unique moAb capable of potentiating the complement regulator FH, presumably by increasing the binding to C3b. Applying this moAb as a therapeutic could prove to be an invaluable strategy in the treatment of diseases linked to decreased complement regulation.

21. Hemostatic phenotype of processed solvent detergent plasma

Brinkman HJM[1], Pitkänen H[2], Jouppila A[2], Mowinckel MC[3], Lemponen M[2], Patiwael S[1], Sandset PM[3], Lassila R[2]
1) Sanquin Research, Plasma Proteins, Amsterdam, The Netherlands
2) Helsinki University, Helsinki, Finland
3) University of Oslo, Oslo, Norway

Background
Standardized solvent/detergent (S/D)-treated plasma has been developed as an improved alternative to fresh frozen-plasma (FFP) in the management of severe bleeds.

Aim
Exploring compositional modifications that may influence the general applicability of S/D-treated plasma.

Methods
Compositional differences between FFP and S/D-treated plasmas were correlated with hemostatic and fibrinolytic characteristics.

Results
Procoagulant factors, antithrombin, TFPI, protein C and protein S antigen were within the normal range, but S/D-treated plasmas had only 12-14% intact functional protein S. Thrombin generation was subsequently increased, especially at low tissue factor concentration (1 pM). Plasma coagulation times in PT and APTT were normal, but 1.5-fold reduced in thromboelastography at low TF (1 pM). Alpha-2-antiplasmin was reduced with a concomitant 3-4 fold shortened clot lysis time measured by thromboelastography in the presence of TF (10 pM) and plasminogen activator (0.2 µg/ml). Enhanced fibrin degradation could be normalised with tranexamic acid.

Conclusions
S/D-treatment seems to induce a procoagulant phenotype that results from a strongly reduced level of intact single chain protein S. Whether this may correct the apparent haemostatic imbalance as suggested from the increased fibrinolysis remains to be established. Our findings may bear implications in patients with protein S deficiency.

22. The effectiveness of an allogeneic single donor fibrin sealant on functional knee recovery, a multicenter randomized controlled trial

Van Hilten JA[1], Verra WC[2], Honohan A[1], De Wildt-Eggen J[1], Nelissen RGHH[2]

1) Sanquin Research, Clinical Transfusion Research, Leiden, The Netherlands

2) Orthopedics, Leiden University Medical Center, Leiden, The Netherlands

Background

Fibrin sealant in total knee replacement (TKR) may be an effective hemostatic to improve post-operative recovery.

Aim

To quantify the postoperative efficacy of functional knee recovery by an allogeneic single donor fibrin sealant spray applied in TKR.

Methods

A multi-center, randomized, single blinded trial of 498 eligible patients undergoing primary TKR. Patients were randomly assigned to receive standard care or CryoSeal (CS) produced from Parvo-free, fresh frozen plasma by the Sanquin Blood Bank. The primary end point of the study was the overall change in extension (CE) measured at both 2 and 6 weeks after surgery, compared to pre-operative extension.

Results

From 430 patients at week 2 and 454 patients at week 6 the overall mean difference in CE was: 0.7^0 (95%CI; -0.6^0 to 2.0^0) and stratified for drain use 2.6^0 (95% CI; 0.7^0 to 4.6^0) favoring CS compared to -0.5^0 (95% CI; -2.2^0 to 1.3^0) in the group without the drain.

Conclusion

Although there was no difference in change in knee extension between patients treated with CS and standard care, in the subgroup in whom a drain was placed, there was significant difference in CE of 2.6^0; by CS.

23. Factor Xa overcomes inhibition by the direct factor Xa-inhibitors following insertion of a structural element from the serine protease domain of snake venom Factor Xa

Verhoef D[1], Schreuder M[2], Van der Sluijs CF[2], Cheung KL[2], Yang XY[2], Reitsma PH[2], Bos MHA[2]

1) Leiden University Medical Center, Thrombosis and Hemostasis, Leiden, The Netherlands

2) Leiden University Medical Center, Leiden, The Netherlands

Venom Factor Xa (FXa) from the Australian snake P. textilis is highly homologous to human coagulation FXa, but shows minimal sensitivity to inhibition by direct FXa-inhibitors such as Rivaroxaban and Apixaban. Strikingly, venom FXa comprises a unique 13-residue insertion between His*91*-Tyr*99* (chymotrypsin numbering) in the serine protease domain.

To investigate the functionality of recombinant human FXa that comprises the unique structural element derived from venom FXa. We constructed, stably expressed and purified three human FXa variants (FX-A, FX-B, FX-C) that incorporate the insertion from three different venom-FXa isoforms.

All variants were able to sustain prothrombin conversion at significantly higher FXa-inhibitor concentrations (IC_{50} 100-600nM) compared to plasma-derived FXa (pdFXa; IC_{50} 2nM). FXa-initiated (5nM) thrombin generation (TG) in human plasma demonstrated near normal TG profiles for the FXa variants. While apixaban (2μM) dramatically prolonged the lag time and reduced peak thrombin in pdFXa-initiated TG, these parameters were unperturbed with the FXa variants present.

Insertion of venom-FXa regions into human FXa enables protease function in the presence of an excess of direct FXa-inhibitors. As such, these chimeric proteases have the potential to serve as rescue therapeutic agents to overcome the effect of FXa-inhibitors in case of potential life-threatening bleeding events or emergency surgical interventions.

Social Program

Network reception - Thursday 23 April 17.10 - 19.00
Drinks and finger food will be served in the beautiful main hall of the Royal Tropical Institute. This enables you to discuss the sessions and posters while having a drink and snack.

Farewell drinks - Friday 24 April 16.30 - 17.30
On Friday after the last session a farewell reception will be organized in the main hall of the Royal Tropical Institute. We would like to invite you for this reception.

General Information

Coffee/tea and lunch
During the official seminar breaks coffee and tea will be available. On both Seminar days an extended lunch will be served.

Accreditation
The following societies have rewarded accreditation points.
All participants will receive a certficate of attendance by e-mail.
You may be asked to sign a list of attendance for the society in question.

- Dutch Society for Internal Medicine: 10 points for 2 days
- Dutch Society for Clinical Chemistry: applied for
- Dutch Society for Immunology: Accredited by the Dutch College of Medical Immunologists: 6 points for 2 days

Badges
All participants will receive a personal badge upon registration.
You are kindly requested to wear your name badge when attending any meeting or social gathering during the conference.

Registration desk
The registration desk will be open at the following times:
Thursday, 23 April 2015 08.30 - 18.00 hours
Friday, 24 April 2015 08.30 - 16.00 hours

Shops
Most shops in Amsterdam are open from 09.00 to 18.00 hours.
On Thursdays, shops are open till 21.00 hours.

Public transport
Amsterdam has an extended public transport network. It is the easiest and cheapest way to travel in Amsterdam. Should you need any assistance please come to the registration desk.

Taxis
Numerous taxi stands are located throughout Amsterdam.
The telephone number of the central taxi service is
020 - 777 77 77 (country code 31).

Weather
While April may offer lovely spring weather, it can be quite unpredictable and might be chilly in the evening. Temperatures range from 8 to 14°C. As showers might occur, we advise you to bring raincoat or umbrella.

WiFi
Free WiFi is available at the conference venue. The code may be obtained at the registration desk.

Sanquin
Plesmanlaan 125
Po Box 9892
1006 AN Amsterdam
Telephone +31 20 - 512 30 00
www.sanquin.nl/sss